IOSR Journal Of Pharmacy *www.iosrphr.org*
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 6, Issue 6 Version. 3 (June 2016), PP. 08-38

The pharmacology of *Crocus sativus*- A review

Prof Dr Ali Esmail Al-Snafi
Department of Pharmacology, College of Medicine, Thi qar University, Iraq.

Abstract: Saffron contained more than 150 volatile and several nonvolatile compounds, approximately 40–50 constituents have already been identified. It contained apocarotinoid glycosides: in particular crocin (crocetin-betadigentiobioside), colored intensive yellow orange; picrocrocin (glycosidic bitter principle, up to 4%): the apocarotinoids and picrocrocin were presumably breakdown products of a carotinoid-digentiobioside-diglucoside (protocrocin); volatile oil (0.4 to 1.3%): [(4,5-dehydro-betacyclocitral (safranal), 4-hydroxy-beta-cyclocitral (breakdown products of the picrocrocin)]; carotinoids: lycopene, alpha-, beta-, gamma-carotene; fatty oil and starch. The previous pharmacological studies revealed that saffron possessed antidepressant, anticonvulsant, antianxiety, memory improvement, for the treatment of tremor and morphine-withdrawal syndrome, antidiabetic, antioxidant, dermatological, immunological, cardiovascular, respiratory, reproductive, gastrointestinal, smooth muscle relaxation, anticancer, antiparasitic, anti-inflammatory, analgesic, protective (hepatic, renal, CNS) and many other pharmacological effects. This review was designed to highlight the chemical constituents and pharmacological effects of *Crocus sativus*.

Keywords: *constituents, pharmacology, Crocus sativus, saffron*

I. INTRODUCTION

The knowledge of plant properties was acquired by ancient civilization that passed down from generation to generation until today. In the last few decades there has been an exponential growth in the field of herbal medicine. Plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives [1-70]. Saffron contained more than 150 volatile and several nonvolatile compounds, approximately 40–50 constituents have already been identified. It contained apocarotinoid glycosides: in particular crocin (crocetin-betadigentiobioside), colored intensive yellow orange; picrocrocin (glycosidic bitter principle, up to 4%): the apocarotinoids and picrocrocin were presumably breakdown products of a carotinoid-digentiobioside-diglucoside (protocrocin); volatile oil (0.4 to 1.3%): [(4,5-dehydro-betacyclocitral (safranal), 4-hydroxy-beta-cyclocitral (breakdown products of the picrocrocin)]; carotinoids: lycopene, alpha-, beta-, gamma-carotene; fatty oil and starch. The previous pharmacological studies revealed that saffron possessed antidepressant, anticonvulsant, antianxiety, memory improvement, for the treatment of tremor and morphine-withdrawal syndrome, antidiabetic, antioxidant, dermatological, immunological, cardiovascular, respiratory, reproductive, gastrointestinal, smooth muscle relaxation, anticancer, antiparasitic, anti-inflammatory, analgesic, protective (hepatic, renal, CNS) and many other pharmacological effects. This review was designed to highlight the chemical constituents and pharmacological effects of *Crocus sativus.*

II. PLANT PRPFILE

Synonyms: *Crocus officinalis* Martyn [71].

Taxonomical classification:

Kingdom: Plantae; **Division**: Magnoliophyta; **Class**: Liliopsida; **Order**: Asparagales; **Family**: Iridaceae; **Genus**: *Crocus;* **Species**: *Crocus sativus*[72-73].

Nomenclature and common names:

The name saffron is derived from Arabic za´-faran, be yellow. The spice was known to the Greeks as Krokos (as mentioned by Homer in the Iliad), but the name is pre-Greek and possibly of Babylonian-Assyrian origin. Common names were: **Arabic**: Za'faran**; Chinese**: Fan hong hua, Xi hong hua (medicinal name); **English**: autumn crocus, saffron, saffron crocus; **French**: Safran, Safran cultivé; **German**: Safran, Safran-Krokus, Echter Safran, Saffran, Echter Saffran; **Hindi**: Zaafraan, Kesar**; Portuguese**: açafrão; **Spanish**: azafrán; **Swedish**: saffranskrokus [72-73].

Distribution

The plant was believed to have originated in the eastern Mediterranean, probably Asia Minor and Persia [74]. Commercial saffron was produced in Spain, Italy, Greece, Turkey, and Morocco in the Mediterranean region,

Iran in Central Asia, and Kashmir and Nepal in the Himalayas. It was also produced, in very small quantities, in Australia, China, Austria, Switzerland, France, California, Myanmar, and the Argentine [75-76].

Description

Flower and Fruit: The lily-like flowers have two 2 bracts at the base. There is a pale violet-veined calyx, yellow anthers and a white filament. The thread-like style is 10 mm long. The stigma is bright orange. The plant is non-fruit-bearing. Leaves, Stem and Root: It is a perennial that grows 8 to 30 cm high. There is a large squat tuber, surrounded by reticulate and fibrous sheaths. The leaves are erect or splayed, narrow, and have a ciliate margin and keel. Saffron is produced by drying the brown-red stigma over fire[77].

Traditional uses:

Saffron has long been used as both spice and medicine by a number of cultures. It was mentioned that saffron stigma was used as a medicine over 3,600 years ago [78]. In the Middle East, saffron was considered as carminative, antispasmodic, thymoleptic, cognition enhancer, aphrodisiac, and emmenagogue [79]. In traditional Chinese medicine, it was used in menorrhagia, amenorrhea, high-risk deliveries and postpartum lochiostasis. In India, Saffron was used for bronchitis, sore throat, headache, vomiting and fever [77].

Parts used: The medicinal parts were the stigma and style [77].

Chemical constituents:

Saffron contained more than 150 volatile and several nonvolatile compounds, approximately 40–50 constituents have already been identified [80-84].

The plant contained apocarotinoid glycosides: in particular crocin (crocetin-betadigentiobioside), colored intensive yellow orange; picrocrocin (glycosidic bitter principle, up to 4%): the apocarotinoids and picrocrocin were presumably breakdown products of a carotinoid-digentiobioside-diglucoside (protocrocin); volatile oil (0.4 to 1.3%): [(4,5-dehydro-betacyclocitral (safranal), 4-hydroxy-beta-cyclocitral (breakdown products of the picrocrocin)]; carotinoids: lycopene, alpha-, beta-, gamma-carotene; fatty oil and starch [71, 77, 80, 85-86].

Twelve components were isolated saffron stigmas: crocin-1, crocin-2, crocin-3, picrocroein, acid form of picrocrocin, HTCC-diglycosil-kaempferol, trans-crocin-4, trans-crocin-2, trans-crocin-3, safranal, crocetin and cis-crocin-3 [87]. However, chemical analysis of pure saffron stigmas obtained from four different countries, Azerbaijan, Spain, India and Iran, showed that they were contained (milligrams per gram of total extract): picrocrocin 3.92-32.45, hydroxy-trimethyl- carboxaldehyde-ciclohexene (HTCC) 0.42-2.09; kaempferol 0.26-1.14; (crocin 1) 6.76-48.75; (crocin 2) 3.67-37.34; (crocin 3) 0.23-2.70; (crocin 4) 0.35-2.33; safranal 2.81-8.59; (crocin 5) 1.26-12.73; (crocin 6) 0.55-5.62 [88].

New monoterpenoid compounds, crocusatins F, G, H, I, J, K, L together with a new naturally occurring acid, (3S),4-dihydroxybutyric acid, were isolated from an aqueous and ethanolic extracts of the stigmas of *Crocus sativus* (saffron) [89-91].

The phenolic and flavonoid compounds of saffron stigma were examined using reversed phase (RP)-HPLC. The total phenolics value for methanolic saffron extract was 6.54 ± 0.02 mg gallic acid equivalent (GAE)/g dry weight (DW), and the total flavonoids were 5.88 ± 0.12 mg rutin equivalent/g DW [92].

Total phenolic content (TPC) of the methanolic extract of *Crocus sativus* flowers was 86.65 mg/g gallic acid equivalents [93].

Saffron petal also contained protein (10.20%), fat (5.3%), ash (7.00%), fiber (8.80%), sodium (25.75 mg/100 g), potassium (542.13 mg/100 g), calcium (486.25 mg/100 g), copper (0.87 mg/100 g), iron (17.99 mg/100 g), magnesium (2.93 mg/100 g), zinc (1.80 mg/100 g) and phosphorus (209.90 mg/100 g) [94].

Pharmacological effects:

Pharmacokinetics:

Orally administered crocin is not absorbed either after a single dose or repeated doses. It was excreted largely through the intestinal tract following oral administration. Plasma crocetin concentrations did not tend to accumulate with repeated oral doses of crocin and the intestinal tract serves as the important site for the crocin hydrolysis. It has been observed that orally administered crocins were hydrolysed to crocetin before or during intestinal absorption, and the absorbed crocetin was partly metabolized to mono- and diglucuronide conjugates [95-96].

Central nervous effects:

Antidepressant:

The antidepressant properties of stigmas and corms of *Crocus sativus* was studied experimentally. The aqueous ethanol extract of *Crocus sativus* corms was fractionated on the basis of polarity. Among the different fractions, the petroleum ether and dichloromethane fractions at doses of 150, 300, and 600 mg/kg showed significant antidepressant-like activities in dose-dependent manners, by means of behavioral models of depression. The immobility time in the forced swimming test and tail suspending test was significantly reduced by the two fractions, without accompanying changes in ambulation when assessed in the open-field test. By means of a gas

chromatography-mass spectrometry technique, twelve compounds of the petroleum ether fraction were identified. Aqueous stigmas extract also exerted antidepressive effects in the behavioral models. Crocin 1 and crocin 2 of the aqueous stigmas extract were identified by a reversed-phase HPLC analysis. The data indicated that antidepressant-like properties of aqueous stigma extracts attributed to crocin 1[97].

The efficacy of hydroalcoholic extract of *Crocus sativus* (stigma) in comparison with fluoxetine in the treatment of mild to moderate depression was studied in a 6-week double-blind, randomized trial. Forty adult outpatients who met the Diagnostic and Statistical Manual of Mental Disorders, fourth edition for major depression based on the structured clinical interview for DSM-IV and with mild to moderate depression were participated in the trial. Patients were randomly assigned to receive capsules of saffron 30 mg/day (BD) (Group 1) and capsule of fluoxetine 20 mg/day (BD) (Group 2) for a 6-week study. Saffron at this dose was found to be effective similar to fluoxetine in the treatment of mild to moderate depression ($F = 0.13$, d.f. = 1, $P = 0.71$). There were no significant differences between the two groups in terms of observed side effects [98].

The efficacy of petal of *Crocus sativus* was compared with fluoxetine in the treatment of depressed outpatients in an 8-week pilot double-blind randomized trial. Forty adult outpatients who met the DSM- IV criteria for major depression based on the structured clinical interview for DSM- IV were participated in the trial. Patients have a baseline Hamilton Rating Scale for Depression score of at least 18. In this double-blind and randomized trial, patients were randomly assigned to receive either capsule of petal of *Crocus sativus* 15 mg bid (morning and evening) or fluoxetine 10 mg bid (morning and evening) for a 8-week. At the end of trial, petal of *Crocus sativus* was found to be effective similar to fluoxetine in the treatment of mild to moderate depression ($F=0.03$, d.f.=1, $P=0.84$). In addition, in the both treatments, the remission rate was 25%. There were no significant differences in the two groups in terms of observed side effects [99].

The non selective serotonin (5-HT) receptor agonist mCPP is known to induce obsessive–compulsive disorder (OCD-like) behavior (excessive self-grooming) in rodents and exacerbated symptoms in patients with OCD. Crocins (30 and 50 mg/kg, ip) in rats attenuated mCPP-induced excessive self-grooming. The results also indicated that the effects of crocins on an animal model of OCD cannot be attributed to changes in locomotor activity, the effect could be attributed to interaction between crocins and the serotonergic system [100].

In a randomized, double-blind study, 30 mg of saffron extract (in capsules) given for 6 weeks resulted in significant alleviation of depression compared to placeb group, and no side effects were recorded. Many follow-up double blind trials carried out on saffron preparation compared with imipramine and fluoxetine; showed that saffron possessed antidepressant effects [98,101-103].

The molecular mechanism of antidepressant effect of aqueous extract of saffron and its effect on the levels of brain-derived neurotrophic factor (BDNF), VGF neuropeptide, cyclic-AMP response element binding protein (CREB) and phospho-CREB (p-CREB) in rat hippocampus, were investigated. The aqueous extract of saffron (40, 80 and 160 mg/kg/day) and imipramine 10 mg/kg/day were injected intraperitoneally (ip) for 21 days to rats. The FST (forced swimming test) was performed on the days 1^{st} and 21^{st}. The results of FST showed that saffron reduced the immobility time. The protein levels of BDNF, CREB and p-CREB were significantly increased in saffron treated rats. VGF protein expression was also increased, but not significantly. The transcript levels of BDNF was also significantly increased. No significant changes in CREB and VGF transcript levels were observed. The authors concluded that aqueous extract of saffron has antidepressant effects and the mechanism of its antidepressant effect may be due to increasing the levels of BDNF, VGF, CREB and P-CREB in rat hippocampus [104].

Anticonvulsant

The anticonvulsant activity of the aqueous (0.08-0.8 g/kg) and ethanolic extracts (20-40 mg/kg) of *Crocus sativus* stigma (CSS) was studied in mice using pentylenetetrazole (PTZ) and the maximal electroshock seizure (MES) tests. In the PTZ test, CSS delayed the onset of tonic convulsions, but failed to produce complete protection against mortality. In the MES test, both extracts decreased the duration of tonic seizures [105].

The anticonvulsant activities of *Crocus sativus* stigma constituents, safranal and *crocin*, were studied using pentylenetetrazole (PTZ)-induced convulsions in mice. Safranal (0.15 and 0.35 mg/kg body weight, ip) reduced the seizure duration, delayed the onset of tonic convulsions, and protected mice from death. Crocin (22 mg/kg, ip) did not show anticonvulsant activity [106].

Safranal is an effective anticonvulsant, it was an agonist at $GABA_A$ receptors, and the nose to brain delivery via nanoparticle formulation improved its brain delivery [107].

Antianxiety:

The anxiolytic and hypnotic effects of saffron aqueous extract and its constituents, crocin and safranal were studied in mice. Agents were administered intraperitoneally in mice before the experiments for the evaluation of hypnotic activity (induced by sodium pentobarbital, 30 mg/kg, ip), anxiolytic activity (elevated plus maze test), locomotor activity (open field test) and motor coordination (Rotarod test). The aqueous extract reduced the locomotor activity dose dependently. At low doses, saffron showed a significant increase in the time on the open arms of the maze. When using the Rotarod method, the aqueous extract showed considerable effect on

motor coordination of the mice. In the hypnotic test, only a dose of 0.56 g/kg of saffron increased the total sleep. Crocin showed no anxiolytic, hypnotic or myorelaxation effects. Safranal, in higher doses, 0.15 and 0.35 ml/kg, showed anxiolytic effects. Safranal increased the total sleep time dose dependently. This constituent at lower doses (0.05 and 0.15 ml/kg) decreased some locomotion activity parameters. Safranal demonstrated no effects on motor coordination. Based on the results, saffron aqueous extract and safranal showed anxiolytic and hypnotic effects [108].

Intragastric administration of 125–250 mg/kg bw of a 50% ethanol extract of the stigmas showed tranquillizing effect and potentiated the sedative effects of barbiturates in mice [109].

The anxiolytic properties of crocins was investgated in rodents via light/dark test. Crocins, at a dose which did not influence animals' motor activity (50 mg/kg), or diazepam (1.5 mg/kg), increased the rats latency to enter the dark compartment and prolonged the time spent in the lit chamber. Lower doses of crocin (15-30 mg/kg) did not modify animals behavior [110].

Antianxiety-like behavior of aqueous, ethanolic and acetonitrile *Crocus sativus* extracts have been investigated in forced-swimming stress in rats. Different doses of extracts (10, 30, 60 mg/kg) were injected intraperitoneally (ip) in a 9-day period, meanwhile, swimming stress was performed for 15 minutes in four sessions (days 3, 5, 7 and 9). The time performing the followings: immobility, swimming and struggling was measured. Moreover, free fatty acids, glucose, corticosterone and HSP70 were also measured. The outcomes demonstrated that saffron decreased stress significantly by prolonging immobility and decreasing the active behavior swimming, without much effect on struggling. The extracts also showed significant reduction in levels of the stress biomarkers. Acetonitrile was identified as the most effective extract in reducing anxiety. The saffron extracts probably proved anti-stress and sedative properties, partly due to distinct proportion and synergistic impact of the active constituents. On the other hand, crocin and safranal have anti-oxidant and anti-inflammatory powers that may aid to mediate this protective central impact [111].

The effects of saffron water extract and its constituent, safranal was studied on the behavioral and metabolic signs induced by electroshock stress in male Wistar. Animals were received intra-amygdala (1, 5, and 10 µg/rat) or intraperitoneal (1, 5, and 10 mg/kg) of the extract, safranal, or saline 5 or 30 min before stress induction. The results showed that stress elevated the corticosterone plasma concentration (115 nmol/l) in the control and intra-amygdala (1, 5, and 10 µg/rat)-treated groups but not in groups received extract or safranal (55 nmol/l) intraperitoneally (1, 5, and 10 mg/kg). Moreover, anorexia was reduced only in groups received the extract (1, 5, and 10 mg/kg) or safranal (1, 5, and 10 mg/kg) intraperitoneally (50 sec). Stress increased sniffing, rearing, locomotion, and coping time, which were decreased by intraperitoneal (1, 5, and 10 mg/kg) but not by intra-amygdala (1, 5, and 10 µg/rat) administration of saffron extract and safranal. The results revealed that saffron water extract and safranal had an important impact on the reduction of both metabolic and behavioral signs of stress in male rats [112].

For the treatment of memory impairment:

The recent behavioural and electrophysiological studies have demonstrated that saffron extract affected learning and memory in experimental animals. Saffron extract improved ethanol-induced impairments of learning behaviours in mice, and prevented ethanol-induced inhibition of hippocampal long-term potentiation, a form of activity-dependent synaptic plasticity that may underly learning and memory. Accordingly, saffron extract or its active constituents, crocetin and crocin, could be useful as a treatment for neurodegenerative disorders accompanying memory impairment [113].

Alzheimer's disease was characterized pathologically by deposition of amyloid beta-peptide (Abeta) fibrils. Oxidation was thought to promote Abeta fibril formation and deposition. To identify agents inhibiting the pathogenesis of Alzheimer's disease, the antioxidant properties of extract of *Crocus sativus* stigmas and its effect on Abeta (1-40) fibrillogenesis was investigated in vtro. The antioxidant properties were determined by measuring the ferric-reducing antioxidant power and Trolox-equivalent antioxidant capacity, while its effects on Abeta-aggregation and fibrillogenesis were studied by thioflavine T-based fluorescence assay and by DNA binding shift assay. The water: methanol (50:50, v/v) extract of *Crocus sativus* stigmas possessed good antioxidant properties, higher than those of tomatoes and carrots, and inhibited Abeta fibrillogenesis in a concentration and time-dependent manner. The main carotenoid constituent (trans-crocin-4) the digentibiosyl ester of crocetin, inhibited Abeta fibrillogenesis at lower concentrations than dimethylcrocetin, revealing that the action of the carotenoid was enhanced by the presence of the sugars. The result suggest the possible use of *Crocus sativus* stigma constituents for inhibition of aggregation and deposition of Abeta in the human brain [114].

Saffron extract was investigated in preventing D-galactose and $NaNO_2$ induced memory impairment and improving learning and memory deficits in amnestic mice. The learning and memory functions in ovariectomized mice were examined by the one way passive and active avoidance tests. In active avoidance test, training in amnestic treated (AT) and amnestic prophylaxis (AP) groups, was improved, there was a significant difference between them and the amnestic control (AC) group. In passive avoidance test, animal's step through latency, as an index for learning, in all test groups was significantly greater than control group.

Total time spent in dark room (DS), which opposed the memory retention ability, in AC was significantly greater than AT group at 1 and 2 hours after full training, while there was no significant difference in this parameter between AP and AT [115].

