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Antiobesity Effect of Terminalia Chebula Fruit Extract on High Fat Diet Induced Obese Animal Model

Mujeeba Rehman[1], S.B Tiwari[2]
[1,2]*Department of Pharmaceutical sciences, Division of pharmacology, Mahatma jyotiba phule Rohilkhand university, Bareilly, India*
Corresponding Author: Mujeeba Rehman

Abstract: The aim of the present investigation was to determine whether the methanolic extract of terminalia chebula has an effect on body weight in obese animal model induced by high fat diet. Terminalia chebula methanolic extract was orally administered (500mg/kg) for 49 days to treated group rats to determine it's antiobesity potential as compared to other groups of normal, obese control and standard group. The effect was evaluated on the basis of various parameters (body weight, weight of liver and fatty tissue, lipid profile and histopathological examination of adipose tissue in liver). Our data promises the use of terminalia chebula in further investigation as an anti-obesity agent.

Keywords: Orlistat, Terminalia chebula, adipose tissue

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I. INTRODUCTION

On global scale, obesity has reached epidemic proportions and is a major contributor to the global burden of chronic disease and disability. Currently, more than one billion adults worldwide are overweight and at least 300 million of them are clinically obese (WHO, 2009) [1]. Obesity and overweight recognised as chronic conditions which has emerged as one of the most serious global problem worldwide. The recent report of WHO (2012) estimated that worldwide 1.5 billion adults are overweight, among them over 200 million men and almost 300 million women are obese. In India, obesity is the most prevalent in urban populations (male: 5.5%, female: 12.6%), followed by the urban slums (male: 1.9%, female: 7.2%) and least in rural populations (male: 1.6%, female: 3.8%). Obesity has reached epidemic proportions in India with morbid obesity affecting 5% of the country's population [2].The word obesity comes from the Latin word obesitas, which means stout, fat, or plump. Medically, obesity is a condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and increased health problem. The World Health Organization described obesity as an epidemic hazard worldwide, based on the data analysis of body mass index (BMI). Person with BMI of less than 18.5 is referred to as underweight, 18.5-24.9 normal weight, 25.0-29.9 is overweight, 30.0-34.9 is class I obesity, 35.0-39.9 is class II obesity and over40.0 is class III obesity.

Indeed obesity facilitates the development of metabolic disorders (e.g. diabetes, hypertension), and cardiovascular diseases in addition to chronic diseases (e.g .stroke, osteoarthritis, sleep apnoea, cancers and inflammation based pathologies) [3]. A streak of evidence indicates that serotonin, histamine, dopamine and their associated receptor activities as well as role of leptin, ghrelin and neuropeptides in obesity regulation.[4] Overweight of a person is recognised when body mass index (BMI) of a person is 25-30kg/m^2 and above 30kg/m^2 represents obesity. Various social and economic factors are responsible for causing obesity some of them including lack of physical ability, chronic alcoholism, overconsumption of food and sedentary lifestyle. Data from U.S. National health and nutrition examination survey published in 1998 revealed a U-shaped relationship between BMI and mortality. Significantly increased mortality was only associated with either extreme of BMI [5]. The primary treatment of obesity is dieting and physical exercise. If this fails, antiobesity drugs or surgery is performed to reduce appetite or inhibit fat absorption [6]. Among the treatments of obesity along with the physical work and the drugs used a low fat diet must be recommended. A low-energy diet recommended for the treatment of obesity should be low fat (30%), high carbohydrate (55% of daily energy intake), high protein (up to 25% of daily energy intake), and high fiber (25 g/day). A high-carbohydrate low-fat energy deficient diet is usually recommended for weight management by medical societies and health authorities [7]. Currently drugs available in market for treatment of obesity are divided into two major classes one is Orlistat which reduces fat absorption through inhibition of pancreatic lipase and second is Sibutramine which is an anorectic or appetite suppressent but it's prolonged use have toxic effects including increase in headache, dry mouth, insomnia and constipation. Orlistat acts locally in gastrointestinal tract to inhibit

pancreatic and gastric lipases by forming covalent bond with active serine residue site of gastric and pancreatic lipase leading to the inactivation of enzymes. As a result of this the inactivated enzymes are unable to hydrolyse dietary fat in the form of triglycerides to absorbable free fatty acids and monoglycerides. Undigested triglycerides are not absorbed, leading to caloric deficit which ultimately leads to weight control[8]. Another drug sibutramine influences both sides of energy balance. Along with the reduction in food (energy) intake by enhancing satiety (fullness) it also reduces decline in metabolic rate that leads to weight loss [9]. In 1990 fenfluramine and dexfenfluramine were withdrawn from market because of heart valve damage. Similarly in feburary 2011 contrive popularly known as combination of buproprion/naltrexone were rejected by US FDA due to cardiovascular risks [10]. Therefore, keeping in view about high cost and side effects the need for development of natural products against obesity have come into working proposition for safe, effective antiobesity drug with no/minimal side effects.