The acute effects of an alcohol extract of *Crocus sativus* (CS-extract) were studied on learning and memory in step through (ST) and step down (SD) tests in normal, trained and memory-impaired mice. A single oral administration of CS-extract had no effects on memory registration, consolidation or retrieval in normal mice. CS-extract reduced the ethanol-induced impairment of memory registration both in ST and SD tests and the ethanol-induced impairment of memory retrieval in SD test. CS-extract decreased the motor activity (MA) and prolonged the sleeping time induced by hexobarbital [109].

Long-term potentiation (LTP) was thought as a generative mechanism underlying learning and memory via storing information in central nervous system. Electro-neurophysiological assay for LTP was generally used in screening the drugs that can facilitate learning and memory. Methanol extract of saffron (MES) being able to facilitate LTP-induction, and can antagonize the inhibiting effect of 30% ethanol on LTP induction (30 pulses/60 Hz) [116].

The effects of *Crocus sativus*, and its active constituent crocin was evaluated on learning and memory loss and the induction of oxidative stress in the hippocampus by chronic stress. Rats were injected with saffron extract, crocin or vehicle over a period of 21 days while being exposed to chronic restraint stress (6 h/day). Then, animals were trained and tested on a water-maze spatial memory task. They performed four trials per day for 5 consecutive days, and this was followed by a probe trial two days later. At the end of the behavioral testing, several parameters of oxidative stress in the hippocampus were measured. Treatment with saffron extractor crocin blocked the ability of chronic stress to impair spatial learning and memory retention. Relative to controls that received vehicle, stressed animals that received saffron extract or crocin had significantly higher levels of lipid peroxidation products, significantly higher activities of antioxidant enzymes including glutathione peroxidase, glutathione reductase and superoxide dismutase and significantly lower total antioxidant reactivity capacity. Crocin significantly decreased plasma levels of corticosterone, as measured after the end of stress. These results indicated that saffron and its active constituent crocin can prevent the impairment of learning and memory as well as the oxidative stress damage to the hippocampus induced by chronic stress [117].

The effect of aqueous extracts of saffron was investigated in morphine-induced memory impairment. On the training trial, the mice were received an electric shock when the animals were entered into the dark compartment. Twenty-four and forty-eight hours later, the time latency for entering the dark compartment was recorded and defined as the retention trial. The mice were divided into (1) control, (2) morphine which received morphine before the training in the passive avoidance test, (3-5) three groups treated by 50, 150 and 450 mg/kg of saffron extract before the training trial, and (6 and 7) the two other groups received 150 and 450 mg/kg of saffron extract before the retention trial. The time latency in morphine-treated group was lower than control ($p<0.01$). Treatment of the animals by 150 and 450 mg/kg of saffron extract before the training trial increased the time latency at 24 and 48 hours after the training trial ($p<0.05$ and $p<0.01$). Administration of both 150 and 450 mg/kg of the extract before retention trials also increased the time latency ($p<0.01$). The results revealed that the saffron extract attenuated morphine-induced memory impairment [118].

Inhibitors of acetylcholine breakdown by acetylcholinesterase (AChE) constituted the main therapeutic modality for Alzheimer's disease. The inhibition of AChE activity of saffron extract and its constituents was studied by *in vitro* enzymatic and molecular docking studies. Saffron extract showed moderate AChE inhibitory activity (up to 30%), but IC_{50} values of crocetin, dimethylcrocetin, and safranal were 96.33, 107.1, and 21.09 μM, respectively. Kinetic analysis showed mixed-type inhibition, which was verified by in silico docking studies. Safranal interacted only with the binding site of the AChE, but crocetin and dimethylcrocetin bind simultaneously to the catalytic and peripheral anionic sites [119].

The efficacy of *Crocus sativus* was studied in the treatment of patients with mild-to-moderate Alzheimer's disease. Fifty-four Persian adults, 55 years of age or older were participated in a 22-week, double-blind study of parallel groups of patients with AD. The main efficacy measures were the change in the Alzheimer's Disease Assessment Scale-cognitive subscale and Clinical Dementia Rating Scale-Sums of Boxes scores compared with baseline. Adverse events (AEs). Participants were randomly assigned to receive a capsule saffron 30 mg/day (15 mg twice per day) or donepezil 10 mg/day (5 mg twice per day). Saffron at this dose was found to be effective similar to donepezil in the treatment of mild-to-moderate AD after 22 weeks. The frequency of AEs was similar between saffron extract and donepezil groups with the exception of vomiting, which occurred significantly more frequently in the donepezil group [120].

In the treatment of tremor:

The protective and therapeutic effects of aqueous and ethanolic extracts of *Crocus sativus*, and its active consistent, safranal, was studied in the harmaline-induced tremor in mice. To induce tremor, harmaline (30 mg/kg) was injected intraperitoneally. Test groups were also given the aqueous and ethanolic extracts of saffron (40, 80, and 160 mg/kg) or safranal (0.1, 0.3, and 0.5 ml/kg), intraperitoneally, 10 min before harmaline administration (prophylactic study) or 10 min after the onset of tremors (curative study). The latency of onset,

duration, and intensity of tremor were recorded. The extracts (80 and160 mg/kg) dose dependently attenuated duration of harmaline-induced tremors as did reference drug (propranolol 2 and 5 mg/kg). Only the highest dose of extracts (160 mg/kg) attenuated intensity of harmaline-induced tremors. Safranal at the doses of (0.1 and 0.3 ml/kg) but not 0.5 ml/kg attenuated duration and intensity of tremor. Onset of tremor increased with the extracts (80 and 160 mg/kg) in prophylactic study, as the effect observed with propranolol at the dose of 5 mg/kg. Safranal did not affect the latency of tremor [121].

Treatment of morphine withdrawal syndrom

The effects of aqueous and ethanolic extracts of *Crocus sativus* stigma and its constituents were evaluated on morphine-withdrawal syndrome in mice. Dependence was induced using subcutaneous injections of morphine for 3 days. On day 4, morphine was injected 0.5 h prior the interaperitoneal injections of the extracts, crocin, safranal, clonidine (0.3 mg/kg) or normal saline. Naloxone was injected (5 mg/kg ip) 2 h after the final dose of morphine and the number of episodes of jumping during 30 mm was considered as the intensity of the withdrawal syndrome. Clonidine, the aqueous and ethanolic extracts of saffron reduced the jumping activity. Safranal injected (sc) 30 mm prior and 1 and 2 h after the injection of morphine potentiated some signs of withdrawal syndrome. The aqueous extract decreased the movement in all doses (80, 160, 320 mg/kg) and the ethanolic extract decreased it in the dose of 800 mg/kg in open field test. But crocin and the dose of 400 mg/kg ethanolic extract showed no effect on activity in this test. The authors concluded that the extracts and crocin may interacted with the opioid system to reduce withdrawal syndrome [122].

CNS protective effects:

The protective effect of aqueous saffron extract on neurotoxicity induced by aluminuim chloride ($AlCl_3$) was evaluated in mice. Balb/c and C57BL/6 mice were injected with $AlCl_3$, 40 mg/kg/day for 45 days. Each mice strain was divided into four groups: $AlCl_3$ treated group, $AlCl_3$ plus water saffron extract group (administered with saffron extract at 200 mg/kg bw once a day for 45 days, $AlCl_3$ plus honey syrup group (administered with honey syrup at 500 mg/kg bw for 45 days). The control group received no treatment. Oxidative stress and antioxidant status were estimated in the brain and differential display was performed for both mice strains to scan the mRNA in the treated and non treated groups. In addition, the up and down regulated genes were isolated, cloned and sequenced. The sequence analysis was performed and compared with the other genes cited on GenBank. The results showed that there was a decrease in the activity of the antioxidant enzymes ($p \leq 0.001$) such as superoxide dismutase, catalase, and glutathione peroxidase in the $AlCl_3$ groups of both mice strains. The level of brain thiobarbituric acid reactive substances showed a significant increase ($p \leq 0.001$) of lipid peroxidation in the $AlCl_3$ groups. There was an indication of carcinogenicity in the $AlCl_3$ treated group representing an increase in serum tumor markers such as arginase and a-l-fucosidase. More than 350 band patterns were obtained and about 22 different up-down regulated genes were observed. The sequence analysis of the three selected up-regulated genes revealed that they were similar to B-cell lymphoma 2 (Bcl-2), R-spondin and the inositol polyphosphate 4-phosphatase genes (INPP4B), respectively. The R-spondin gene was up-regulated in all examined animals except the control ones but the other two genes were only induced in the animals treated with $AlCl_3$ and honey syrup. The authors conclude that the biochemical and molecular studies revealed the neurotoxicity of $AlCl_3$ in the brains of mice. In addition, there was an ameliorative change with saffron extract and honey syrup against $AlCl_3$ neurotoxicity. The obtained molecular results suggested that $AlCl_3$ made induction for BCL-W gene, which was an anticancer gene or belonged to the DNA repair system in the brain cells, as well as for R-spondin and inositol polyphosphate 4-phosphatase genes, which helped in cell proliferation [123].

The possible reversal effects of saffron against established aluminum (Al)-toxicity was investigated in adult mice. Groups used included Control, Al-treated (50 mg $AlCl_3$/kg/day diluted in the drinking water for 5 weeks) and Al+saffron (Al-treatment +60 mg saffron extract/kg/day intraperitoneally for the last 6 days). Learning/ memory, the activity of acetylcholinesterase [AChE, salt-(SS)/detergent-soluble(DS) isoforms], butyrylcholinesterase (BuChE, SS/DS isoforms), monoamine oxidase (MAO-A, MAO-B), the levels of lipid peroxidation (MDA) and reduced glutathione (GSH), in whole brain and cerebellum were assessted. Brain Al and crocetin, the main active metabolite of saffron, were determined in brain after intraperitoneal saffron administration by HPLC. Al caused memory impairment, significant decrease of AChE and BuChE activity, activation of brain MAO isoforms but inhibition of cerebellar MAO-B, significant elevation of brain MDA and significant reduction of GSH content. Although saffron extract co-administration had no effect on cognitive performance of mice, it reversed significantly the Al-induced changes in MAO activity and the levels of MDA and GSH. AChE activity was further significantly decreased in cerebral tissues of Al+saffron group. The biochemical changes support the neuroprotective potential of saffron under toxicity [124].

The effect of ethanol extract of *Crocus sativus* was evaluated in the treatment of experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mice. EAE was induced by immunization of 8 week old mice with MOG(35-55) with complete Freunds adjuvant. Therapy with saffron was started on the day of immunization. After daily oral dosage the saffron significantly reduced the clinical symptoms in C57BL/6 mice with EAE. Also, treated mice displayed a delayed disease onset compared with control mice. TAC production was

significantly elevated in saffron treated mice. Effect of saffron on serum NO production was not significant. Typical spinal cord leukocyte infiltration was observed in control mice compared with saffron treated mice. The results suggested that saffron was effective in the prevention of symptomatic EAE by inhibition of oxidative stress and leukocyte infiltration to CNS and may be potentially useful for the treatment of multiple sclerosis (MS) [125].

The neuroprotective effect of saffron extract, its active component crocin and gamma-glutamylcysteinylglycine (GSH) was studied in glucose-induced neurotoxicity, using PC12 cells as a suitable *in vitro* model of diabetic neuropathy. Cell viability was quantitated by MTT assay. ROS was measured using DCF-DA by flow cytometry analysis. The result showed that glucose (13.5 and 27 mg/ml) reduced the viability of PC12 cells after 4 days. Saffron extract (5 and 25 mg/ml), crocin (10 and 50 muM) and GSH (10 muM) decreased this toxicity. Glucose toxicity was associated with increased ROS production which reduced by saffron, crocin and GSH pretreatment. The results suggested that saffron and its carotenoid crocin could be potentially useful in diabetic neuropathy treatment [126].

The preventive effect of the aqueous extract of saffron was studied against diazinon (DZN) -induced rise of several specific inflammation, oxidative stress and neuronal damage in rats. The saffron extract inhibited the effect of DZN on these biomarkers levels [127].

The modifying effects of *Crocus sativus* (CS) stigma extract on neurobehavioral activities, malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase, glutathione reductase, glutathione S-transferase, superoxide dismutase (SOD), catalase (CAT), and Na^+,K^+-ATPase activities, and glutamate (Glu) and aspartate (Asp) content were examined in the middle cerebral artery (MCA) occlusion (MCAO) model of acute cerebral ischemia in rats. The right MCA of male Wistar rats was occluded for 2 hours using intraluminal 4-0 monofilament, and reperfusion was allowed for 22 hours. MCAO caused significant depletion in the contents of GSH and its dependent enzymes, with significant elevation of MDA, Glu, and Asp. The activities of Na^+,K^+-ATPase, SOD, and CAT were decreased significantly by MCAO. The neurobehavioral activities (grip strength, spontaneous motor activity, and motor coordination) were also decreased significantly in the MCAO group. All the alterations induced by ischemia were significantly attenuated by pretreatment with CS (100 mg/kg of body weight, po) 7 days before the induction of MCAO and correlated well with histopathology by decreasing the neuronal cell death following MCAO and reperfusion [128].

A rat model of chronic cerebral hypoperfusion was used to determine the effect of saffron extract and crocin on vascular cognitive impairment. Male adult Wistar rats were administered different doses of an aqueous solution of crocin or hydroalcohol extract of saffron intraperitoneally (ip), 5 days after permanent occlusion of the common carotid arteries. Spatial learning and memory were assessed in training trials, 7-11 days after common carotid artery ligation using the Morris water maze. The results showed that the escape latency time was significantly reduced from 24.64s in the control group to 8.77 and 10.47s by crocin (25 mg/kg) and saffron extract (250 mg/kg). The traveled distance to find the platform was also changed from 772 cm in the control group to 251 and 294 cm in the crocin (25 mg/kg) and saffron extract (250 mg/kg) groups. The percentages of time spent in the target quadrant, in comparison with the control group (24.16%), was increased to 34.25% in the crocin (25 mg/kg) and 34.85% in the saffron extract (250 mg/kg) group. Accordingly, saffron extract and crocin improved spatial cognitive abilities following chronic cerebral hypoperfusion, the effect which may be related to the antioxidant effects of these compounds [129].

The ameliorative effect of saffron aqueous extract on hyperglycemia, hyperlipidemia, and oxidative stress was studied in diabetic encephalopathy in streptozotocin induced diabetes mellitus in rats. Saffron at 40 and 80 mg/kg significantly increased body weight and serum TNF-α and decreased blood glucose levels, glycosylated serum proteins, and serum advanced glycation endproducts (AGEs) levels. Furthermore, significant increase in HDL and decrease ($P<0.05$) in cholesterol, triglyceride, and LDL were observed after 28 days of treatment. At the end of experiments, the hippocampus tissue was used for determination of glutathione content (GSH), superoxide dismutase (SOD), and catalase (CAT) activities. Saffron significantly increased GSH, SOD, and CAT in the the hippocampus tissue, but remarkably decreased cognitive deficit, serum TNF-α, and induced nitric oxide synthase (iNOS) activity in hippocampus tissue. Accordingly saffron extract reduced hyperglycemia and hyperlipidemia risk and also reduced the oxidative stress in diabetic encephalopathy rats [130].

Other CNS effects:

The effects of saffron ethanolic extract and its constituent, safranal, on the acquisition and expression of morphine-induced place preference (CPP) were investigated in male Swiss Webster mice. An unbiased place conditioning method was applied for assessment of morphine reward properties. The saffron extract and safranal were administered intraperitoneally during (acquisition) or after induction (expression) of morphine CPP. In a pilot study, the extract and safranal were alone administered to the animals to assess if they have any reward properties. Subcutaneous of morphine (4 and 8 mg/ kg) and extract (50 mg/ kg; ip) induced CPP. Extract (10, 50 and 100 mg/ kg; ip) reduced the acquisition and expression of morphine- induced place preference. The same results were obtained when safranal (1, 5 and 10 mg/ kg, ip) was used [131].

The effect of hydroethanolic saffron extract (CSE) and trans-crocetin was investigated on synaptic transmission. Postsynaptic potentials (PSPs) were elicited by focal electrical stimulation and recorded using intracellular placed microelectrodes in pyramidal cells from rat cingulate cortex. CSE (10-200 μg/ml) inhibited evoked PSPs as well as the isolated NMDA and non-NMDA component of PSPs. Glutamate (500 μM) added into the organ bath induced membrane depolarization. CSE decreased glutamate-induced membrane depolarization. Additionally, CSE at 100 μg/ml decreased NMDA (20 μM) and kainate (1 μM)-induced depolarization, whereas AMPA (1 μM)-induced depolarization was not affected. Trans-crocetin (1-50 μM) showed inhibition of evoked PSPs and glutamate-induced membrane depolarization comparable to CSE. Trans-crocetin at 10 μM decreased NMDA (20 μM)-induced membrane depolarization, but did not inhibit the isolated non-NMDA component of PSPs. The authors conclude that trans-crocetin was involved in the antagonistic effect of CSE on NMDA but not on kainate receptors [132].

Antioxidant effect:

The extract of *Crocus sativus* showed high superoxide dismutase activity by cytochrome c reduction, nitro blue tetrazolium reduction, and pyrogallol autoxidation methods [133].

Antioxidant activity of saffron was tested by Folin-Ciocalteu (F-C) reagent and various free radical species produced in cell-free or cell model systems. Oregano and turmeric methanol extracts were used as reference antioxidants. In the human monocyte system, saffron extracts or free crocetin were found to reduce ROS production as effectively as the phenolic antioxidants [134].

The antioxidant activity of saffron stigmas was evaluated after extraction with different solvents. Results showed that saffron stigma possessed antioxidant activity. The free radical scavenging and ferric reducing power activities were higher for the methanolic extract of saffron stigma at a concentration of 300 μg/ml, with values of 68.2% and 78.9%, respectively, as compared to the corresponding boiling water and ethanolic extracts, but the activities were lower than those of antioxidant standards such as BHT and α-tocopherol [92].

The radical scavenging activities of *Crocus sativus* petals, stamens and entire flowers, which were waste products in the production of the saffron, were determined by employing ABTS radical scavenging method. The high variety of glycosylated flavonoids found in the metabolic profiles and the radical scavenging activities, gave value to *Crocus sativus* petals, stamens and entire flowers as antioxidant therapy [135].

The antioxidant activity of the extract of *Crocus sativus*, and some of its bioactive constituents (crocin, safranal) was studied. Methanol extract of *Crocus sativus* exhibited high antioxidant activity. In trying to approximate a structure-activity relationship, two bioactive constituents of saffron extract were tested, namely crocin and safranal. Crocin showed high radical scavenging activity (50% and 65% for 500 and 1,000 ppm solution in methanol, respectively), followed by safranal (34% for 500 ppm solution). All the tested samples showed high radical scavenging activity, probably due to the ability to donate a hydrogen atom to the DPPH radical [136].

The antioxidant activity of methanolic extract of *Crocus sativus* flowers was evaluated by total phenolic contents (TPC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity (ABTS) and the reducing power. The results showed that TPC was 86.65 mg/g gallic acid equivalents, while DPPH and ABTS for 1 mg/ml concentration were 92.41 and 86.87, respectively. In the reducing power experiment, the IC_{50} value was 231.75 [93].

The antioxidants crocin and kaempferol were purified by flash column chromatography, and identified by thin layer chromatography (TLC), HPLC-DAD, infrared (IR), and nuclear magnetic resonance (^{1}H &^{13}C NMR) spectroscopy. The antioxidant activity was determined with the ABTS and DPPH tests. The antioxidant activities were mainly attributed to carotenoid and flavonoid compounds, notably glycosides of crocin and kaempferol. Crocin and kaempferol in dried petals were 0.6% and 12.6 (w/w), respectively. Petals of *Crocus sativus* have commercial potential as a source for kaempferol and crocetin glycosides, natural compounds with antioxidant activity that were considered to be the active ingredients in saffron-based herbal medicine [86].

The effect of aqueous saffron extract (*Crocus sativus*) and its active constituent, crocin on oxidative stress was evaluated following renal ischemia-reperfusion injury (IRI) in rats. The left kidney was exposed to warm ischemia for 60 min followed by reperfusion for 90 min. The macerated aqueous extract of saffron (with doses of 5, 20 and 80 mg/kg, ip) and crocin (with doses of 50, 200 and 400 mg/kg, ip) were administrated prior to induction of ischemia. Normal saline (10 ml/kg, ip) was injected to control group and a sham group that did not have ischemia-reperfusion. Ischemia-reperfusion (IR) caused a significant increase in thiobarbituric acid reactive species (TBARS) levels ($p<0.001$) and decrement in both antioxidant power (FRAP value) ($p<0.05$) and total thiol concentration ($p<0.001$) in kidney homogenate samples. In crocin pretreated groups, a reduction in TBARS levels (from 85.8 ± 5.4 to 20.9 ± 1.5 nmol/g tissue, $p<0.001$; 400 mg/kg) and elevation in antioxidant power (FRAP value) (from 3.05 ± 0.16 to 4.15 ± 0.16 micromol/g tissue, $p<0.001$; 400 mg/kg) and total thiol concentrations (from 0.38 ± 0.03 to 0.62 ± 0.03 mM, $p<0.001$; 200 mg/kg), as compared with control group, were recorded. The aqueous extract also reduced lipid peroxidation products (from 85.8 ± 5.4 to 15.9 ± 2.6 nmol/g tissue, $p<0.001$; 80 mg/kg) and increased antioxidant power (from 2.98 ± 0.11 to 5.97 ± 0.56 micromol/g tissue, $p<0.001$; 80 mg/kg) in ischemia-reperfusion injured rat kidneys [89].

Protocatechuic acid, kaempferol, and kaempferol 7-O-beta-d-glucopyranoside isolated from *Crocus sativus* were more effective in scavenging alpha, alpha-diphenyl-beta-picrylhydrazyl (DPPH) radicals than alpha-tocopherol [91].

Cardiovascular effects:

The effect of saffron was investigated against acute myocardium damage by anthracyclines compared with electrolysis as a free radical generating system. The Langendorff isolated rabbit heart model was used in this study. In one set of experiments, ROS was generated by electrolysis of the perfused heart solution (3 mA for 30 min) in the presence and absence of saffron extracts at the optimal dose (10 µg/ml). In another set, the heart was perfused with anthracycline, i.e. 30 µM doxorubicin (Doxo) in the presence and absence of 10 µg/ml saffron extracts. Cardiodynamics was evaluated in addition to biochemical and pathological parameters, to emphasize the effectiveness of the treatment with saffron extract using the optimal dose of catalase (150 IU) as a positive control. ROS generated, respectively, by electrolysis and by Doxo significantly ($p<0.05$) affected cardiovascular function; it decreased ventricular pressure (45.02 and 40.41%), heart rate (36.31 and 22.39%) and coronary flow (50.98 and 36.67%). Increased lipid peroxidation of the myocardium was also observed (118.22 and 56.58%), while superoxide dismutase activity decreased (48.33 and 38.70%). The myocardial architecture was altered and the intercellular spaces increased. Saffron perfused during electrolysis helped trap ROS and significantly improved myocardial function; however, saffron was less effective against Doxo, thus suggesting that mechanisms other than oxidative stress underlie Doxo cardiotoxicity [137].

The cardioprotective effect of *Crocus sativus* (saffron) aqueous extract and safranal, the major constituent of the essential oil of saffron was evaluated on lipid peroxidation, biochemical parameters and histopathological findings in isoproterenol (ISO)-induced myocardial infarction in Wistar rats. The saffron extract (20, 40, 80 and 160 mg/kg/day ip) or control were administered for 9 days along with ISO (85 mg/kg, sc at 24 hr interval) on 8^{th} and 9^{th} day in rats. ISO administration induced a statistically significant increase ($p<0.001$) in serum activities of lactate dehydrogenase (LDH), creatine kinase-muscle, brain (CK-MB) and a significant increase ($P< 0.001$) in the levels of thiobarbituric acid reactive substances (TBARs) in the heart as compared to vehicle control rats. Saffron pretreatment (20, 40, 80 and 160 mg/kg ip) or safranal pretreatment (0.025, 0.050, 0.075 ml/kg ip) for 8 days, significantly decreased ($p<0.001$) the serum LDH and CK-MB and myocardial lipid peroxidation as compared to ISO- induced rats. Histological findings of the heart sections confirmed myocardial injury with ISO administration and preserved nearly normal tissue architecture with saffron or safranal pretreatment [138].

The cardioprotection effect of saffron was evaluated in isoproterenol-induced myocardial damage. Male Wistar rats were divided into five groups: control, isoproterenol (ISO) and three saffron (200, 400 and 800 mg/kg) treatment groups. Aqueous extract of saffron or vehicle was administered orally to rats for four weeks. On days 28 and 29, the animals in ISO and saffron treatment groups were administered ISO (85 mg/kg, sc) at an interval of 24 h. On day 30, after recording hemodynamics and left ventricular functions, animals were sacrificed for biochemical, histopathological and electromicroscopical examinations. Isoproterenol challenged animals showed depressed hemodynamics and left ventricular functions as evident by decreased left ventricular rate of peak positive and negative pressure change and elevated left ventricular end-diastolic pressure. Structural and ultrastructural studies further confirmed the damage which was reconfirmed by increased thiobarbituric acid reactive substances ($p<0.001$) and decreased creatine kinase-MB and lactate dehydrogenase ($p<0.001$). In addition, significant reduction in superoxide dismutase and catalase ($p<0.001$) was observed in ISO group. Saffron at all the doses exerted significant cardioprotective effect by preserving hemodynamics and left ventricular functions, maintaining structural integrity and augmenting antioxidant status. Among the different doses used, saffron at 400mg/kg exhibited maximum protective effects which could be due to maintenance of the redox status of the cell which reinforcing its role as an antioxidant [139].

The effect of *Crocus sativus* on Ca^{2+} influx in isolated rat aortas was investigated by using ^{45}Ca as a radioactive tracer. Ca^{2+} uptake in isolated rat aorta rings in normal physiological status was not markedly altered by these drugs, whereas the Ca^{2+} influxes induced by norepinephrine of 1.2 mmol/l and KCl of 100 mmol/l were significantly inhibited by crocus in a concentration-dependent manner. The results showed that extracellular Ca^{2+} influx through receptor-operated Ca^{2+}channels and potential dependent Ca^{2+}channels can be blocked by crocus [140].

The effect of the hydroalcohol extract effects of *Crocus sativus* (saffron) was studied on (i) the basic and rate-dependent electrophysiological properties of the AV node, (ii) remodeling of the AV node during experimental atrial fibrillation (AF) and (iii) the role of nitric oxide (NO) in the effects of saffron on the AV node. Stimulation protocols in isolated AV node were used to quantify AV nodal recovery, facilitation and fatigue in four groups of rabbits. In addition, the nodal response to AF was evaluated at multiple cycle lengths and during AF. Saffron had a depressant effect on AV nodal rate-dependent properties; further, it increased Wenckebach block cycle length, functional refractory period, facilitation and fatigue ($p < 0.05$). NO-synthase inhibitor (L-NAME) prevented the depressant effects of saffron on the AV node ($p < 0.05$). Saffron increased the zone of concealment in experimental AF ($p < 0.05$). It appeared that the depressant effects of saffron were mediated by endogenous NO [141].

The effects of an aqueous-ethanol extract from *Crocus sativus* on heart rate and contractility were examined on isolated guinea-pig hearts. Heart rate and contractility were determined in the presence of four concentrations of the extract (0.1, 0.5, 1.0 and 5.0 mg%) and diltiazem (0.1, 1, 10 and 100 microm) in perfused heart with: (1) ordinary Krebs solution (group 1) and calcium-free Krebs solution (group 2). In group 1, three higher concentrations of diltiazem (1, 10 and 100 microm), but only the highest (5.0 mg%) and two higher concentrations (1.0 and 5.0 mg%) of the extract caused significant reduction in heart rate and contractility, respectively ($p < 0.05$ to $p < 0.001$). In group 2, the highest (100 microm), two higher concentrations (10 and 100 microm) of diltiazem ($p<0.05$ to $p<0.01$), and the highest concentration of the extract showed significant reductions in the heart rate and contractility ($p < 0.05$ to $p < 0.01$). There were significant negative correlations between concentrations of the extract and diltiazem and their effects in both groups ($p<0.01$ to $p<0.001$). The results suggested a potent inhibitory effect of aqueous-ethanol extract from *Crocus sativus* on the calcium channel of guinea-pig heart [142].

The effects of *Crocus sativus* petals' extract on blood pressure was evaluated on anaesthetized rats. Aqueous and ethanol extracts of *Crocus sativus* petals reduced the blood pressure in a dose-dependent manner. Administration of 50mg/100 g of aqueous extract changed the blood pressure from 133.5±3.9 to 117±2.1 (mmHg) [143].

The effects of saffron (*Crocus sativus*) stigma aqueous extract and two active constituents, crocin and safranal, were investigated on blood pressure of normotensive and desoxycorticosterone acetate-induced hypertensive rats. Three doses of crocin (50, 100 and 200 mg/kg), safranal (0.25, 0.5 and 1 mg/kg) and the aqueous extract (2.5, 5 and 10 mg/kg) were administered intravenously in different groups of normotensive and hypertensive animals and their effects on mean arterial blood pressure (MABP) and heart rate (HR) were evaluated. The aqueous extract of saffron stigma, safranal and crocin reduced the MABP in normotensive and hypertensive anaesthetized rats in a dose-dependent manner. Administrations of 10 mg/kg of aqueous extract, 1 mg/kg of safranal and 200 mg/kg of crocin caused 60 ± 8.7, 50 ±5.2 and 51 ± 3.8 mmHg reductions in MABP, respectively. Accordingly, the aqueous extract of saffron stigma had hypotensive properties which appear to be attributable, in part, to the actions of two major constitutes of this plant, crocin and safranal, and safranal was more important than crocin for lowering the blood pressure of rats [144].

The effects of saffron (*Crocus sativus*) stigma aqueous extract was studied on blood pressure of normotensive and desoxycorticosterone acetate (DOCA)-salt induced hypertensive rats. Five weeks administration of three doses saffron aqueous extract (10, 20 and 40 mg/Kg/day) and spironolactone (50 mg/Kg/day) in different groups of normotensive and hypertensive rats (at the end of 4 weeks treatment by DOCA-salt) showed that chronic administration of saffron aqueous extract reduced the MSBP in DOCA salt treated rats in a dose dependent manner. It did not decrease the MSBP in normotensive rats. The data also showed that the antihypertensive effects of saffron did not persist [145].

The influence of *Crocus sativus* (Saffron) aqueous extracts administration on blood pressure, pressure-rate product (PRP) and electrocardiogram (ECG) indices was studied in rat. Animals were divided to control group that orally received tap water, aqueous extracts of saffron 50, 100 and 200 mg/kg/day respectively for seven days. Different doses of saffron had no significant effect on blood pressure and PRP. Higher dose (200 mg/kg) of saffron significantly increased the PR interval, P duration, QT interval ($p<0.01$), QRS interval, QTcn (normalized corrected QT) ($p<0.001$), and JT interval ($p<0.05$) of ECG compared to the control group. In addition, the two other doses only significantly prolonged the QT, QTcn and JT intervals of ECG versus the control group. The SAF200 group also showed a notable increase in RR interval which only was significant compared to the SAF50. There was no significant difference among ST height and T amplitude ranges of different groups. Accordingly, the results revealed that high dose of saffron definitely slowed the electrical conduction velocity in both atrium and ventricle [146].

The synergic effects of saffron and electromagnetic field (EMF) on vascular endothelial growth factor receptor (VEGFR2) gene expression in MCF7 cells were investigated. MCF7 cells were grown in RPMI 1640 medium supplemented with 10% FBS and incubated at 37°C with 5% CO_2. After 24 hr, cells were treated with saffron extract at concentrations of 100, 200, 400 and 800 µg/ml. Forty eight hr after treatment, all flasks were exposed with EMF (50 Hz, 0.004 T). Then total RNA was extracted and cDNA was synthetized using specific primer. Synthetized products were analyzed by real time PCR to determine expression level of VEGFR2. A critical inhibitory effect on VEGFR2 gene expression wasrecorded (20%) at 400 µg/ml. Synergic use of EMF and saffron extract showed most reduction (38%) at 100 µg/ml. On the other hand synergic use of 200, 400 and 800 µg/ml saffron aqua-extract and EMF declined noticeably the VEGFR2 level of gene expression to 29, 35 and 36%, respectively. EMF itself also reduced VEGFR2 up to 25% in comparison with control group which was remarkable at $p<0.001$. Results indicated a decrease in the expression of vascular endothelial growth factor receptor in the samples treated with saffron extract compared to control. This reduction in VEGFR2 level induced by synergic treatment of saffron and EMF revealed an inhibitory effects of saffron on angiogenesis and could be also considered as a promising chemotherapeutic agent in breast cancer treatment [147].

The vasomodulatory effects of crocetin was analyzed in hypertension. Myographical experiments were performed to compare the relaxation induced by acetylcholine (ACH) on aortic rings from normotensive (Wistar) and hypertensive (SHR) rats, incubated with or without crocetin or saffron extract and L-NAME or indomethacin. Extracts were also assayed in deendothelialized rings. Crocetin enhanced the ACH relaxations in aorta from hypertensive (strongly) and normotensive rats (weakly). Crocetin plus L-NAME abolished the relaxant response in SHR but not in Wistar aorta. Crocetin plus indomethacin did not modify the indomethacin response in either SHR or Wistar aorta. Crocetin in rubbed segments did not modify the ACH responses. In contrast, saffron increased this response in rubbed segments from SHR but not Wistar rats. Accordingly, crocetin exerts healthy vasomodulatory effects in hypertension, strongly improving endothelium-dependent ACH relaxations via endothelial nitric oxide but not the cyclooxygenase pathway [148].

Serum triglycerides, total-, LDL-, cholesterol, fecal excretion of fat and cholesterol were significantely inhibited by crocin (100 mg/kg/day) compared to the control group [149].

Crocetin, was administered to rabbits to determine its effect on the development of atherosclerosis. New Zealand white rabbits were given three different diets for eight weeks: a standard diet, a high lipid diet (HLD), or a high lipid + crocetin diet. The HLD group developed hypercholesterolemia and atherosclerosis, while the crocetin-supplemented group decreased the negative health effects of a high lipid diet. However, the results did not show a significant difference in the plasma lipid levels (total, low density lipoprotein (LDL), and high density lipoprotein (HDL) cholesterol) between the HLD and crocetin groups but showed significant decrease in the aorta cholesterol deposits, atheroma, foam cells, and atherosclerotic lesions. The authors suggested that nuclear factor kappa B (NF-κB) activation in the aorta was suppressed by crocetin which in turn decreased the vascular cell adhesion molecule-1 (VCAM-1) expression [150].

Administration of a monthly intramuscular injection of crocetin reduced serum cholesterol concentrations by 50%, and the severity of atherosclerosis by 30% in rabbits fed an atherosclerosis-inducing diet [151].

Crocin exerted antiatherosclerotic effects through decreasing the level of Ox-LDL that plays an important role in the initiation and progression of atherosclerosis [152].

Fifty milligrams of saffron dissolved in 100 ml of milk was administered twice a day to human subjects, the significant decrease in lipoprotein oxidation susceptibility in patients with coronary artery disease (CAD) indicated the potential of saffron as an antioxidant [153].

A hot aqueous extract 10–100 mg/ml, prolonged partial thromboplastin and prothrombin times, and inhibited platelet aggregation in human platelets induced by adenosine diphosphate and collagen *in vitro* [154].

The inhibitory activity of saffron extract was studied on human platelets. Platelet aggregation and lipid peroxidation were evaluated with platelet rich plasma (PRP) and platelet membranes obtained from blood of healthy human volunteers. Human platelets were subjected to stimulation with a variety of agonists like ADP (61 microM), epinephrine (76 microM), collagen (11 microg/ml), calcium ionophore A 23187 (6 microM) and ristocetin (1.25 microg/ml) in the presence and absence of saffron extract. The inhibitory effect was dose dependent with concentrations varying between 0.16 to 0.80 mg and time dependent. A significant decrease was observed in malondialdehyde (MDA) formed, one of the end products of arachidonic acid metabolism and of serotonin released from dense granules of platelets at respective IC_{50}. Lipid peroxidation in platelet membranes induced by iron-ascorbic acid system was inhibited by saffron extract significantly with IC_{50} of 0.33 mg. Hence, it may be said that aqueous extract of saffron may have component(s), which protect platelets from aggregation and lipid peroxidation [155].

Respiratory effects:

The prophylactic effect of the extract of *Crocus sativus* and its constituent, safranal on lung pathology and total and differential white blood cells (WBC) of sensitized guinea pigs was examined. Guinea pigs were sensitized with injection and inhalation of ovalbumin (OA). One group of sensitized guinea pigs were given drinking water alone (group S) and three groups were given drinking water containing three concentrations of safranal (S+SA1, S+SA2 and S+SA3 groups), three groups, drinking water containing three concentrations of extract (S+CS1, S+CS2 and S+CS3 groups) and one group drinking water containing one concentration of dexamethasone (S+D group). Treatment of S animals with dexamethasone, all concentrations of the extract and safranal significantly improved lung pathological changes, WBC and serum histamine levels compared to group S ($p<0.05$-0.001). Treatment of S group with first concentration of safranal also decreased total WBC. Treatment with safranal was more effective in improvement of most pathological changes, total and differential WBC count as well as serum histamine level ($p<0.05$-0.001). These results indicated a preventive effect of the extract of *Crocus sativus* and its constituent safranal on lung inflammation of sensitized guinea pigs [156].

The effect of the extract of *Crocus sativus* and one of its constituents, safranal, on the inflammatory changes of sensitized guinea pigs was examined. Eight groups of sensitized guinea pigs to ovalbumin were studied. One group was given drinking water alone (group S), while other 7 groups were received drinking water containing; three concentrations of safranal (4, 8 and 16 μg/ml), three concentrations of extract (0.1, 0.2 and 0.4 mg/ml) and one concentration of dexamethasone (S+D group). Total and differential white blood cell (WBC) counts in blood were evaluated. Total blood WBC number, eosinophyl and lymphocyte percentage in blood were

increased, but neutrophil decreased in sensitized animals compared to those of control groups (P<0.05 to P<0.001). Treatment of animals with dexamethasone, all concentrations of the extract and safranal significantly improved most types of WBCs but total WBC number was only decreased in treated groups with dexamethasone and high concentration of the extract compared to group S (P<0.05 to P<0.001). Safranal was more effective in the improvement of eosinophil and lymphocyte compared to the extracts (P<0.001 for both cases). However, the preventive effect of the extract of *Crocus sativus* on total WBC count was more prominent than that of the safranal (p<0.01) [157].

The effect of the extract of *Crocus sativus* and its constituent, safranal on inflammatory markers in sensitized guinea pigs was examined. Ovalbumin (OA) sensitized guinea pigs were given drinking water alone (group S), or drinking water containing three concentrations of safranal, three concentrations of extract and one concentration of dexamethasone, (n=6, for all groups) and serum levels of endotheline-1 (ET-1) and total protein (TP) were assessed. Serum levels of group S were significantly higher than control group (P<0.01 for ET-1 and P<0.001 TP). Treatment of group S animals with dexamethasone, most concentrations of the extract and safranal significantly reduced serum levels of ET-1 and TP compared to group S (P <0.01 to P <0.001). The effects of one concentration of the extract and safranal were significantly higher than dexamethasone (P <0.05 to P <0.01) [158].

The prophylactic effect of saffron (*Crocus sativus*) in asthma was examined in ovalbumin sensitized rats. The sensitized rats were pretreated with three different concentrations of extract, 50, 100, and 200 mg/kg. Total WBC number, eosinophil and neutrophil percentage in blood were increased, but lymphocyte decreased in sensitized animals compared with those of control group (p<0.05 to p<0.001). In addition to elevation of RBC and platelet counts after sensitization in the asthma group. Pretreatment of sensitized rats in all concentrations decreased WBC count which was significant in first two concentrations (p<0.01 compared with asthma group). All concentrations of extract decreased eosinophil percentage significantly (p<0.001 compared with asthma group), however, for neutrophil percentage this improvement was not significant. Lymphocyte percentage increased in group asthma +100EX compared with asthma group (p<0.05). Moreover, in all concentrations, the extract reduced RBC and platelet count in pretreated sensitized rats compared with group of asthma (p<0.01 to p<0.001) [159].