At the turn of century, approx. 170 herbal drugs were officially recognised in U.S.P. and National Formulary (N.F). In India 70% of populations are reported using traditional medicines for primary health care and needs [11]. Terminalia chebula Retz (combretaceae) medium sized up to 25m tall, deciduous tree of variable appearance with usually short cylindrical bole of 5-10m length, 60-80cm in diameter, crown rounded with spreading branches, leaves alternate ovate or elliptic ovate, flowers in axillary 5-7cm long spikes, simple or sometimes branched, yellowish white and unpleasantly scented, fruit an obovoid or oblong ellipsoid drupe, 2.5-5cm long, yellow to orange-brown when ripe glabrous [12]. Ethanobotanical information regarding Terminalia chebula reveals the following medicinal uses antibacterial, antihyperglycemic, antioxidant, anti-nociceptive, hypercholestermic, anti-caries anti-arthritic, cytoprotective, antihyperglycemic, neuropharmacological activities[13,14,15,16]. More future research is necessary to discover new drug therapies that can be used to reduce the prevalence of obesity. Our present investigation is designed to determine whether the plant Terminalia chebula selected for study has an effect on body weight in HFD induced obese animal model.

II. MATERIALS AND METHODS

2.1 Chemical and reagents

Orlistat, gallic acid and quercetin was procured from Sigma Aldrich Chemicals Co., Ltd, St. Louis, MO, USA. Folin ciocalteau reagent was procured from CDH fine chemicals (P) Ltd., Darya ganj, New Delhi, India. Other analytical grade chemicals, reagents and solvents were obtained from Lobachem and Merck laboratories.

2.2 Collection and authentication of plant material

The fruits of terminalia chebula were collected from local firms in Bareilly. The fruits were identified and authenticated by Dr. Alok srivastava , Professor of Department of Plant sciences, Faculty of science, M.J.P. Rohilkhand University, Bareilly.

2.3 Preparation of the plant extract

Fruits of the plant were baked into finely grounded powder using mechanical grinder. Preparation of the extract was done by maceration method [17]. Powdered fruit sample (300grams) was soaked in 750 ml of methanol for 16 days. It was then filtered through cotton plug followed by whattman filter paper no.1. Extract was then concentrated with rotary evaporator and it gave 7.20 gram of methanolic extract.

2.4 Phytochemical screening of extract:

The phytochemical screening as well as identification of the plant extract was done by standard chemical methods. The results of the phytochemical test are shown in Table 1**.**

2.4.1 Saponins: Powdered drug extract on shaking vigorously with water results into frothing which indicates the presence of saponins.

2.4.2 Alkaloids: Extracts were treated with few drops of dilute hydrochloric acid and filtered. The filtrate obtained when treated with dragendorff reagent (brown precipitate), Wagner reagent (red colour precipitate) and Hager reagent (yellow precipitate) indicated the presence of alkaloids.

2.4.3 Flavonoids**:** Extract treated with few drops of10% lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Alkaline reagent test: Extract treated with sodium hydroxide solution shows yellow colouration which decolourises on addition with few drops of dilute hydrochloric acid indicates the presence of flavonoids[18]

2.4.4 Glycosides**:** Extract treated with glacial acetic acid containing one drop of 2% ferric chloride solution, add concentrated H_2SO_4dropwise. Brown ring appeared at the interface indicated the presence of glycosides

2.4.5 Phenols**:** Plant extract dissolved in 2 ml distilled water and treated with few drops of 10% ferric chloride solution. Bluish black colour indicates the presence of phenols [19].

2.4.6 Tannins: Extract treated with 1% gelatin solution containing sodium chloride. White coloured precipitate indicates the presence of flavonoids.

2.4.7 Triterpenes: Extract treated with few drops of acetic anhydride boiled and cooled, concentrated sulphuric acid added to the sides of tube dropwise. Appearance of brown ring at the junction of the two layers and the red coloured lower layer confirmed the presence of triterpenoid.