The effects of *Crocus sativus* extract on total and differential white blood cells (WBC) count in lung lavage fluid (LLF) was studied in ovalbumin-sensitized rats. Total WBC count, neutrophil, and eosinophil percentage in LLF were increased in sensitized animals compared with the control group (p<0.001). Treatment of sensitized animals with all doses of the extract significantly reduced WBC number and the percentage of neutrophil and eosinophil compared with the sensitized animals (p<0.01-0.001). According to the results, the extract of *Crocus sativus* could be effective on alleviating lung inflammatory cells specially eosinophils in lung lavage of sensitized animals which may indicate a preventive effect against lung inflammation in asthma [160].

The preventive effects of the extract of *Crocus sativus* on tracheal responsiveness and plasma levels of IL-4, IFN-γ, total NO and nitrite were examined on sensitized guinea pigs. Methacholine and ovalbumin (OVA) sensitized guinea pigs, were given drinking water containing three concentrations of the extract of *Crocus sativus*. The TR to both methacholine and OVA significantely increased the levels of serum IL-4, total NO and nitrite, but that of IFN-γ and IFN-γ/IL-4 ratio (Th1/Th2 balance) were decreased compared to the controls (p<0.05 to p<0.001). In the treated animals with dexamethasone and all concentrations of the extract, TR to both methacholine and OVA, IL-4, total NO and nitrite were significantly decreased but IFN-γ and IFN -γ/IL-4 ratio increased (p<0.05 to p<0.001). The effects of the highest concentration of the extract was greater than those of other concentrations and dexamethasone (p<0.05 to p<0.01) [161].

The potential of saffron to induce cytotoxic and apoptotic effects in lung cancer cells (A549) was studied. The caspase-dependent pathways activation of saffron-induced apoptosis against the A549 cells was also investigated. The proliferation of the A549 cells were decreased after treatment with saffron in a dose- and time-dependent manner. The percentage of apoptotic cells was increased with the increase in saffron concentration [162].

Many studies have indicated relaxant, inhibitory effect on histamine (H1) and muscarinic receptors, and stimulatory effect on β-drenoceptor of *Crocus sativus* on guinea pig tracheal chains [157].

The relaxant effects of aqueous-ethanolic extracts of *Crocus sativus* and one of its main constituents, safranal, were examined on guinea-pig tracheal chains. The relaxant effects of four cumulative concentrations of aqueous-ethanolic extract (0.15, 0.3, 0.45, and 0.60 g %) and safranal (0.15, 0.30, 0.45, and 0.60 ml 0.2 mg/ml solution) in comparison with saline, as negative control, and four cumulative concentrations of theophylline (0.15, 0.30, 0.45, and 0.60 mM), as positive control, were examined using guinea-pig precontracted tracheal chains. The tracheal chains had been precontracted by three different methods. Group 1 had been precontracted using 10 microM methacholine. The other two groups had been precontracted using 60 mM KCl at two different conditions: non-incubated tissues (group 2) and tissues incubated with 1 microM propranolol, 1 microM chlorpheniramine and 1 microM atropine (group 3). In group 1 all concentrations of

theophylline, extract and safranal showed significant relaxant effects compared with saline ($p<0.05$ to $p<0.001$). In group 2 theophylline, extract and safranal showed concentration-dependent relaxant effects also compared with saline ($p<0.05$ to $p<0.001$ for different concentrations except two low concentrations of safranal). However, in group 3 the extracts of *Crocus sativus* showed a weak relaxant effect ($p<0.05$ only for the highest concentration). The effects of the last concentration of safranal (0.60 ml 0.2 mg/ ml solution) in group 1, and all its concentrations in group 2 were significantly lower than those of theophylline ($p<0.05$ to $p<0.001$). In addition, the effects of safranal 0.45 and 0.60 ml 0.2 mg/ ml solution in groups 1 and 2 were significantly lower than that of *Crocus sativus* extract. There were significant correlations between the relaxant effects and concentrations for extract, safranal and theophylline in all experimental groups ($p<0.001$ for all cases). The authors concluded that *Crocus sativus* induced potent relaxant effect on tracheal chains of guinea-pigs that was comparable to or even higher than that of theophylline at the concentrations used. The results also indicated that safranal was, at least in part, responsible for the relaxant effect of *Crocus sativus* [163].

The antitussive activity of *Crocus sativus* stigma and petal extracts and its components, safranal and crocin, was evaluated using the nebolized solution of citric acid 20% in guinea pigs. They were injected intraperitoneally. The ethanolic extract of *Crocus sativus* (100-800 mg/kg) and safranal (0.25-0.75 ml/kg) reduced the number of cough. The ethanolic and aqueous extracts of petal and crocin did not show antitussive activity [164].

Anticancer :

Extract of saffron (*Crocus sativis*) inhibited colony formation and cellular DNA and RNA synthesis by HeLa cells *in vitro* [165-166].

The anti-proliferative effect of *Crocus sativus* extract and its major constituent, crocin, was studied on three colorectal cancer cell lines (HCT-116, SW-480, and HT-29). The cell growth inhibition effect was compared to that of non-small cell lung cancer (NSCLC) cells. In addition, *Crocus sativus* effect on non-cancer cells was also evaluated. Significant concentration-related inhibitory effects of the extract on all three colorectal cancer cell lines were observed ($p<0.01$). The proliferation was reduced most significantly in HCT-116 cells (to 45.5%) at 1 mg/ml and (to 6.8%) at 3 mg/ml. Crocin at 1 mM, significantly reduced HCT-116, SW-480, and HT-29 cell proliferation to 2.8%, 52%, and 16.8%, respectively ($p<0.01$). Since 3 mg/ml *Crocus sativus* extract contained approximately 0.6 mM crocin, the observed effects suggest that crocin was the major responsible constituent in the extract. Significant anti-proliferative effects were also observed in non-small cell lung cancer cells. However, *Crocus sativus* extract did not significantly affect the growth of non-cancer young adult mouse colon cells [167].

The potential of the ethanolic extract of saffron to induce antiproliferative and cytotoxic effects was tested in cultured carcinomic human alveolar basal epithelial cells in comparison with non-malignant (L929) cells. Both cells were cultured in Dulbecco's modified Eagle's medium and treated with the ethanolic extract of saffron at various concentrations for two consecutive days. The results showed that the ethanolic extract of saffron decreased cell viability in malignant cells in a concentration and time-dependent manner. The IC_{50} values against the lung cancer cell line were determined as 1500 and 565 µg/ml after 24 and 48 h, respectively. However, the extract at different concentrations could not significantly decrease the cell viability in L929 cells. Morphology of MCF7 cells treated with the ethanolic extract confirmed the MTT results [168].

In order to examine saffron's anti-proliferative and pro-apoptotic effects in colorectal cancer cells, two p53 isogenic HCT116 cell lines (HCT wildtype and HCT p53-/-) were treated with different doses of the drug and analyzed cell proliferation and apoptosis in a time-dependent manner. Saffron extract induced a p53-dependent pattern of cell cycle distribution with a full G2/M stop in HCT116 p53 wildtype cells. However, it induced a remarkable delay in S/G2 phase transit with entry into mitosis in HCT116 p53 -/- cells. The apoptotic Pre-G1 cell fraction as well as Annexin V staining and caspase 3 cleavage showed a more pronounced apoptosis induction in HCT116 p53 wildtype cells. Obviously, the significantly higher DNA-damage, reflected by γH2AX protein levels in cells lacking p53, was coped by up-regulation of autophagy. The saffron-induced LC3-II protein level was a remarkable indication of the accumulation of autophagosomes, a response to the cellular stress condition of drug treatment [169].

The cytotoxic and apoptotic effects of the ethanolic extract of saffron were evaluated on carcinomic human alveolar basal epithelial cells (A549), a commonly used cell culture system for *in vitro* studies on lung cancer. The cells were cultured in Dulbecco's modified Eagle's medium with 10% fetal bovine serum and treated with different concentrations of the ethanolic extract of saffron for two consecutive days. Cell viability was quantitated by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay. Apoptotic cells were determined using annexin V-fluorescein isothiocyanate by flow cytometry. Saffron decreased the cell viability in the malignant cells as a concentration- and time-dependent manner. The IC_{50} values against the A549 cell lines were determined as 1,200 and 650 µg/ml after 24 and 48 h, respectively [170].

The cytotoxic effect of saffron extract was evaluated on HepG2 and HeLa cell lines. Malignant and non-malignant cells (L929) were cultured in DMEM medium and incubated with different concentrations of

ethanolic saffron extract. Cell viability was quantitated by MTT assay. Apoptotic cells were determined using PI staining of DNA fragmentation by flow cytometry (sub-G1 peak). ROS was measured using DCF-DA by flow cytometry analysis. Saffron decreased cell viability in malignant cells in a concentration and time-dependent manner. The IC_{50} values against HeLa and HepG2 were determined as 800 and 950 microg/ml after 48 h, respectively. Saffron induced a sub-G1 peak in flow cytometry histogram of treated cells compared to control, which indicated that apoptotic cell death was involved in saffron toxicity. This toxicity was also independent of ROS production [171].

The cytotoxic effect of aqueous extract of saffron was evaluated in human transitional cell carcinoma (TCC) and mouse non-neoplastic fibroblast cell lines. After 24 hours, morphological observations showed growth inhibitory effects at saffron extract concentrations higher than 200 microg/ml for mouse non-neoplastic fibroblast (L929) cells and at concentrations of 50 to 200 microg/ml for the TCC cells. These changes became more prominent after 48 hours. However, significant growth inhibitory effects of the extract were shown at concentrations of 400 and 800 microg/ml. Higher concentrations of saffron correlated inversely with cell population of both cell lines. Significant reduction of the survived cells was seen at concentrations of 400 and 2000 microg/ml for TCC and L929 cell lines, respectively. After 120 hours, decrease in the percentage of survived cells at higher concentrations of saffron extract was seen in both cell lines. At a concentration of 800 microg/ml, the survived L929 cells plummeted to less than 60% after 120 hours, while no TCC cells survived at this time. No L929 cells survived at 2000 microg/ml [152].

In order to compare the sensitivity of malignant and non-malignant cells to saffron, the effect of the extract was studied on macromolecular synthesis in three human cell lines: A549 cells (derived from a lung tumor), WI-38 cells (normal lung fibroblasts) and VA-13 cells (WI-38 cells transformed *in vitro* by SV40 tumor virus). It appeared that the malignant cells were more sensitive than the normal cells to the inhibitory effects of saffron on both DNA and RNA synthesis. There was no effect on protein synthesis in any of the cells [165].

The anticancer activity of saffron extract (dimethyl-crocetin) against a wide spectrum of murine tumors and human leukemia cell lines was studied. Dose-dependent cytotoxic effect to carcinoma, sarcoma and leukemia cells *in vitro* were noted. Saffron delayed ascites tumor growth and increased the life span of the treated mice compared to untreated controls by 45-120%. In addition, it delayed the onset of papilloma growth, decreased incidence of squamous cell carcinoma and soft tissue sarcoma in treated mice. It appeared that saffron (dimethyl-crocetin) disrupted DNA-protein interactions e.g. topoisomerases II, important for cellular DNA synthesis [166-173].

The mutagenic, antimutagenic and cytotoxic effects of saffron and its main components were studied on the growth of different human malignant cells *in vitro*. Colony formation assay was used to determinate the cytotoxic activity of saffron extract and its components on human tumor cells *in vitro*. Mutagenicity and antimutagenicity assays were performed by the Ames method. Saffron was non-mutagenic, non-antimutagenic and non-comutagenic. Saffron extract itself and some of its ingredients displayed a dose-dependent inhibitory activity against different types of human malignant cells *in vitro*. HeLa cells were more susceptible to saffron than other tested cells [87].

The antiproliferative effects of saffron extract (SE) and its major constituent crocin was investigated on 5 different malignant and 2 nonmalignant prostate cancer cell lines. All cells were incubated with different concentrations of SE or crocin for 48 h. Cell cycle and apoptosis were also evaluated. In a time- and concentration-dependent manner, both SE and crocin reduced cell proliferation in all malignant cell lines with IC_{50} values ranging between 0.4 and 4 mg/ml for SE and 0.26 and 0.95 mM/ml for crocin. Nonmalignant cells were not affected. Flow cytometry profiles revealed that most cells were arrested at G0/G1 phase with a significant presence of apoptotic cells. Western blot analysis revealed that the expression of Bcl-2 was strikingly downregulated, whereas Bax was upregulated. Analysis of caspase activity indicated a caspase-dependent pathway with involvement of caspase-9 activation, suggesting an intrinsic pathway [174].

The beneficial effect of saffron (*Crocus sativus*) aqueous extract (SAE) on the 1-Methyl -3- nitro -1-nitrosoguanidine (MNNG)-induced gastric cancer was investigated in rats. MNNG was used to induce gastric cancer and then, different concentrations of SAE were administered to rats. After sacrificing, the stomach tissue was investigated by both pathologist and flow cytometry, and several biochemical parameters was determined in the plasma (or serum) and stomach of rats. Pathologic data indicated that the induction of cancer at different stages from hyperplasia to adenoma in rats, was inhibited by SAE administration; 20% of cancerous rats treated with higher doses of SAE was completely became normal at the end of experiment and there was no rat with adenoma in the SAE treated groups. In addition, the results of the flow cytometry/ propidium iodide staining showed that the apoptosis/proliferation ratio was increased in the SAE treated cancerous rats. Moreover, the significantly increased serum LDH and decreased plasma antioxidant activity due to cancer induction fell backwards after treatment of rats with SAE. But changes in the other parameters (Ca^{2+}), tyrosine kinase activity and carcino-embryonic antigen) were not significant [175].

The potential of saffron to induce cytotoxic and apoptotic effects in lung cancer cells (A549) and the caspase-dependent pathways activation of saffron-induced apoptosis against the A549 cells were investigated. A549

cells were incubated with different concentrations of saffron extract; then cell morphological changes, cell viability, and apoptosis were determined. The proliferation of the A549 cells were decreased after treatment with saffron in a dose- and time-dependent manner. The percentage of apoptotic cells were increased with saffron concentrations. Saffron induced morphological changes, decreased percentage of viable cells, and induced apoptosis. Saffron induced apoptosis in the A549 cells and activate caspase pathways. The levels of caspases involved in saffron-induced apoptosis in the A549 cells indicating caspase-dependent pathway were induced by saffron. The anticancer activity of the aqueous extract of saffron could be attributed partly to its inhibition of the cell proliferation and induction of apoptosis in cancer cells through caspase-dependent pathways activation [162].

Antitumor activity of saffron (*Crocus sativus*) extract was studied against intraperitoneally transplanted sarcoma-180 (S-180), Ehrlich ascites carcinoma (EAC) and Dalton's lymphoma ascites (DLA) tumours in mice. Oral administration of 200 mg/kg bw of the extract increased the life span of S-180, EAC, DLA tumour bearing mice to 111.0%, 83.5% and 112.5%, respectively. The same extract was found to be cytotoxic to P38B, S-180, EAC and DLA tumour cells *in vitro*. Thymidine uptake studies indicated that the effect was mediated via inhibition of DNA synthesis [176].

Saffron treatments were given both before and after the induction of skin carcinogenesis. Standard histological examination of mice skin demonstrated that saffron ingestion inhibited the formation of skin papillomas and reduced their size also. The inhibition of skin carcinoma of early saffron treatment was attributed to the induction of cellular defense systems in mice [177].

To investigate the mechanism of saffron-induced cytotoxicity, the role of caspases and Bax protein in saffron induced apoptosis in MCF-7 cells, a commonly used cell culture system for *in vitro* studies on breast cancer, was investigated . Cells were incubated with different concentrations of saffron extract. Cell viability was quantitated by MTT assay. Apoptotic cells were determined using PI staining of DNA fragmentation by flow cytometry (sub-G1 peak). Role of caspase were studied using the pancaspase inhibitor. Bax protein expression was analysed by western blotting. Saffron extract (200-2000 g/ml) decreased cell viability in MCF-7 cells as a concentration- and time dependent manner with an IC_{50} of 400 ± 18.5 microg/ml after 48 h. Analysis of DNA fragmentation by flow cytometry showed apoptotic cell death in MCF-7 cell treated with saffron extract. Saffron-induced apoptosis could be inhibited by pan-caspase inhibitors, indicating caspase-dependent pathway was induced by saffron in MCF-7 cells. Bax protein expression was also increased in saffron-treated cells [178].

MTT assay was performed to detect the inhibitory action of crocin on the proliferation of ovarian cancer HO-8910 cells. Flow cytometry was used to test the cell cycle distribution and apoptosis rate of ovarian cancer HO-8910 cells. Western blot analysis was utilized to measure the levels of apoptotic proteins such as p53, Fas/APO-1, and Caspase-3. MTT analysis revealed that crocin significantly inhibited the growth of HO-8910 cells. Additionally, flow cytometry illustrated that crocin raised the proportion of HO-8910 cells in the G0/G1 phase and increased their apoptosis rate. Furthermore, Western blot analysis revealed that crocin up-regulated the expression of p53, Fas/APO-1, and Caspase-3. Accordingly, crocin significantly inhibited the growth of HO-8910 cells and arrest them in the G0/G1 phase. Crocin also promoted ovarian cancer HO-8910 cell apoptosis, most likely by increasing p53 and Fas/APO-1 expression, and activating the apoptotic pathway regulated by Caspase-3 [179].

Anti-inflammatory and analgesic effects:

The preventive effect of the aqueous extract of saffron was studied against diazinon (DZN) -induced rise of several specific inflammation, oxidative stress and neuronal damage in rats. Vitamin E (200 IU/kg) and the aqueous extract of saffron at doses 50, 100 and 200 mg/kg were injected intraperitoneally three times per week alone or with DZN (20 mg/kg/day, orally) for 4 weeks. Red blood cell (RBC) cholinesterase activity was inhibited by DZN and this effect was not affected by vitamin E or saffron plus DZN. The levels of serum tumor necrosis factor-α (inflammation marker), direct 8-iso-prostaglandin F2α (oxidative stress marker) and soluble protein-100 β (S100β, neuronal damage marker) were increased significantly by DZN. The saffron extract inhibited the effect of DZN on these biomarkers levels. However, vitamin E was able to only reduce 8-iso-prostaglandin F2α and S100β levels [127].

The antinociceptive and anti-inflammatory activity of saffron extracts were evaluated in mice using aqueous and ethanolic maceration extracts of *Crocus sativus* stigma and petals. Antinociceptive activity was examined using the hot plate and writhing tests. The effect of extracts against acute inflammation was studied using xylene induced ear edema in mice. The activity of the extracts against chronic inflammation was assessed by formalin-induced edema in the rat paw. In the hot plate tests, intraperitoneal injection of both extracts showed no significant antinociceptive activity in mice. The extracts exhibited antinociceptive activity against acetic acid induced writhing. Naloxone partially blocked only the antinociceptive activity of the stigma aqueous extract. Only the stigma extracts showed weak to moderate effect against acute inflammation. In chronic inflammation, both aqueous and ethanolic stigma extracts, as well as ethanolic petal extract, exerted anti-inflammatory effects [180].

Effect on reproductive systems:

The aphrodisiac activities of *Crocus sativus* stigma aqueous extract and its constituents, safranal and crocin, were evaluated in male rats. The aqueous extract (80, 160 and 320 mg/kg bw), crocin (100, 200 and 400 mg/kg bw), safranal (0.1, 0.2 and 0.4 ml/kg), sildenafil (60 mg/kg bw, as a positive control) and saline were administered intraperitoneally to male rats. Mounting frequency (MF), intromission frequency (IF), erection frequency (EF), mount latency (ML), intromission latency (IL) and ejaculation latency (EL) were evaluated. Crocin, at all doses, and the extract, especially at doses 160 and 320mg/kg body wt., increased MF, IF and EF behaviors and reduced EL, IL and ML parameters. Safranal did not show aphrodisiac effects [181].

A randomized, parallel-group, double-blind, placebo-controlled trial was designed to investigate the effects of *Crocus sativus* gel on erectile dysfunction in diabetic men. Patients were randomly allocated to 2 equal groups (with 25 patients each). The intervention group was treated with topical saffron, and the control received a similar treatment with placebo. The 2 groups were assessed using the international index of erectile function questionnaire before the intervention and 1 month after the intervention. Compared to placebo, the prepared saffron gel significantly improved erectile dysfunction in diabetic patients ($P < .001$) [182].

The effects of different concentrations of saffron (*Crocus sativus*) aqueous extract (SAE), was evaluated in *in vitro* maturation (IVM) of immature mouse oocytes. Cumulus-oocyte complexes (COCs) were collected from 6-8 weeks old female mice ovaries. COCs were cultured in IVM medium supplemented with 0 (control), 5, 10, 20 and 40 µg/ml of (*Crocus sativus*) aqueous extract (SAE) in 5% CO2 at 37℃. The rates of maturation, fertilization and development were recorded. The maturation rate was significantly higher in all groups treated with different concentrations of SAE compared with the control group ($p<0.05$). However, the lower concentrations of SAE (10 and 5 µg/ml in maturation medium) increased the fertilization rate of oocytes and *in vitro* developmental competence when compared with the control group ($p<0.05$). The authors conclude that addition of appropriate amounts of SAE to maturation medium improved oocyte maturation and embryo development [183].

The effects of different concentrations of saffron (*Crocus sativus*) aqueous extract (SAE) and its ingredient, crocin, were evaluated on the improvement of *in vitro* maturation (IVM) and subsequent *in vitro* fertilization (IVF) and embryo development of mouse oocytes. Cumulus oocyte complexes were collected from ovaries, and germinal vesicle oocytes were cultured in the presence of SAE and crocin. SAE was added at dosages of 5, 10, and 40 µg/m and crocin 50, 100, and 400 µg/ml. All dosages were added to maturation medium and a group without SAE or crocin was considered as the control group. Both SAE and crocin improved the rate of IVM, IVF, and *in vitro* culture. Addition of 40 µg/ml SAE to maturation medium significantly increased the rate of IVM, IVF, and *in vitro* culture ($p < 0.05$). Furthermore 100 µg/ml crocin significantly increased the IVM rate compared to the control group ($p < 0.05$) [184].

A double-blind and placebo-controlled trial was designed to investigate the effect of saffron (stigma of *Crocus sativus*) on the symptoms of premenstrual syndrome. The study was carried out on women aged 20–45 years with regular menstrual cycles and experience of PMS symptoms for at least 6 months. Women were randomly assigned to receive capsule saffron 30 mg/day (15 mg twice a day; morning and evening) or capsule placebo (twice a day) for two menstrual cycles. The primary outcome measure was the daily symptom report, and secondary outcome measure was the Hamilton depression rating scale. The trial showed that saffron was effective in relieving symptoms of PMS. A significant difference was observed in efficacy of saffron in the total premenstrual daily symptoms and Hamilton depression rating scale [185].

Protective effects:

The protective effects of saffron extract and crocin was evaluated in chronic - stress induced oxidative stress damage of the brain, liver and kidneys in rats. Rats were injected with a daily dose of saffron extract (30 mg/kg, ip) or crocin (30 mg/kg, ip) during a period of 21 days following chronic restraint stress (6 h/day). In order to determine the changes of the oxidative stress parameters following chronic stress, the levels of the lipid peroxidation product, malondialdehyde (MDA), the total antioxidant reactivity (TAR), as well as antioxidant enzyme activities glutathione peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD) were measured in the brain, liver and kidneys tissues after the end of chronic stress. In the stressed animals that receiving saline, the levels of MDA, and the activities of GPx, GR, and SOD were significantly higher ($P<0.0001$) and the TAR capacity was significantly lower than those of the non-stressed animals ($P<0.0001$). Both saffron extract and crocin were able to reverse these changes in the stressed animals as compared with the control groups ($P<0.05$). These observations indicate that saffron and its active constituent crocin can prevent chronic stress-induced oxidative stress damage of the brain, liver and kidneys [186].

The protective effects of hydroalcoholic extract from *Crocus sativus* petals (CSP) against Acetaminophen (APAP) -induced hepatotoxicity was evaluated in male rats. Rats were treated with either low dose (10 mg /kg) or high dose (20 mg/kg) of CSP before receiving APAP (600 mg/kg, iv). The APAP treatment resulted in higher levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin, along with lower total protein and albumin concentration than the control group. The administration of CSP with a dose of 20 mg/kg resulted in lower levels of AST, ALT and bilirubin, with a significant higher concentration of total

protein and albumin. The histopathological results regarding liver pathology, revealed cell swelling, severe inflammation and necrosis in APAP-exposed rats, which was quiet contrasting compared to the control group. The pre-treated rats with low doses of CSP showed hydropic degeneration with mild necrosis in centrilobular areas of the liver, while the same subjects with high doses of CSP appeared to have only mild hepatocyte degeneration. It was appeared that the antioxidant property of CSP resulted in reducing the oxidative stress complications of toxic levels of APAP in intoxicated rats, and 20 mg/kg of CSP ameliorates APAP-induced acute liver injury in rats [187].

The potential protective effect of saffron ethanol extract (SEE) in a rat model upon hepatic ischemia-reperfusion (IR) injury was studied. Caspases 3 and terminal deoxynucleotidyl transferase-mediated dUTP biotin nick end labeling (TUNEL) results showed increased cell death in the IR samples; reversely, minor apoptosis was detected in the SEE/IR group. Pretreatment with SEE significantly restored the content of antioxidant enzymes (SOD and catalase) and remarkably inhibited the intracellular ROS concentration in terms of reducing p47 phox translocation. Proteome tools revealed that 20 proteins were significantly modulated in protein intensity between IR and SEE/IR groups. Particularly, SEE administration attenuate the carbonylation level of several chaperone proteins [188].

The protective effect of ethanolic extract of *Crocus sativus* stigma (EECSL.S) was evaluated against rifampin-induced hepatotoxicity in the rats in comparison with standard drug silimarin. Male Wistar rats with were randomly assigned into 5 groups. Group I as normal control received normal saline (10 ml/kg) and group II as toxicant control received rifampin (500 mg/kg). Group III as positive control received silymarin plus rifampin (500 mg/kg) and groups IV and V (50 mg/kg) received EECSL.S at 40 and 80 mg/kg plus rifampin, respectively. All the treatments were given through gavage for 1 month. At the end of experiment, levels of liver function marker enzymes (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase), total bilirubin, albumin and total proteins were assessed in serum of the rats. In rifampin-treated rats, silymarin and EECSL.S (40 and 80 mg/kg) were significantly decreased the levels of serum biomarker of hepathic injury and total bilirubin and elevated the levels of albumin and total proteins. Histopathologically, silymarin and EECSL.S ameliorated rifampin induced hepatic injury [189].

Crocin possessed hepatoprotective effects against aflatoxin B1 hapatotoxicity via the reduction of hepatic (AST, ALT, ALP and γ-GGT) and via its antioxidant activity in rats [190].

The effect of aqueous extract of *Crocus sativus* stigmas (CSE) and crocin (trans-crocin 4) was examined on methyl methanesulfonate (MMS)-induced DNA damage in multiple mice organs using the comet assay. Adult male NMRI mice in different groups were treated with either physiological saline (10 ml/kg, ip), CSE (80 mg/kg, ip), crocin (400 mg/kg, ip), MMS (120 mg/kg, ip), and CSE (5, 20, and 80 mg/kg, ip) 45 min prior to MMS administration or crocin (50, 200, and 400 mg/kg, ip) 45 min prior to MMS administration. Mice were sacrificed about 3 h after each different treatment, and the alkaline comet assay was used to evaluate the effect of these compounds on DNA damage in different mice organs. A significant increase in the % tail DNA was seen in nuclei of different organs of MMS-treated mice. In control groups, no significant difference was found in the % tail DNA between CSE- or crocin-pretreated and saline-pretreated mice. The MMS-induced DNA damage in CSE-pretreated mice (80 mg/kg) was decreased between 2.67-fold (kidney) and 4.48-fold (lung) compared to those of MMS-treated animals alone ($p<0.001$). This suppression of DNA damage by CSE was found to be depended on the dose, pretreatment with CSE (5 mg/Kg) only reduced DNA damage by 6.97%, 6.57%, 7.27%, and 9.90% in liver, lung, kidney, and spleen, respectively ($p>0.05$ as compared with MMS-treated group. Crocin also significantly decreased DNA damage (between 4.69-fold for liver and 6.55-fold for spleen, 400 mg/Kg) [191].

The genotoxic potential of anti-tumor drugs limits their efficacy in the treatment of cancers. The chemoprotective potential of saffron against the genotoxicity of three well-known anti-tumor drugs-cisplatin (CIS), cyclophosphamide (CPH) and mitomycin-C (MMC)—was studied using comet assay. Three doses of saffron (20, 40 and 80 mg/kg b.w.) were orally administered to mice for five consecutive days prior to the administration of anti-tumor drugs under investigation. Pre-treatment with saffron significantly inhibited anti-tumor drugs induced cellular DNA damage (strand breaks) as revealed by decreased comet tail length, tail moment and percent DNA in the tail. These findings, suggest a potential role for saffron as an anti-genotoxic, anti-oxidant and chemopreventive agent and could be used as an adjuvant in chemotherapeutic applications [192].

Experiments were carried out to ascertain whether or not saffron (dried stigmas of *Crocus sativus* L.), exert modulatory effects on the *in vivo* genotoxicity of cisplatin (CIS), cyclophosphamide (CPH), mitomycin C (MMC) and urethane (URE). Swiss albino mice were pretreated for five consecutive days with three doses (20, 40 and 80 mg/kg body weight) of the aqueous extract of saffron. Genotoxic effects were assessed in the mouse bone marrow micronucleus test. The results showed that pretreatment with saffron significantly inhibited the genotoxicity of CIS, CPH, MMC and URE. This inhibitory effect was not always dose-dependent. In addition, the hepatic glutathione S-transferase (GST) activity was assessed in the control and treated animals. No

significant change in GST activity was observed after pretreatment with saffron alone. Treatment with the genotoxins alone significantly inhibited GST activity [193].

Crocus sativus (CSE) (250 mg/kg, po) was also effective in preventing acetaldehyde-induced inhibition of LTP in the dentate gyrus of anesthetized rats. These results suggest that CSE can prevent aversive effects induced by ethanol and its metabolite acetaldehyde [194].

The modifying effects of the aqueous extract of saffron (dried stigmas of *Crocus sativus*) on cisplatin (CIS), cyclophosphamide (CPH), mitomycin-C (MMC) and urethane (URE) induced alterations in lipid peroxidation and antioxidant status were investigated in Swiss albino mice. Three doses of saffron (20, 40 and 80 mg/kg body weight) were orally administered to mice for 5 consecutive days prior to administration of genotoxins. A significant reduction in the extent of lipid peroxidation with a concomitant increase in the liver enzymatic (SOD, CAT, GST, GPx) and non-enzymatic antioxidants were observed in saffron pretreated animals compared with the genotoxins alone treated animals. However, the modulatory effects were not always dose dependent. Data suggested that saffron may exerted its chemopreventive effects by modulation of lipid peroxidation, antioxidants and detoxification systems [195].

Saffron (dried stigmas of *Crocus sativus*), was evaluated in the mouse bone marrow micronucleus test for its possible protective effects against chromosomal damage induced by cisplatin (CIS), mitomycin-C (MMC) and urethane (URE). Three doses of saffron (25, 50 and 100 mg/kg body weight) were orally administered to mice for five consecutive days prior to administration of genotoxins under investigation. From the results obtained, it was evident that the administration of 50 and 100 mg saffron/kg bw significantly inhibited the *in vivo* genotoxicity of these genotoxins. However, all the three doses of saffron were effective in exerting a protective effect against urethane [196].

It appeared that concurrent administration of *Crocus sativus* reduced the toxicity of cisplatin in rats. When cisplatin was administered ip for 5 alternate days as 3 mg/kg, *Crocus sativus* stigmas (50 mg/kg) significantly reduced blood urea nitrogen (BUN) and serum creatinine levels as well as cisplatin-induced serum total lipids increases. In contrast, the protective agents given together (Cysteine, vitamin E, *Crocus sativus* and *Nigella sativa*) with cisplatin led to an even greater decrease in blood glucose than that seen with cisplatin alone. The serum activities of alkaline phosphatase, lactate dehydrogenase, malate dehydrogenase, aspartate aminotransferase and alanine aminotransferase of cisplatin-treated rats were significantly decreased, whereas the activities of glutathione reductase and isocitrate dehydrogenase were significantly increased. Addition of cysteine and vitamin E, *Crocus sativus* and *Nigella sativa* in combination with cisplatin partially prevented many changes in the activities of serum enzymes. In cisplatin-treated rats, the liver activities of isocitrate dehydrogenase and aspartate aminotransferase were significantly increased, whereas much greater changes were found in the kidneys, with increased activity of glucose-6-phosphate dehydrogenase and decreased activities of alkaline phosphatase, isocitrate dehydrogenase, malate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, sorbitol dehydrogenase and γ -glutamyl transferase, as well as a decreased phosphorylation to oxidation ratio in the mitochondria, indicating reduced adenosine triphosphate production. Also, administration of cysteine and vitamin E, *Crocus sativus* and *Nigella sativa* together with cisplatin partially reversed many of the kidney enzymes changes induced by cisplatin. Cysteine together with vitamin E, *Crocus sativus* and *Nigella sativa* tended to protect from cisplatin-induced falls in leucocyte counts, haemoglobin levels and mean osmotic fragility of erythrocytes and also prevented the increase in haematocrit [197-198].

The protective effect of *Crocus sativus* on gentamicin nephrotoxicity was investigated in rats. Male rats were treated with saffron (40 or 80 mg/k/day) for 10 days, or saffron (40 or 80 mg/ kg/day) for 10 days and gentamicin 80 mg/kg/day for five days, starting from day 6. At the end of treatment, blood samples were taken for measurement of serum creatinine (SCr) and BUN. The left kidney was prepared for histological evaluation and the right kidney for malondialdehyde (MDA) measurement. Gentamicin 80 (mg/k/day) increased SCr, BUN and renal tissue levels of MDA and induced severe histological changes. Saffron at 40 mg/kg/day significantly reduced gentamicin-induced increases in BUN and histological scores ($p<0.05$). Gentamicin-induced increases in BUN, SCr and MDA and histological injury were significantly reduced by treatment with saffron 80 mg/k/d ($p<0.05$, $p<0.001$, $p<0.05$, and $p<0.001$ respectively) [199].

The protective effect of saffron aqueous extracts in the cytogenetic and testicular damage induced by the antiepileptic drug, sodium valporate (SVP) was investigated in albino rats. Animals given SVP and saffron showed an improvement in chromosomal aberrations, mitotic index, DNA damage and testicular alterations caused by SVP. Moreover, MDA decreased and CAT and RAP increased. The authors concluded that the ameliorative effects of saffron extract against SVP-induced cytogenetic and testicular damage in albino rats could be attributed to one or more antioxidant components of saffron [200].

The extract of *Crocus sativus* stigmas partially prevented the decreases in body weight, hemoglobin levels and leucocyte counts caused by 2 mg/kg of cisplatin ip for 5 days in mice. Treatment with the *Crocus sativus* extract also significantly prolonged the life span of cisplatin-treated mice almost three-fold [201].

Gastrointestinal effects:

An aqueous suspension of saffron was subjected to evaluate its gastric antiulcer activity induced by pylorus ligation (Shay rats), indomethacin and various necrotizing agents including (80% ethanol, 0.2 M NaOH and 25% NaCl) in rats. Gastric wall mucus and non-protein sulfhydryl contents were estimated in rats. Histopathological assessment of rat stomach was also carried out. The saffron aqueous suspension at doses (250 and 500 mg/kg) decreased gastric secretion and ulcer index in Shay rats and indomethacin treated groups. Gastric wall mucus was enhanced. A large margin of safety was observed in animals after acute and chronic treatment [202].

The effects of an ethanol and aqueous extract of saffron *Crocus sativus* and its constituents safranal and crocin was investigated on stress-induced reduction in food intake, weight gain and anorexic time in mice. Male albino mice were irregularly exposed to a trial of electroshock stress for 7 days. Then, the anorexic time as well as the animal's food intake and weight were recorded. Intraperitoneal administration of the aqueous but not the ethanol extract (10, 50 and 100 mg/kg) significantly reduced the anorexic time. The results were similar for crocin (1, 5 and 10 mg/kg; ip). In addition, a reduction in weight gain was observed in the controls as well as in the groups that received alcohol extract or safranal. The plasma corticosterone level did not increase in the aqueous extract and crocin treated animals. The authors concluded that the saffron aqueous extract and its constituent crocin reduce side effects of electroshock stress in mice [203].

The beneficial effect of saffron (*Crocus sativus*) aqueous extract (SAE) on the 1-Methyl -3- nitro -1-nitrosoguanidine (MNNG)-induced gastric cancer was investigated in rats. Different concentrations of SAE were administered to rats. After sacrificing, the stomach tissue was investigated by both pathologist and flow cytometry, and several biochemical parameters were determined in the plasma (or serum) and stomach of rats. Pathologic data indicated the induction of cancer at different stages from hyperplasia to adenoma in rats was inhibited by SAE administration; 20% of cancerous rats treated with higher doses of SAE was completely became normal at the end of experiment and there was no rat with adenoma in the SAE treated groups [175].

Effect on overweight:

Healthy, mildly overweight women (N = 60) participated in a randomized, placebo-controlled, double-blind study to evaluated the efficacy of satiereal supplementation (Inoreal Ltd, Plerin, France), a novel extract of saffron stigma, on body weight changes over an 8-week period. They took twice capsule of satiereal (176.5 mg extract per day or a matching placebo. Caloric intake was left unrestricted during the study. At baseline, both groups were homogeneous for age, body weight, and snacking frequency. Satiereal caused a significantly greater body weight reduction than placebo after 8 weeks ($p<0.01$). The mean snacking frequency was significantly decreased in the satiereal group as compared with the placebo group ($P < 0.05$). Other anthropometric dimensions and vital signs remained almost unchanged in both groups. No subject withdrawal attributable to a product effect was reported throughout the trial, suggesting a good tolerability to satiereal [204].

Effects on smooth muscles:

Many studies have indicated relaxant, inhibitory effect on histamine (H1) and muscarinic receptors, of *Crocus sativus* on guinea pig tracheal chains [157].

The effects of *Crocus sativus* petals' extract was studied on responses of the isolated rat vas deferens and guinea-pig ileum induced by electrical field stimulation (EFS). EFS of the isolated rat vas deferens and guinea-pig ileum evoked contractions were decreased by aqueous and ethanol extracts of *Crocus sativus* petals. The aqueous extract (560 mg/ml) significantly reduced the contractile responses of vas deferens to epinephrine (1 microM) without any change in contraction induced by KCl (300 mM). The results suggested that the relaxatory action of *Crocus sativus* petals' extract on contraction induced by EFS in the rat isolated vas deferens was a postsynaptic effect [143].

Immunological effects:

The effects of three concentrations of macerated extract of *Crocus sativus*, dexamethasone, and saline were evaluated on cell viability and production of cytokines, including interleukin (IL)-4, IL-10, and interferon-γ (IFN-γ) were evaluated. In cells stimulated with phytohemagglutinin (PHA), different concentrations of the extract significantly inhibited cell viability of lymphocytes ($P<.001$ for all concentrations). High concentrations of the extract (500 μg/mL) also inhibited secretion of IFN-γ in stimulated cells and IL-10 secretion in both stimulated and nonstimulated cells ($p<0.05$ for all cases). The effects of high and low concentrations of the extract (500 and 50 μg/ml, respectively) on IL-4 secretion were lower than that of dexamethasone ($P<.05$ to $P<.001$). The extract showed a stimulatory effect on IFN-γ and IL-4 secretion in nonstimulated cells. The ratios of IFN-γ to IL-4 in the presence of all concentrations of saffron on stimulated cells were significantly higher than for the control group ($P<.05$ to $P<.01$) [205].