2.5 In-vitro studies:

2.5.1 Determination of total phenol content [20]

The amount of total phenolics in extract was determined with folin-ciocalteau reagent. Gallic acid was used as standard and the total phenolics were expressed as milligram/gram gallic acid equivalents.(GAE). For this purpose, the calibration curve of gallic acid was drawn. (Figure 1). 1ml of standard solution of concentration 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid prepared in methanol. Extract solution of 1mg/ml concentration was also prepared. 0.5ml of this solution was introduced into test tubes and mixed with2.5ml of a 10 fold dilute Folin- Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature then absorbance was read at 760 nm spectrometrically

2.5.2 Determination of flavonoid content [20]

Aluminum chloride method was used for flavonoid determination with some modifications using quercetin as standard and measured as quercetin equivalent. For the preparation of standard calibration curve of quercetin, different concentration aliquots from 1ml of standard solution of quercetin (0.02, 0.04, 0.06, 0.08, 0.1 mg/l) was taken into 10ml volumetric flask, containing 4ml of distilled water. 0.3ml of 5%$NaNO_2$ added to the flask. After 5min, 0.3ml 10%$AlCl_3$ was added to the mixture. At the 6th min 2ml of 1M NaOH was added and volume made up to 10ml with distilled water. (Figure 2). Extract solution of 1mg/ml was also prepared and repeated the same process then the absorbance was noted at 510nm spectrometrically.

2.6 Experimental animlals

Healthy male adult albino wistar rats 7-8 weeks old weighing 140-160 gram have been housed at polypropylene confines and kept up in standard environment [12 hour light and 12 hour dark cycle, (25±3) degree Celsius. Animals of control group were fed on a standard chow diet and water ad libitum, whereas animals used for evaluation of obesity are feed on a High fat diet (HFD) and water ad libitum. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in M.J.P. Rohilkhand university, Bareilly (CPCSEA Approval No. 1884/Go/Re/S/16/CPCSEA)

2.7 High fat diet (HFD) formula (gm/kg) [21]

Corn starch-186.8, Sugar-167.5, Lard -180, Vitamin mixture-10, Mineral mixture-35, Casein-290, Cellulose powder-50.6, Butter oil-99

2.8 Experimental design:

In this experiment total 24 rats were used. They were separated into four groups of 6 rats each. The total experimental period was of 49 days.

Group I: Control rats (Normal pellet chow and water for 49 days)

Group II: Obese control group (HFD for 49 days)

Group III: Treated group (HFD + extract 500 mg/kg body weight orally from 16^{th} to 49^{th} day)

Group IV: Standard group (HFD + standard drug orlistat 32mg/kg body weight orally from 16^{th} to 49^{th} day)

2.9 Evaluation parameters:

2.9.1 Body weight: The body weight (gram) of each rat in all four groups was recorded on day one and then on every week continued till whole experimental period of 49 days using digital weighing balance.

2.9.2 Weight of organs and fat pads: On 50^{th} day of experiment all the animals in each group were sacrificed by cervical dislocation and organs like liver and visceral fat pads were carefully taken out from the body, washed in normal saline to remove blood dried on filter paper and weighed.

2.9.3 Biochemical estimation: The blood samples of each and every animal in all four groups were collected into dry centrifugation tubes and allowed to stand for 30 minutes at 20-25 degree Celsius. The clear serum separated at 2500 rpm for 10 minutes using centrifuge. Following parameters were evaluated using commercial kits available:-

- Blood glucose: Fasting blood glucose was estimated by using a commercial glucometer and test strips (Accucheck Sensor test meter).
- Total cholesterol(TC) : Total cholesterol present in serum was determined by (Autospan liquid Gold cholesterol) diagnostic kit.

- High density lipid (HDL): It was measured by CHOD-PAP method using Span diagnostic in vitro kit.
- Triglycerides (TG): Serum triglyceride(TGL) was determined by GPO/PAP method using in vitro diagnostic kit
- Low density lipids(LDL): The Friedewald formula was used to calculate low density lipoprotein lipase

  LDL= C- TGL-(HDL+ TG/5)
- Very low density lipids (VLDL): Estimation of very low density lipid protein (VLDL) was estimated by liquid gold span diagnostic reagent kit.
- Lipase enzyme test: This test was performed by modified assay method using in vitro Span diagnostic kit.

2.9.4 Histopathological studies: The liver tissues of the rats were preserved in 10% formalin. Liver sections (5µm) were acquired and stained by hematoxylin-eosin and viewed under electron microscope for further parameters.