The effect of the extract of *Crocus sativus* and one of its constituents (safranal) on the inflammatory changes was exaimined in sensitized guinea pigs was examined. Treatment of animals with dexamethasone, all concentrations of the extract and safranal significantly improved most types of WBCs but total WBC number was only decreased in treated groups with dexamethasone and high concentration of the extract compared to control group ($p<0.05$ to $p<0.001$). Safranal was more effective in the improvement of eosinophil and

lymphocyte compared to the extracts (P<0.001 for both cases). However, the preventive effect of the extract of *Crocus sativus* on total WBC count was more prominent than that of the safranal (P<0.01) [157].
The effects of *Crocus sativus* extract on total and differential white blood cells (WBC) count in lung lavage fluid (LLF) was studied in ovalbumin-sensitized rats. Total WBC count, neutrophil, and eosinophil percentage in LLF were significantly increased in sensitized animals compared with the control group (p<0.001). Treatment of sensitized animals with all doses of the extract significantly reduced WBC number and the percentage of neutrophil and eosinophil compared with the sensitized animals (p<0.01-0.001) [160].
The immunomodulatory activity of *Crocus sativus* was studied on Th1 and Th2 limbs of the immune system. Oral administration of alcoholic extract of *Crocus sativus* (ACS) at graded dose levels (1.56-50 mg/kg, po), potentiated the Th2 response of humoral immunity, causing significant increases in agglutinating antibody titre in mice at a dose of 6.25 mg/kg and an elevation of CD19(+) B cells and IL-4 cytokine, a signature cytokine of Th2 pathway. Appreciable elevation in levels of IgG-1 and IgM antibodies of the primary and secondary immune response was also observed. However, ACS showed no appreciable expression of the Th1 cytokines IL-2 (growth factor for CD4(+) T cells) and IFN-γ (signature cytokine of Th1 response). A significant modulation of immune reactivity was observed in all the animal models [206].

Antidiabetic effects:

The ameliorative effect of saffron aqueous extract on hyperglycemia and oxidative stress on diabetic encephalopathy was studied in streptozotocin induced diabetes mellitus in rats. Saffron at 40 and 80 mg/kg significantly increased body weight and serum TNF-α and decreased blood glucose levels, glycosylated serum proteins, and serum advanced glycation endproducts (AGEs) levels which triggered oxidative reaction [130].
Advanced glycation end products (AGEs) were causally correlated with diabetic vascular complications. AGEs triggered oxidative reaction then accelerated endothelial cell apoptosis which was a critical event in the process of vascular complications. Exposure of bovine endothelial cells (BEC) to 200 g/ml AGEs for 48h resulted in a significant increase in apoptotic rate, compared with control. Crocetin (a metabolite of crocin) prevented AGEs-induced BEC apoptosis, which correlates with crocetin attenuation of AGEs mediated increase of intracellular reactive oxygen species (ROS) formation and elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) level (P < 0.01 versus AGEs group). These results demonstrate that crocetin prevents AGEs-induced BEC apoptosis through ROS inhibition and ($[Ca^{2+}]i$) stabilization and suggest that crocetin exerted a beneficial effect in preventing diabetes-associated vascular complications [207].

Dermatological effects:

The efficacy of pollen of saffron extract cream was evaluated in the treatment of thermal induced burn wounds and to compare its results with silver sulfadiazine (SSD) in rats. Animals were divided into four groups and administrated a topical cream including control, base, saffron (20%) or SSD (1%) at 24 hour after a burn injury that was induced by hot water. Animal's weight, wound size, as well as skin histopathology were determined in different groups under topical treatments. On day 25, average size of wound was 5.5, 4, 0.9 and 4.1 cm^2 in control, base, saffron and SSD groups. The wound size of saffron group was significantly smaller than other groups. Histological comparison has shown that saffron significantly increased re-epithelialization in burn wounds, as compared to other cream-treated wounds [208].
Saffron treatments were given both before and after the induction of skin carcinogenesis. Standard histological examination of mice skin demonstrated that saffron ingestion inhibited the formation of skin papillomas and reduced their size. Saffron extract inhibited skin carcinoma due to the induction of cellular defense systems in mice [177].

Antiparasitic effect:

The effectiveness of *Crocus sativus* and its apoptotic activity against *Leishmania major* (MRHO/IR/ 75/ER) promastigotes was studied using MTT assay to find viability of *L. major* promastigotes and the results were explicated as IC_{50} (50% inhibitory concentration). ED_{50} (50% effective doses) for *L. major* amastigotes were also analyzed. Annexin-V FLUOS staining was performed to study the cell death properties of saffron by using FACS analysis. Qualitative analysis of the DNA fragmentations was accomplished by agarose gel electrophoresis, and light microscopy was used to observe morphological changes of promastigotes. The results revealed that *L. major* promastigotes and amastigotes are sensitive to saffron at different concentrations and time dependent manner, with apoptotic features including DNA laddering, cytoplasmic shrinkage, and externalization of phosphatidylserine. IC_{50} and ED_{50} of this extract after 48 h of incubation was 0.7 and 0.5 mg/ml respectively [209].
Safranal isolated from *Crocus sativus* extract exhibited insecticidal and pesticidal effect. This fact could present saffron as safe and effective herbal insecticide and pesticide which was more environment friendly than other synthetic insecticides [210].

Other effects:

The diuretic activity of aqueous extract of dried saffron (stigma of *Crocus sativus*) was studied in rat. Aqueous extracts of saffron were administered to experimental rats orally as 60, 120 and 240 mg/kg bw and compared with hydrochlorothiazide (10 mg/kg bw, intraperitoneally), as positive control and normal saline solution as

placebo. The measured parameters for diuretic activity were total urine volume, urine electrolytes concentration such as sodium and potassium, creatinine and urea concentration. The treated rats with aqueous extract of saffron of 120 and 240 mg/kg bw showed higher urine output when compared to the control group. Also, the extract possessed significant dose-dependent increase in the excretion of electrolytes when compared to the control group [211].

The effect of *Crocus sativus* on the continuous light exposure in the retina was evaluated in rats. Experimental animals were prefed either saffron or beta-carotene (1 mg extract/kg/day) before exposure to bright continuous light (BCL) for 24 hours. Flash electroretinograms (fERGs) were recorded in control and treated rats the day before and 1 week after light exposure. At the end of the second recording session, the animals were killed and the retinas were quickly removed, fixed, cryosectioned, and the thickness of the outer nuclear layer (ONL) was analyzed. Changes in protein level and cellular localization of fibroblast growth factor (FGF)2 were determined by Western blot analysis and retinal immunohistochemistry, respectively. In a second series of experiments, rats were killed at the end of light exposure, and the amount of apoptotic figures in the ONL was assessed by terminal transferase-mediated deoxyuridine triphosphate (d-UTP)-biotin nick-end labeling (TUNEL). BCL induced DNA fragmentation, characteristic of dying cells, almost exclusively in the photoreceptor layer. The rate of photoreceptor death induced by BCL is expressed as the frequency of TUNEL-positive profiles per millimeter. The photoreceptor layer was largely preserved in saffron-treated animals. In addition, the rate of photoreceptor death induced by BCL appeared drastically reduced in treated animals. In beta-carotene prefeeding experiments, morphologic analysis showed preservation of the ONL similar to that obtained with saffron prefeeding, whereas the fERG response was unrecordable. Western blot analysis showed that exposure to light induced a strong upregulation of FGF2 in control and beta-carotene-treated rats, but no change was noted in saffron-treated rats. The results revealed that saffron may protect photoreceptors from retinal stress, maintaining both morphology and function and probably acting as a regulator of programmed cell death [212].

Saffron extract prevented selenite-induced cataract formation in Wistar rats, possibly through the reinforcement of antioxidant status, reduction of the intensity of lipid peroxidation, protection of the sulfhydryl groups, and inhibition of proteolysis of the the water-soluble fraction (WSF) of lens proteins [200].

Crocin analogs isolated from saffron significantly increased the blood flow in the retina and choroid as well as facilitated retinal function recovery and it could be used to treat ischemic retinopathy and/or age-related macular degeneration [213].

The effects of saffron ethanolic extract and its constituents, crocin and safranal, were evaluated in skeletal muscle during ischemia-reperfusion (I/R) injury. Hind limb ischemia was induced using clamping the common femoral artery and vein. After 2 h ischemia, the clamp of the femoral vessels of animals was taken off and the animal underwent 1h reperfusion. Muscle injuries were evaluated by recording of the electromyographic (EMG) potentials and performing some biochemical analysis including thiobarbituric acid reactive substances (TBARS), total sulfhydryl (SH) groups and antioxidant capacity of muscle (using FRAP assay). The ethanolic extract of saffron (5, 20 and 80 mg/ kg), crocin (50, 200 and 400 mg/ kg), safranal (0.1, 0.25 and 0.5 ml/ kg) and normal saline (10 ml/ kg) were administered intraperitoneally 1 h prior reperfusion. The average peak-to-peak amplitude during I/R was significantly increased in extract, crocin and safranal groups in comparison with control-ischemic group. Following saffron, crocin and safranal administration, the total SH contents and antioxidant capacity were elevated in muscle flap. The MDA level was declined significantly in test groups[214].

The methanolic extract of *Crocus sativus* flowers showed a significant inhibitory effect on tyrosinase activity of 28.22% [23]. The tyrosinase inhibitory activities of 25 compounds isolated from the stigmas of *Crocus sativus* were evaluated *in vitro* using mushroom tyrosinase. Among them, crocusatin H, crocin-1, and crocin-3 showed significant tyrosinase inhibitory activity [90]. Crocusatin-K, crocusatin-L, and 4-hydroxy-3,5,5-trimethylcyclohex-2-enone also showed significant antityrosinase activity [91].

Toxicity, side effects and contra-indications:

The LD_{50} for stigma croci was reported to be 20.7 g/kg bw in rodents [123]. The LD_{50} of a 95% ethanol extract of the stigmas was > 600 mg/kg bw in mice [126]. No haematological or biochemical toxic effects were recorded after intragastric administration of up to 50 mg/kg bw in mice [173].

The effects of saffron petal extract (SPE) on blood parameters, immune system, and spleen histology were studied using five concentrations, 0, 75, 150, 225, and 450 mg/kg body weight of SPE. The SPE was injected intraperitoneally to rats for 14 days. Results showed no significant difference between treatments and control group regarding the amount of RBC, HGB, HCT, and PLT. The level of IgG at 75 mg/kg was significantly increased in comparison with other groups. No changes were observed in spleen histology [215].

The possible toxic effects of ethanolic extract of *Crocus sativus* stigma on liver, kidney and some hematological parameters was evaluated in rats. Wistar rats were given 0.35, 0.70 and 1.05 g/kg of ethanolic extract of *Crocus sativus* stigma, daily for 2 weeks intraperitoneally. Body weight of the animals were recorded on the first, seventh and final days of the experiment. The haematological studies included total RBC count, total WBC count, Hb, %HCT, MCV, MCH and MCHC. Biochemical studies included ALT, AST, urea, uric

acid and creatinine. Tissue specimens of the liver and kidneys were subjected to histological examination using standard hematoxyline-eosin staining. The extract caused significant reductions in the Hb, HCT levels and total RBC count, however, these effects were not dose-dependent effect. Total WBC count showed significant dose-dependent increases in extract treated rats. Significant dose-dependent increased values of AST, ALT, urea, uric acid and creatinine were recorded. Microscopically, there were mild to severe hepatic and renal tissue injuries which supported the biochemical analysis. The results indicated that extract of *Crocus sativus* stigma was toxic in high doses [216].

Crocus sativus stigma tablets were evaluated for short-term safety and tolerability in healthy adult volunteers. The study was a double-blind, placebo- controlled design consisting of a 1 week treatment of saffron tablets. Volunteers were divided into 3 groups of 10 each (5 males and 5 females). Group I received placebo; groups 2 and 3 received 200 and 400 mg saffron tablets, respectively, for 7 days. General measures of health were recorded during the study, including hematological, biochemical and electrocardiographic parameters in pre-and post-treatment periods. Clinical examination showed no gross changes in all volunteers after intervention. Saffron with higher dose (400 mg) decreased standing systolic blood pressure and mean arterial pressures significantly. Saffron decreased slightly some hematological parameters such as red blood cells, hemoglobin, hematocrit and platelets. Saffron increased sodium, blood urea nitrogen and creatinine. Accordingly, saffron tablets may change some hematological and biochemical parameters, but these alterations were in normal ranges and they were not important clinically [217].

At doses of 5 g or more, it cause serious adverse reactions. Overdose of stigma croci (12–20 g/day) may be fatal [218-219]. However, the lethal dose is 20 g, smaller doses may cause vomiting, uterine bleeding, bloody diarrhoea, haematuria, bleeding from the nose, lips and eyelids, vertigo, numbness and yellowing of the skin and mucous membranes [220].

Oral administration of 5 g in human, resulted in localized skin haemorrhages, marked thrombocytopenia, and abnormalities of blood clotting [221]. Stigma croci may induce uterine contractions and is therefore contraindicated during pregnancy [220].

III. CONCLUSION

This review discusses the chemical constituent, pharmacological and therapeutic effects of *Crocus sativus* as promising source of many drugs because of its safety and effectiveness.

REFERENCES

[1] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of plants with anticancer activity (part 1) . Int J of Pharmacy 2015; 5(3): 104-124.

[2] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of plants with anti-inflammatory, antipyretic and analgesic activity (part 1). Int J of Pharmacy 2015; 5(3): 125-147.

[3] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of their immunological effects (part 1). Asian Journal of Pharmaceutical Research 2015; 5(3): 208-216.

[4] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of their gastro-intestinal effects (part 1) Ind J of Pharm Sci & Res 2015; 5(4): 220-232.

[5] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of their antiparasitic, antiprotozoal, molluscicidal and insecticidal activity (part 1). J of Pharmaceutical Biology 2015; 5(3): 203-217.

[6] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of plants with antidiabetic effects (part 1). J of Pharmaceutical Biology 2015; 5(3): 218-229.

[7] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of plants with antifungal activity (part 1). Int J of Pharm Rev & Res 2015; 5(3):321-327.

[8] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of their respiratory effects (part 1). International Journal of Pharmacological Screening Methods 2015; 5(2):64-71.

[9] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of plants with hypolipidemic, hemostatic, fibrinolytic and anticoagulant effects (part 1). Asian Journal of Pharmaceutical Science & Technology 2015; 5(4): 271-284.

[10] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of their effect on reproductive systems (part 1). Ind J of Pharm Sci & Res 2015; 5(4): 240-248.

[11] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of their dermatological effects (part 1) Int J of Pharm Rev & Res 2015; 5(4):328-337.

[12] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of plants affected smooth muscles functions (part 1). Int J of Pharmacy 2015; 5(2): 90-97.

[13] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of their detoxification capacity and protective effects (part 1). Asian Journal of Pharmaceutical Science & Technology 2015; 5(4): 257-270.

[14] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of their antibacterial activity (part 1). International Journal of Pharmacology and Toxicology 2015; 6(3): 137-158.

[15] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of plants with antioxidant activity (part 1). International Journal of Pharmacology and Toxicology 2015; 6(3): 159-182.
[16] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of their antiviral activity (part 1). International Journal of Pharmacological Screening Methods 2015; 5(2): 72-79.
[17] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of plants with cardiovascular effects (part 1). Int J of Pharmacology & Toxicology 2015; 5(3): 163-176.
[18] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of medicinal plants with central nervous effects (part 1). Int J of Pharmacology & Toxicology 2015; 5(3): 177-192.
[19] Al-Snafi AE. Pharmacological effects of *Allium* species grown in Iraq. An overview. International Journal of Pharmaceutical and health care Research 2013;1(4):132-147.
[20] Al-Snafi AE. Galactagogue action of the crude phenolic extracts of grape seeds (*Vitis vinifera*). International Journal of Biological & Pharmaceutical Research 2015; 6(8): 577-580.
[21] Al-Snafi AE. Mammary gland stimulating effects of the crude phenolic extracts of green tea (*Camellia sinensis*). International Journal of Biological & Pharmaceutical Research 2015; 6(7): 573-576.
[22] Al-Snafi AE. The pharmacological Importance of *Antirrhinum majus* - A review. Asian J of Pharm Sci & Tech 2015; 5(4): 313-320.
[23] Al-Snafi AE. Chemical constituents and pharmacological effects of *Astragalus hamosus* and *Astragalus tribuloides* grown in Iraq. Asian J of Pharm Sci & Tech 2015; 5(4): 321-328.
[24] Al-Snafi AE. The Pharmacological Importance of *Ballota nigra* –A review. Ind J of Pharm Sci & Res 2015; 5(4): 249-256.
[25] Al-Snafi AE. Chemical constituents and pharmacological importance of *Bidens tripartitus* - A review. Ind J of Pharm Sci & Res 2015; 5(4): 257-263.
[26] Al-Snafi AE. The pharmacological importance of *Brassica nigra* and *Brassica rapa* grown in Iraq. J of Pharm Biology 2015; 5(4): 240-253.
[27] Al-Snafi AE. The chemical constituents and pharmacological importance of *Celosia* cristata – A review. J of Pharm Biology 2015; 5(4): 254-261.
[28] Al-Snafi AE. The pharmacological importance of *Centaurea cyanus*- A review. Int J of Pharm Rev & Res 2015; 5(4): 379-384.
[29] Al-Snafi AE. The chemical constituents and pharmacological importance of *Chrozophora tinctoria*. Int J of Pharm Rev & Res 2015; 5(4): 391-396.
[30] Al-Snafi AE. Medicinal plants with anti-urolithiatic effects (part1). Int J of Pharmacy 2015; 5(2): 98-103.
[31] Al-Snafi AE, Allahwerdi, IY. and Jawad IA. Using of topical 5% urtica dioica ointment in treatment of psoriasis. European Journal of Biomedical and Pharmaceutical Sciences 2015; 2(4):103-111.
[32] 32-Al-Snafi AE. Cardiovascular effects of *Carthamus tinctorius*: A mini-review. Asian Journal of Pharmaceutical Research 2015; 5(3): 199-209.
[33] Al-Snafi AE. The pharmacological activities of *Cuminum cyminum* - A review. IOSR Journal of Pharmacy 2016; 6(6): 46-65.
[34] Al-Snafi AE. Medical importance of *Cupressus sempervirens*- A review. IOSR Journal of Pharmacy 2016; 6(6): 66-76.
[35] Al-Snafi AE. The contents and pharmacology of *Crotalaria juncea*- A review. IOSR Journal of Pharmacy 2016; 6(6): 77-86.
[36] Al-Snafi AE. The medical importance of *Cydonia oblonga*- A review. IOSR Journal of Pharmacy 2016; 6(6): 87-99.
[37] Al-Snafi AE. The chemical constituents and pharmacological importance of *Carthamus tinctorius* - An overview. Journal of Pharmaceutical Biology 2015; 5(3): 143-166.
[38] Al-Snafi AE. Clinically tested medicinal plant: A review (Part 1). SMU Medical Journal 2016; 3(1): 99-128.
[39] Al-Snafi AE. The pharmacological importance of *Artemisia campestris*- A review. Asian Journal of Pharmaceutical Research 2015;5(2): 88-92.
[40] Al-Snafi AE. Chemical constituents and pharmacological effects of *Asclepias curassavica* – A review. Asian Journal of Pharmaceutical Research 2015; 5(2): 83-87.
[41] Al-Snafi AE. The pharmacological importance of *Asparagus officinalis* - A review. Journal of Pharmaceutical Biology 2015; 5(2): 93-98.
[42] Al-Snafi AE. The medical importance of *Betula alba* - An overview. Journal of Pharmaceutical Biology 2015; 5(2): 99-103.
[43] Al-Snafi AE. Bioactive components *and* pharmacological effects of *Canna indica*- An overview. International Journal of Pharmacology and toxicology 2015; 5(2):71-75.
[44] Al-Snafi AE. The chemical constituents and pharmacological effects of *Capsella bursa-pastoris* - A review. International Journal of Pharmacology and toxicology 2015; 5(2):76-81.

[45] Al-Snafi AE. The pharmacological importance of *Ailanthus altissima*- A review. International Journal of Pharmacy Review and Research 2015; 5(2):121-129
[46] Al-Snafi AE. *Alhagi maurorum* as a potential medicinal herb: An Overview. International Journal of Pharmacy Review and Research 2015; 5(2):130-136.
[47] Al-Snafi AE. The pharmacological importance of *Aloe vera*- A review. International Journal of Phytopharmacy Research 2015; 6(1) : 28-33.
[48] Al-Snafi AE. The constituents and biological effects of *Arundo donax* - A review. International Journal of Phytopharmacy Research 2015; 6(1): 34-40.
[49] Al-Snafi AE. The nutritional and therapeutic importance of *Avena sativa* - An Overview. International Journal of Phytotherapy 2015; 5(1): 48-56.
[50] Al-Snafi AE. The Pharmacological importance of *Bellis perennis* - A review. International Journal of Phytotherapy 2015; 5(2): 63-69.
[51] Al-Snafi AE. The chemical constituents and pharmacological effects of *Capparis spinosa* - An overview. Indian Journal of Pharmaceutical Science and Research 2015; 5(2): 93-100.
[52] Al-Snafi AE. The chemical constituents and pharmacological effects of *Carum carvi* - A review. Indian Journal of Pharmaceutical Science and Research 2015; 5(2): 72-82.
[53] Al-Snafi AE. The pharmacological importance of *Casuarina equisetifolia* - An Overview. International Journal of Pharmacological Screening Methods 2015; 5(1): 4-9.
[54] Al-Snafi AE. The chemical constituents and pharmacological effects of *Chenopodium album* - An overview. International J of Pharmacological Screening Methods 2015; 5(1): 10-17.
[55] Al-Snafi AE. Chemical constituents and pharmacological effects of *Clerodendrum inerme*- A review. SMU Medical Journal 2016; 3(1): 129-153.
[56] Ali Esmail Al-snafi. Chemical constituents and pharmacological effects of *Citrullus colocynthis* - A review. IOSR Journal Of Pharmacy 2016; 6(3): 57-67.
[57] Ali Esmail Al-Snafi. Medical importance of *Cichorium intybus* – A review IOSR Journal Of Pharmacy 2016; 6(3): 41-56.
[58] Ali Esmail Al-Snafi. Pharmacological importance of *Clitoria ternatea* – A review IOSR Journal Of Pharmacy 2016; 6(3): 68-83.
[59] Ali Esmail Al-Snafi. The medical Importance of *Cicer arietinum* - A review IOSR Journal Of Pharmacy 2016; 6(3): 29-40.
[60] Ali Esmail Al-Snafi. Medical importance of *Antemis nobilis* (*Chamaemelum nobilis*)- A review. Asian Journal of Pharmaceutical Science & Technology 2016; 6(2): 89-95.
[61] Ali Esmail Al-Snafi. *Adonis aestivalis*: pharmacological and toxicological activities- A revew. Asian Journal of Pharmaceutical Science & Technology 2016; 6(2): 96-102.
[62] Al-Snafi AE. Study of drugs prescribing pattern of specialists and general practitioners in Tikrit city. Med J Tikrit Univ 1997; 3: 12-17 .
[63] Al- Snafi AE. Antimicrobial drugs. Al Diaa Publication house, Iraq 2013.
[64] Al- Snafi AE. Pharmacology and therapeutics. Al Diaa Publication house, Iraq 2013.
[65] Al-Snafi, AE. The best lysosomal stabilizing and hypolipoproteinemic mono/ polyunsaturated fatty acids combination . Med. J Tikrit Univer 2002, 8:148-153 .
[66] Al-Snafi AE. Chemical constituents and pharmacological importance of *Agropyron repens* – A review. Research Journal of Pharmacology and Toxicology 2015; 1 (2): 37-41.
[67] Al–Snafi AE. Pharmacology and medicinal properties of *Caesalpinia crista* - An overview. International Journal of Pharmacy 2015; 5(2): 71-83.
[68] Al-Snafi AE. The chemical constituents and pharmacological effects of *Calendula officinalis* - A review. Indian Journal of Pharmaceutical Science & Research 2015; 5(3): 172-185.
[69] Al-Snafi AE. The constituents and pharmacological properties of *Calotropis procera* - An Overview. International Journal of Pharmacy Review & Research 2015; 5(3): 259-275.
[70] Al-Snafi AE. The pharmacological importance of *Artemisia campestris*- A review. Asian Journal of Pharmaceutical Research 2015;5(2): 88-92.
[71] Hansel R *et al*., eds. Hagers handbuch der pharmazeutischen Praxis. Bd 4, Drogen A–D, 5th ed. Berlin, Springer 1992.
[72] ITIS, *Crocus sativus* (L.), http://www.itis.gov/servlet/SingleRpt/SingleRpt?search_ topic =TSN&search_value=501794
[73] USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network-(GRIN) National Germplasm Resources Laboratory, Beltsville, Maryland. URL: http://www.ars-grin.gov.4/cgi-bin/npgs/html/taxon.pl?12265 [08 Oct 2015]
[74] Schormüller J. Handbuch der Lebensmittelchemie 1970: Vol. VI.
[75] Vinning G. Saffron: issues in international marketing. SNV Thimpu, Kingdom of Bhutan 2005; No. 9.

[76] Singla RK and Varadaraj BG. Crocin: an overview. Indo Global Journal of Pharmaceutical Sciences 2011; 1(4): 281-286.
[77] PDR for Herbal Medicines. Medical Economics Company, Inc. at Montvale, 2000: 653-654.
[78] Ferrence SC and Bendersky G. Therapy with saffron and the goddess at Thera. Perspect Biol Med 2004;47:199-226.
[79] Rios JL, Recio MC, Giner RM and Manez S.. An update review of saffron and its active constituents. Phytother Res 1996;10:189-193.
[80] Winterhalter P and Straubinger, M. Saffron-renewed interest in an ancient spice. Food Reviews International 2000;16: 39-59.
[81] Iborra JL, Castellar MR, Canovas M and Manjon A. TLC preparative purification of picrocrocin, HTCC and crocin from saffron. J Food Sci 1992; 57: 714-718.
[82] Tarantilis PA, Polissiou M, Mentzafos D, Terzis A and Manfait M. The structure of dimethylcrocetin. J Chemical Crystallogr 1994; 24: 739-742.
[83] Bolhasani A, Bathaie SZ, Yavari I, Moosavi-Movahedi AA and Ghaffari M. Separation and purification of some components of Iranian saffron. Chem Asian J 2005; 17: 725-729.
[84] Caballero-Ortega H, Pereda-Miranda R and Abdullaev FI. HPLC quantification of major active components from 11 different saffron (*Crocus sativus* L.) sources. Food Chemistry 2007; 100: 1126-1131.
[85] Fern´andez JA. Biology, biotechnology and biomedicine of saffron. Recent Res Devel Plant Sci 2004; 2: 127-159.
[86] Zeka K, Ruparelia KC, Continenza MA, Stagos D, Vegliò F and Arroo RR. Petals of *Crocus sativus* L. as a potential source of the antioxidants crocin and kaempferol. Fitoterapia 2015; 107: 128-134.
[87] Abdullaev Jafarova F, Caballero-Ortega H, Riverón-Negrete L, Pereda-Miranda R, Rivera-Luna R, Manuel Hernández J, Pérez-López I and Espinosa-Aguirre JJ. *In vitro* evaluation of the chemopreventive potential of saffron. Rev Invest Clin 2002;54(5): 430-436.
[88] Caballero-Ortega H, Pereda-Miranda R, Riverón-Negrete L, Hernández JM, Medécigo-Ríos M, Castillo-Villanueva A and Abdullaev FI. Chemical composition of saffron (*Crocus sativus* L.) from four countries. Proc. Ist IS on Saffron. Eds: JA Fernández & F Abdullaev, Acta Hort 650, ISHS 2004
[89] Hosseinzadeh H, Sadeghnia HR, Ziaee T and Danaee A. Protective effect of aqueous saffron extract (*Crocus sativus* L.) and crocin, its active constituent, on renal ischemia-reperfusion-induced oxidative damage in rats. J Pharm Pharm Sci 2005; 8(3): 387-393.
[90] Li CY and Wu TS. Constituents of the stigmas of *Crocus sativus* and their tyrosinase inhibitory activity. J Nat Prod 2002; 65(10): 1452-1456.
[91] Li CY, Lee EJ and Wu TS. Antityrosinase principles and constituents of the petals of *Crocus sativus*. J Nat Prod 2004;67(3):437-440.
[92] Karimi E, Oskoueian E, Hendra R and Jaafar HZ. Evaluation of *Crocus sativus* L. stigma phenolic and flavonoid compounds and its antioxidant activity. Molecules 2010; 15(9):6244-6256.
[93] Sariri R, Sabbaghzadeh R and Poumohamad F. *In-vitro* antioxidant and anti-tyrosinase activity of methanol extracts from *Crocus sativus* flowers. Pharmacologyonline 2011; 2: 1205-1215.
[94] Fahim NK, Janati SSF and Feizy J. Chemical composition of agriproduct saffron (*Crocus sativus* L.) petals and its considerations as animal feed. GIDA - Journal of Food 2012; 37(4):197-201.
[95] Asai A, Nakano T, Takahashi M and Nagao A. Orally administered crocetin and crocins are absorbed into blood plasma as crocetin and its glucuronide conjugates in mice. Journal of Agricultural and Food Chemistry 2005; 53: 7302-7306.
[96] Qian Z, Xi L, Du P and Fu J. Pharmacokinetic properties of crocin (crocetin digentiobiose ester) following oral administration in rats. Phytomedicine 2007; 14: 633-636.
[97] Wang Y, Han T, Zhu Y, Zheng CJ, Ming QL, Rahman K and Qin LP. Antidepressant properties of bioactive fractions from the extract of *Crocus sativus* L. J Nat Med 2010; 64(1): 24-30.
[98] Noorbala AA, Akhondzadeh S, Tahmacebi-Pour N and Jamshidi AH. Hydro-alcoholic extract of *Crocus sativus* L. versus fluoxetine in the treatment of mild to moderate depression: a double-blind, randomized pilot trial. J Ethnopharmacol 2005; 97(2):281-284.
[99] Basti AA, Moshiri E, Noorbala A, Jamshidi A, Abbasi SH and Akhondzadeh S. Comparison of petal of *Crocus sativus* L. and fluoxetine in the treatment of depressed outpatients: A pilot double-blind randomized trial. Progress in Neuro-Psychopharmacology & Biological Psychiatry 2007; 31: 439-442.
[100] Georgiadou G, Tarantilis PA and Pitsikas N. Effects of the active constituents of *Crocus sativus* L., crocins, in an animal model of obsessive-compulsive disorder. Neurosci Lett 2012; 528(1): 27-30.
[101] 101-Akhondzadeh S, Tahmacebi-Pour N, Noorbala AA, Amini H, Fallah-Pour H, Jamshidi AH and Khani M. *Crocus sativus* L. in the treatment of mild to moderate depression: a double-blind, randomized and placebocontrolled trial. Phytother Res. 2005; 19: 148 -1 51.

[102] Moshiri E, Basti AA, Noorbala AA, Jamshidi AH, Hesameddin Abbasi S, Akhondzadeh S. *Crocus sativus* L. (petal) in the treatment of mild-to-moderate depression: a double-blind, randomized and placebo controlled trial. Phytomedicine 2006; 13: 607- 611.
[103] Akhondzadeh Basti A, Moshiri E, Noorbala AA, Jamshidi AH, Abbasi SH and Akhondzadeh S. Comparison of petal of *Crocus sativus* L. and fluoxetine in the treatment of depressed outpatients: a pilot double-blind randomized trial. Prog. Neuropsychopharmacol Biol. Psychiatry 2007; 31: 439 -442.
[104] Ghasemi T, Abnous K, Vahdati F, Mehri S, Razavi BM and Hosseinzadeh H. Antidepressant effect of *Crocus sativus* aqueous extract and its effect on CREB, BDNF, and VGF transcript and protein levels in rat hippocampus. Drug Res (Stuttg) 2015; 65(7): 337-343.
[105] Hosseinzadeh H and Khosravan V. Anticonvulsant effects of aqueous and ethanolic extracts of *Crocus sativus* L. stigma in mice. Arch Irn Med 2002; 5 (1): 44-47.
[106] Hosseinzadeh H and Talebzadeh F. Anticonvulsant evaluation of safranal and crocin from *Crocus sativus* in mice. Fitoterapia 2005;76:722–724.
[107] Pathan SA, Alam S, Jain GK, Zaidi SM, Akhter S, Vohora D, Khar RK and Ahmad FJ. Quantitative analysis of safranal in saffron extract and nanoparticle formulation by a validated high-performance thin-layer chromatographic method. Phytochem Anal 2010; 21(3):219-223.
[108] Hosseinzadeh H and Noraei NB. Anxiolytic and hypnotic effect of *Crocus sativus* aqueous extract and its constituents, crocin and safranal, in mice. Phytother Res 2009; 23(6):768-774.
[109] Zhang Y, Shoyama Y, Sugiura M and Saito H. Effects of *Crocus sativus* L. on the ethanol-induced impairment of passive avoidance performances in mice. Biological and Pharmaceutical Bulletin 1994; 17(2):217–221.
[110] Pitsikas N, Boultadakis A, Gergiadou G, Tarantilis PA and Sakellaridis N. Effects of the active constituents of *Crocus sativus* L. in an animal model of anxiety. Phytomedicine. 2008;15:1135-1139.
[111] Mobarakeh JI, Fakhraei N, Sadr1 ZA, Hamidipour A, Mostafavi E, Hosseini RH and Sardari S. Effect of aqueous, ethanolic and acetonitrile *Crocus sativus* L. extracts on stress biomarkers in male rats. Journal of Medicinal Plants Research 2013; 7(44): 3269-3279.
[112] Hooshmandi Z, Rohani AH, Eidi A, Fatahi Z, Golmanesh L and Sahraei H. Reduction of metabolic and behavioral signs of acute stress in male Wistar rats by saffron water extract and its constituent safranal. Pharm Biol 2011; 49(9): 947-954.
[113] Abe K and Saito H. Effects of saffron extract and its constituent crocin on learning behaviour and long-term potentiation. Phytother Res 2000; 14(3): 149-152.
[114] Papandreou MA, Kanakis CD, Polissiou MG, Efthimiopoulos S, Cordopatis P, Margarity M and Lamari FN. Inhibitory activity on amyloid-beta aggregation and antioxidant properties of *Crocus sativus* stigmas extract and its crocin constituents. J Agric Food Chem 2006; 54(23): 8762-8768.
[115] Dashti MH, Zeinali F, Anvari M and Hosseini SM. Saffron (*Crocus sativus* L.) extract prevents and improves D- galactose and $NaNO_2$ induced memory impairment in mice. EXCLI Journal 2012;11:328-337.
[116] He WB, Zhang JL, Xue W, Hu JF, Wu DH and Chen NH. Comparison of the action of isolichenin and methanol extract of saffron on long-term potentiation in hippocampal dentate gyrus *in vivo*. Yao Xue Xue Bao 2009; 44(8): 858-862.
[117] Ghadrdoost B, Vafaei AA, Rashidy-Pour A, Hajisoltani R, Bandegi AR, Motamedi F, Haghighi S, Sameni HR and Pahlvan S. Protective effects of saffron extract and its active constituent crocin against oxidative stress and spatial learning and memory deficits induced by chronic stress in rats. Eur J Pharmacol 2011; 667(1-3): 222-229.
[118] Naghibi SM, Hosseini M, Khani F, Rahimi M, Vafaee F, Rakhshandeh H and Aghaie A. Effect of aqueous extract of *Crocus sativus* L. on morphine-induced memory impairment. Adv Pharmacol Sci 2012; doi: 10.1155/2012/494367.
[119] Geromichalos GD, Lamari FN, Papandreou MA, Trafalis DT, Margarity M, Papageorgiou A and Sinakos Z. Saffron as a source of novel acetylcholinesterase inhibitors: molecular docking and *in vitro* enzymatic studies. J Agric Food Chem 2012; 60(24): 6131-6138.
[120] Akhondzadeh S, Shafiee Sabet M, Harirchian MH, Togha M, Cheraghmakani H, Razeghi S, Hejazi SS, Yousefi MH, Alimardani R, Jamshidi A, Rezazadeh SA, Yousefi A, Zare F, Moradi A and Vossoughi A. A 22-week, multicenter, randomized, double-blind controlled trial of *Crocus sativus* in the treatment of mild-to-moderate Alzheimer's disease. Psychopharmacology (Berl) 2010; 207(4): 637-643.
[121] Amin B, Malekzadeh M, Heidari MR and Hosseinzadeh H. Effect of *Crocus sativus* extracts and its active constituent safranal on the harmaline-induced tremor in mice. Iran J Basic Med Sci 2015; 18(5): 449-458.
[122] Hosseinzadeh H and Jahanian Z. Effect of *Crocus sativus* L. (saffron) stigma and its constituents, crocin and safranal, on morphine withdrawal syndrome in mice. Phytother Res 2010; 24(5): 726-730.

[123] Shati AA, Elsaid FG and Hafez EE. Biochemical and molecular aspects of aluminium chloride-induced neurotoxicity in mice and the protective role of *Crocus sativus* L. extraction and honey syrup. Neuroscience 2011; 175: 66-74.
[124] Linardaki ZI, Orkoula MG, Kokkosis AG, Lamari FN and Margarity M. Investigation of the neuroprotective action of saffron (*Crocus sativus* L.) in aluminum-exposed adult mice through behavioral and neurobiochemical assessment. Food Chem Toxicol 2013; 52: 163-170.
[125] Ghazavi A, Mosayebi G, Salehi H and Abtahi H. Effect of ethanol extract of saffron (*Crocus sativus* L.) on the inhibition of experimental autoimmune encephalomyelitis in C57bl/6 mice. Pak J Biol Sci 2009; 12(9): 690-695.
[126] Mousavi SH, Tayarani NZ and Parsaee H. Protective effect of saffron extract and crocin on reactive oxygen species-mediated high glucose-induced toxicity in PC12 cells. Cell Mol Neurobiol 2010; 30(2):185-191.
[127] Moallem SA, Hariri AT, Mahmoudi M and Hosseinzadeh H. Effect of aqueous extract of *Crocus sativus* L. (saffron) stigma against subacute effect of diazinon on specific biomarkers in rats. Toxicol Ind Health 2014; 30(2): 141-146.
[128] Saleem S, Ahmad M, Ahmad AS, Yousuf S, Ansari MA, Khan MB, Ishrat T and Islam F. Effect of saffron (*Crocus sativus*) on neurobehavioral and neurochemical changes in cerebral ischemia in rats. J Med Food 2006; 9(2): 246-253.
[129] Hosseinzadeh H, Sadeghnia HR, Ghaeni FA, Motamedshariaty VS and Mohajeri SA. Effects of saffron (*Crocus sativus* L.) and its active constituent, crocin, on recognition and spatial memory after chronic cerebral hypoperfusion in rats. Phytother Res 2012; 26(3): 381-386.
[130] Samarghandian S, Azimi-Nezhad M and Samini F. Ameliorative effect of saffron aqueous extract on hyperglycemia, hyperlipidemia, and oxidative stress on diabetic encephalopathy in streptozotocin induced experimental diabetes mellitus. Biomed Res Int. 2014; doi: 10.1155/2014/920857.
[131] Ghoshooni H, Daryaafzoon M, Sadeghi-Gharjehdagi S, Zardooz H, Sahraei H, Tehrani SP, Noroozzadeh A, Bahrami-Shenasfandi F, Kaka GH and Sadraei SH. Saffron (*Crocus sativus*) ethanolic extract and its constituent, safranal, inhibits morphine-induced place preference in mice. Pak J Biol Sci 2011; 14(20): 939-944.
[132] Berger F, Hensel A and Nieber K. Saffron extract and trans-crocetin inhibit glutamatergic synaptic transmission in rat cortical brain slices. Neuroscience 2011; 180:238-247.
[133] Keyhani E and Keyhani J. Comparative study of superoxide dismutase activity assays in *Crocus sativus* L. corms. Prikl Biokhim Mikrobiol 2006; 42(1): 111-116.
[134] Ordoudi SA, Befani CD, Nenadis N, Koliakos GG and Tsimidou MZ. Further examination of antiradical properties of *Crocus sativus* stigmas extract rich in crocins. J Agric Food Chem 2009; 57(8): 3080-3086.
[135] Montoro P, Maldini M, Luciani L, Tuberoso CI, Congiu F and Pizza C. Radical scavenging activity and LC-MS metabolic profiling of petals, stamens, and flowers of *Crocus sativus* L. J Food Sci 2012; 77(8): C893-900.
[136] Assimopoulou AN, Sinakos Z and Papageorgiou VP. Radical scavenging activity of *Crocus sativus* L. extract and its bioactive constituents. Phytother Res 2005; 19(11): 997-1000.
[137] Chahine N, Hanna J, Makhlouf H, Duca L, Martiny L and Chahine R. Protective effect of saffron extract against doxorubicin cardiotoxicity in isolated rabbit heart. Pharm Biol 2013;51(12):1564-1571.
[138] Mehdizadeh R, Parizadeh MR, Khooei AR, Mehri S and Hosseinzadeh H. Cardioprotective effect of saffron extract and safranal in isoproterenol-induced myocardial infarction in wistar rats. Iran J Basic Med Sci 2013; 16(1): 56-63.
[139] Sachdeva J, Tanwar V, Golechha M, Siddiqui KM, Nag TC, Ray R, Kumari S and Arya DS. *Crocus sativus* L. (saffron) attenuates isoproterenol-induced myocardial injury via preserving cardiac functions and strengthening antioxidant defense system. Exp Toxicol Pathol 2012; 64(6): 557-564.
[140] Liu N, Yang Y, Mo S, Liao J and Jin J.Calcium antagonistic effects of Chinese crude drugs: 9155.
[141] Khori V, Alizadeh AM, Yazdi H, Rakhshan E, Mirabbasi A, Changizi S, Mazandarani M and Nayebpour M. Frequency-dependent electrophysiological remodeling of the AV node by hydroalcohol extract of *Crocus sativus* L. (saffron) during experimental atrial fibrillation: the role of endogenous nitric oxide. Phytother Res 2012; 26(6): 826-832.
[142] Boskabady MH, Shafei MN, Shakiba A and Sefidi HS. Effect of aqueous-ethanol extract from *Crocus sativus* (saffron) on guinea-pig isolated heart. Phytother Res 2008; 22(3): 330-334.
[143] Fatehi M, Rashidabady T and Fatehi-Hassanabad Z. Effects of *Crocus sativus* petals' extract on rat blood pressure and on responses induced by electrical field stimulation in the rat isolated vas deferens and guinea-pig ileum. J Ethnopharmacol 2003; 84(2-3): 199-203.

[144] Imenshahidi M, Hosseinzadeh H and Javadpour Y. Hypotensive effect of aqueous saffron extract (*Crocus sativus* L.) and its constituents, safranal and crocin, in normotensive and hypertensive rats. Phytother Res 2010; 24(7): 990-994.
[145] Imenshahidi M, Razavi BM, Faal A, Gholampoor A, Mousavi SM and Hosseinzadeh H. The effect of chronic administration of saffron (*Crocus sativus*) stigma aqueous extract on systolic blood pressure in rats. Jundishapur J Nat Pharm Prod 2013; 8(4): 175-179.
[146] Joukar S . Electrocardiogram alterations following one- week consumption of *Crocus sativus* L.(Saffron). EXCLI Journal 2012; 11: 480-486.
[147] Mousavi M, Baharara J and Shahrokhabadi K. The Synergic Effects of *Crocus sativus* L. and low frequency electromagnetic field on VEGFR2 gene expression in human breast cancer cells. Avicenna J Med Biotechnol 2014; 6(2): 123-127.
[148] Mancini A, Serrano-Díaz J, Nava E, D'Alessandro AM, Alonso GL, Carmona M and Llorens S. Crocetin, a carotenoid derived from saffron (*Crocus sativus* L.), improves acetylcholine-induced vascular relaxation in hypertension. J Vasc Res 2014; 51(5): 393-404.
[149] Sheng L, Qian Z, Zheng S and Xi L. Mechanism of hypolipidemic effect of crocin in rats: crocin inhibits pancreatic lipase. Eur J Pharmacol 2006; 543: 116-122.
[150] Zheng S, Qian Z, Tang F and Sheng L. Suppression of vascular cell adhesion molecule-1 expression by crocetin contributes to attenuation of atherosclerosis in hypercholesterolemic rabbits. Biochem Pharmacol 2005; 70: 1192-1199.
[151] Gainer JW and Chisolm GM. Oxygen diffusion and atherosclerosis. Atherosclerosis 1974; 19:135-13.
[152] He S, Qian Z, Tang F, Wen N, Xu G and Sheng L. Effect of crocin on experimental atherosclerosis in quails and its mechanisms. Life Sciences 2005; 77: 907–921.
[153] Verma SK and Bordia A. Antioxidant property of saffron in man. Indian J Med Sci 1998; 52: 205-207.
[154] Nishio T, Okugawa H, Kato A, Hashimoto Y, Matsumoto K and Fujioka A. Effect of crocus (*Crocus sativus* L, Iridaceae) on blood coagulation and fibrinolysis. Shoyakugaku Zasshi 1987; 41: 271–276.
[155] Jessie SW and Krishnakantha TP. Inhibition of human platelet aggregation and membrane lipid peroxidation by food spice, saffron. Mol Cell Biochem 2005; 278(1-2):59-63.
[156] Boskabady MH, Tabatabaee A and Byrami G. The effect of the extract of *Crocus sativus* and its constituent safranal, on lung pathology and lung inflammation of ovalbumin sensitized guinea-pigs. Phytomedicine 2012;19(10): 904-911.
[157] Bayrami G and Boskabady MH. The potential effect of the extract of *Crocus sativus* and safranal on the total and differential white blood cells of ovalbumin-sensitized guinea pigs. Res Pharm Sci 2012; 7(4): 249-255.
[158] Gholamnezhad Z, Koushyar H, Byrami G and Boskabady MH. The extract of *Crocus sativus* and its constituent safranal, affect serum levels of endothelin and total protein pin sensitized Guinea Pigs. Iran J Basic Med Sci 2013; 16(9): 1022-1026.
[159] Vosooghi S, Mahmoudabady M, Neamati A and Aghababa H. Preventive effects of hydroalcoholic extract of saffron on hematological parameters of experimental asthmatic rats. Avicenna J Phytomed 2013; 3(3): 279-287.
[160] Mahmoudabady M, Neamati A, Vosooghi S and Aghababa H. Hydroalcoholic extract of *Crocus sativus* effects on bronchial inflammatory cells in ovalbumin sensitized rats. Avicenna J Phytomed 2013; 3(4): 356-363.
[161] Byrami G, Boskabady MH, Jalali S and Farkhondeh T. The effect of the extract of *Crocus sativus* on tracheal responsiveness and plasma levels of IL-4, IFN-γ, total NO and nitrite in ovalbumin sensitized guinea-pigs. J Ethnopharmacol 2013; 147(2): 530-535.
[162] Samarghandian S, Borji A, Farahmand SK, Afshari R and Davoodi S. *Crocus sativus* L. (saffron) stigma aqueous extract induces apoptosis in alveolar human lung cancer cells through caspase-dependent pathways activation. Biomed Res Int 2013; doi: 10.1155/2013/417928.
[163] Boskabady MH and Aslani MR. Relaxant effect of *Crocus sativus* (saffron) on guinea-pig tracheal chains and its possible mechanisms. J Pharm Pharmacol 2006; 58(10): 1385-1390.
[164] Hosseinzadeh H and Ghenaati J. Evaluation of the antitussive effect of stigma and petals of saffron (*Crocus sativus*) and its components, safranal and crocin in guinea pigs. Fitoterapia 2006; 77(6): 446-448.
[165] Abdullaev FI and Frenkel GD. The effect of saffron on intracellular DNA, RNA and protein synthesis in malignant and non-malignant human cells. Biofactors 1992; 4(1): 43-45.
[166] Abdullaev FI. Cancer chemopreventive and tumoricidal properties of saffron (*Crocus sativus* L.). Exp Biol Med (Maywood) 2002; 227(1): 20-25.
[167] Aung HH, Wang CZ, Ni M, Fishbein A, Mehendale SR, Xie JT, Shoyama CY and Yuan CS. Crocin from *Crocus sativus* possesses significant anti-proliferation effects on human colorectal cancer cells. Exp Oncol 2007; 29(3): 175-180.

[168] Samarghandian S, Boskabady MH and Davoodi S. Use of *in vitro* assays to assess the potential antiproliferative and cytotoxic effects of saffron (*Crocus sativus* L.) in human lung cancer cell line. Pharmacogn Mag 2010; 6(24): 309-314.
[169] Bajbouj K, Schulze-Luehrmann J, Diermeier S, Amin A and Schneider-Stock R. The anticancer effect of saffron in two p53 isogenic colorectal cancer cell lines. BMC Complement Altern Med 2012; 12: 69-78.
[170] Samarghandian S, Tavakkol Afshari J and Davoodi S. Suppression of pulmonary tumor promotion and induction of apoptosis by *Crocus sativus* L. extraction. Appl Biochem Biotechnol 2011; 164(2): 238-247.
[171] Tavakkol-Afshari J, Brook A and Mousavi SH. Study of cytotoxic and apoptogenic properties of saffron extract in human cancer cell lines. Food Chem Toxicol 2008; 46(11): 3443-3447.
[172] Feizzadeh B, Afshari JT, Rakhshandeh H, Rahimi A, Brook A and Doosti H. Cytotoxic effect of saffron stigma aqueous extract on human transitional cell carcinoma and mouse fibroblast. Urol J 2008; 5(3): 161-167.
[173] Nair SC, Kurumboor SK and Hasegawa JH. Saffron chemoprevention in biology and medicine: a review. Cancer Biother 1995; 10(4): 257-264.
[174] D'Alessandro AM, Mancini A, Lizzi AR, De Simone A, Marroccella CE, Gravina GL, Tatone C and Festuccia C. *Crocus sativus* stigma extract and its major constituent crocin possess significant antiproliferative properties against human prostate cancer. Nutr Cancer 2013; 65(6): 930-942.
[175] Bathaie SZ, Miri H, Mohagheghi MA, Mokhtari-Dizaji M, Shahbazfar AA and Hasanzadeh H. Saffron aqueous extract inhibits the chemically-induced gastric cancer progression in the Wistar albino rat. Iran J Basic Med Sci 2013; 16(1): 27-38.
[176] Nair SC, Pannikar B and Panikkar KR. Antitumour activity of saffron (*Crocus sativus*). Cancer Lett 1991;57(2):109-114.
[177] Das I, Das S and Saha T. Saffron suppresses oxidative stress in DMBA- induced skin carcinoma: A histopathological study. Acta histochemica 2010; 112: 317-327.
[178] Mousavi SH, Afshari JT, Brook A and Anarkooli IJ. Role of caspases and Bax protein in saffron induced apoptosis in MCF-7 cells. Food and Chemical Toxicology 2009; 47:1909-1913.
[179] Xia D. Ovarian cancer HO-8910 cell apoptosis induced by crocin *in vitro*. Nat Prod Commun 2015; 10(2): 249-252.
[180] Hosseinzadeh H and Younesi HM. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. BMC Pharmacol 2002; 2: 7.
[181] Hosseinzadeh H, Ziaee T and Sadeghi A. The effect of saffron, *Crocus sativus* stigma, extract and its constituents, safranal and crocin on sexual behaviors in normal male rats. Phytomedicine 2008; 15(6-7): 491-495.
[182] 182-Mohammadzadeh-Moghadam H, Nazari SM, Shamsa A, Kamalinejad M, Esmaeeli H, Asadpour AA and Khajavi A. Effects of a topical saffron (*Crocus sativus* L) gel on erectile dysfunction in diabetics: A randomized, parallel-group, double-blind, placebo-controlled trial. J Evid Based Complementary Altern Med 2015, doi:10.1177/2156587215583756
[183] Tavana S, Eimani H, Azarnia M, Shahverdi A and Eftekhari-Yazdi P. Effects of saffron (*Crocus sativus* L.) aqueous extract on *in vitro* maturation, fertilization and embryo development of mouse oocytes. Cell J 2012; 13(4): 259-264.
[184] Mokhber Maleki E, Eimani H, Bigdeli MR, Ebrahimi B, Shahverdi AH, Golkar Narenji A and Abedi R. A comparative study of saffron aqueous extract and its active ingredient, crocin on the *in vitro* maturation, *in vitro* fertilization, and *in vitro* culture of mouse oocytes. Taiwan J Obstet Gynecol 2014; 53(1): 21-25.
[185] Agha-Hosseini M, Kashani L, Aleyaseen A, Ghoreishi A, Rahmanpour H, Zarrinara AR and Akhondzadeh S. *Crocus sativus* L. (saffron) in the treatment of premenstrual syndrome: a double-blind, randomised and placebo-controlled trial. International Journal of Obstetrics and Gynaecology 2008; dio:10.1111/j.1471-0528.2007.01652.x
[186] Bandegi AR, Rashidy-Pour A, Vafaei AA and Ghadrdoost B. Protective effects of *Crocus sativus* L extract and crocin against chronic-stress induced oxidative damage of brain, liver and kidneys in rats. Adv Pharm Bull 2014; 4(Suppl 2): 493-499.
[187] Omidi A, Riahinia N, Montazer Torbati MB and Behdani MA. Hepatoprotective effect of *Crocus sativus* (saffron) petals extract against acetaminophen toxicity in male Wistar rats. Avicenna J Phytomed 2014; 4(5): 330-336.
[188] Pan TL, Wu TH, Wang PW, Leu YL, Sintupisut N, Huang CH, Chang FR and Wu YC. Functional proteomics reveals the protective effects of saffron ethanolic extract on hepatic ischemia-reperfusion injury. Proteomics. 2013; 13(15): 2297-2311.

[189] Mohajeri D, Doustar Y, Rezaei A and Mesgari-Abbasi M. Hepatoprotective effect of ethanolic extract of *Crocus sativus* L. (Saffron) stigma in comparison with silymarin against rifampin induced hepatotoxicity in rats. ZJRMS 2010; 12(5): 53-59.
[190] Lin JK and Wang CJ. Protection of crocin dyes on the acute hepatic damage induced by aflatoxin B1 and dimethylnitrosamine in rats. Carcinogenesis. 1986;7:595–599.
[191] Hosseinzadeh H, Abootorabi A and Sadeghnia HR. Protective effect of *Crocus sativus* stigma extract and crocin (trans-crocin 4) on methyl methanesulfonate-induced DNA damage in mice organs. DNA Cell Biol 2008; 27(12): 657-764.
[192] Premkumar K, Thirunavukkarasu C, Abraham SK, Santhiya ST and Ramesh A. Protective effect of saffron (*Crocus sativus* L.) aqueous extract against genetic damage induced by anti-tumor agents in mice. Hum Exp Toxicol 2006; 25(2): 79-84.
[193] Premkumar K, Abraham SK, Santhiya ST, Gopinath PM and Ramesh A. Inhibition of genotoxicity by saffron (*Crocus sativus* L.) in mice. Drug Chem Toxicol 2001; 24(4): 421-428.
[194] Abe K, Sugiura M, Yamaguchi S, Shoyama Y and Saito H. Saffron extract prevents acetaldehyde-induced inhibition of long-term potentiation in the rat dentate gyrus *in vivo*. Brain Res 1999; 851(1-2): 287-289.
[195] Premkumar K, Abraham SK, Santhiya ST and Ramesh A. Protective effects of saffron (*Crocus sativus* Linn.) on genotoxins-induced oxidative stress in Swiss albino mice. Phytother Res 2003; 17(6): 614-617.
[196] Premkumar K, Abraham SK, Santhiya ST and Ramesh A. Inhibitory effects of aqueous crude extract of Saffron (*Crocus sativus* L) on chemical-induced genotoxicity in mice. Asia Pac J Clin Nutr 2003; 12(4): 474-476.
[197] El Daly ES. Protective effect of cysteine and vitamin E, *Crocus sativus* and *Nigella sativa* extracts on cisplatin-induced toxicity in rats. J Pharm Belg 1998; 53(2): 87-93.
[198] El Daly ES. Protective effect of cysteine and vitamin E, *Crocus sativus* and *Nigella sativa* extracts on cisplatin- induced toxicity in rats. Journal of Islamic Academy of Sciences 1996; 9(4): 105-118.
[199] Ajami M, Eghtesadi S, Pazoki-Toroudi H, Habibey R and Ebrahimi SA. Effect of *Crocus sativus* on gentamicin induced Nephrotoxicity. Biol Res 2010; 43: 83-90.
[200] Sakr SA, Zowail ME and Marzouk AM. Effect of saffron (*Crocus sativus* L.) on sodium valporate induced cytogenetic and testicular alterations in albino rats. Anat Cell Biol 2014; 47(3): 171-179.
[201] Nair SC, Salomi MJ, Panikkar B and Panikkar KR. Modulatory effects of *Crocus sativus* and *Nigella sativa* extracts on cisplatin-induced toxicity in mice. J Ethnopharmacol 1991; 31(1): 75-83.
[202] Al-Mofleh LA, Alhaider AA and Mossa JS. Antigastric ulcer studies on 'saffron' *Crocus sativus* L. in rats. Pakistan Journal of Biological Sciences 2006; 9 (6): 1009-1013.
[203] Halataei BA, Khosravi M, Arbabian S, Sahraei H, Golmanesh L, Zardooz H, Jalili C and Ghoshooni H. Saffron (*Crocus sativus*) aqueous extract and its constituent crocin reduces stress-induced anorexia in mice. Phytother Res 2011; 25(12): 1833-1838.
[204] Gout B, Bourges C and Paineau-Dubreuil S. Satiereal, a *Crocus sativus* L extract, reduces snacking and increases satiety in a randomizedplacebo-controlled study of mildly overweight, healthy women. Nutr Res 2010; 30(5): 305-313.
[205] Boskabady MH, Seyedhosseini Tamijani SM, Rafatpanah H, Rezaei A and Alavinejad A. The effect of *Crocus sativus* extract on human lymphocytes' cytokines and T helper 2/T helper 1 balance. J Med Food 2011; 14(12): 1538-1545.
[206] Bani S, Pandey A, Agnihotri VK, Pathania V and Singh B. Selective Th2 upregulation by *Crocus sativus*: A neutraceutical spice. Evid Based Complement Alternat Med 2011; doi: 10.1155/2011/639862.
[207] Xiang M, Yang M, Chenghua Z, Juan L, Wenna L and Zhiyu Q. Crocetin prevents AGEs-induced vascular endothelial cell apoptosis. Pharmacological research 2006; 54: 268-274.
[208] Khorasani G, Hosseinimehr SJ, Zamani P, Ghasemi M and Ahmadi **A.** The effect of saffron *(Crocus sativus)* extract for healing of second-degree burn wounds in rats. Keio J Med 2008; 57 (4): 190-195.
[209] Yousefi E, Eskandari A, Gharavi MJ and Khademvatan S. *In vitro* activity and cytotoxicity of *Crocus sativus* extract against leihmania major (MRHO/IR/75/ER). Infect Disord Drug Targets 2014; 14(1): 56-60.
[210] Leffingwell JC. Saffron. Leffing Well Reports 2002; 2 (5):1-3.
[211] Shariatifar N, Shoeibi S, Sani MJ, Jamshidi AH, Zarei A, Mehdizade A and Dadgarnejad M. Study on diuretic activity of saffron (stigma of *Crocus sativus* L.) aqueous extract in rat. J Adv Pharm Technol Res 2014; 5(1): 17-20.
[212] Maccarone R, Di Marco S and Bisti S. Saffron supplement maintains morphology and function after exposure to damaging light in mammalian retina. Invest Ophthalmol Vis Sci 2008; 49(3): 1254-1261.
[213] Xuan B. Effects of crocin analogs on ocular flow and retinal function. J Ocul Pharmacol Ther 1999; 15: 143–152.

[214] -Hosseinzadeh H, Modaghegh MH and Saffari Z. *Crocus sativus* L. (Saffron) extract and its active constituents (crocin and safranal) on ischemia-reperfusion in rat skeletal muscle. Evid Based Complement Alternat Med 2009; 6(3):343-350.
[215] Babaei A, Arshami J, Haghparast A and Danesh Mesgaran M. Effects of saffron (*Crocus sativus*) petal ethanolic extract on hematology, antibody response, and spleen histology in rats. Avicenna J Phytomed. 2014; 4(2): 103-109.
[216] Mohajeri D, Mousavi G, Mesgari M, Doustar Y and Nouri HK. Subacute toxicity of *Crocus sativus* L. (saffron) stigma ethanolic extract in rats. American Journal of Pharmacology and Toxicology 2007; 2 (4):189-193.
[217] Modaghegha MH, Shahabiana M, Esmaeilib H, Rajbaic Oand Hosseinzadehd H. Safety evaluation of saffron (*Crocus sativus*) tablets in healthy volunteers. Phytomedicine 2008; 15: 1032–1037.
[218] Physician's desk reference for herbal medicines. Montvale, NJ, Medical Economics Co 1998.
[219] Central Council for Research in Ayurveda and Siddha. Experimental cultivation of saffron (kumkum). New Delhi, Ministry of Health and Welfare 1995.
[220] Bisset NG. Herbal drugs and phytopharmaceuticals. Boca Raton, FL, CRC Press 1994.
[221] Frank A. Auffallende purpurea bei artifi ziellem Abort. Deutsche Medizinische Wochenschrift 1961; 86: 1618.