III. RESULTS

3.1 Phytochemical test: Phytochemical test of terminalia chebula was performed and it was evaluated that the methanolic extract of plant shows several phytoconstituents like alkaloids, glycosides, saponins, flavonoids, phenols (Table 1)

Table 1: Phytochemical test of Terminalia chebula extract

Constituents	Test	Methanolic extract
Alkaloids	1.Draggendroff test 2.Wagner test 3.Hager test	++ - ++
Saponins	Foam test	+
Flavonoids	1.Lead acetate test 2.Alkaline reagent test	++ +
Phenols		++
Tannins		++
Triterpenes		-
Glycosides		+

(+) present, (++) moderately present (--) absent

3.2 In-vitro studies: The folin ciocalteau method is one of the oldest methods developed to determine the content of total phenols. Methanolic extract of fruit possess higher amount of phenolics (246 mg/g gallic acid equivalent (Figure1) whereas flavonoid content was (5.47 mg/g quercetin equivalent) (Figure2)

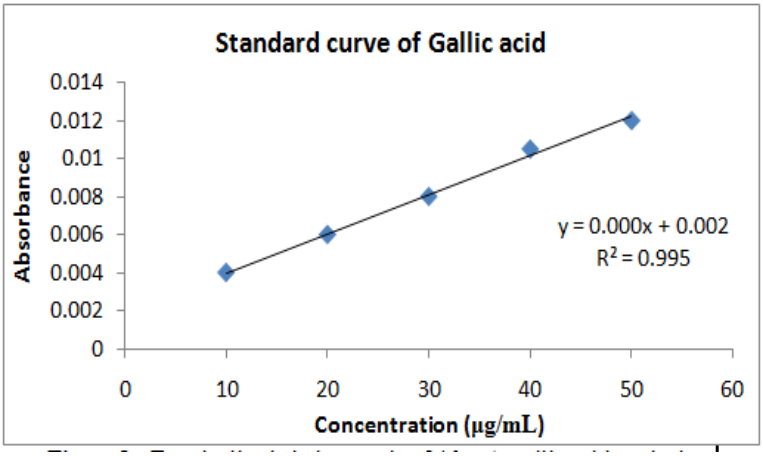

Figure 1: Terminalia chebula contains 246mg/g gallic acid equivalent

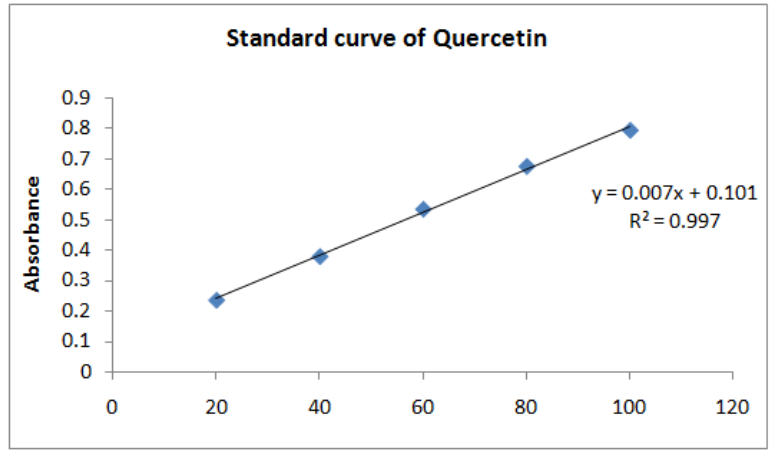

Figure 2 : Terminalia chebula contains 5.47 mg/g of quercetin equivalent

3.3 Effect on body weight: The effect on body weight gain in each of the rats produced by the daily administration of extract from 16th day is illustrated in (table2) and graphical representation in (figure 3). Difference in body weight were statistically significant between normal and obese control whereas extract significantly suppressed body weight from 28th day

Table 2: Changes in body weight

Days	Normal rats (group I)	Obese control (group II)	Treated rats (group III)	Standard rats (group IV)
1	143.3±2.108	143.3±2.108	144.2±2.007	143.8±2.007
7	143.7±2.171	147.7±1.687	147.8±1.515	147.5±2.125
14	143.3±2.275	150.8±1.851*	150.7±1.406	152.1±1.770
21	144.3±2.141	153.8±2.007**	148.8±1.515	148.7±1.926
28	143.5±2.217	156.2±1.990**	146.3±1.726**	145.2±1.641**
35	144±2.082	159.7±1.838***	144.5±1.962***	143.2±1.327****
42	143.3±2.108	162.8±1.612****	142.7±1.909****	140.5±1.335****
49	143.7±2.171	167.3±1.687****	140.2±1.991****	136±1.391****

All values are expressed in (mean ± SEM, n =6) *p<0.05, **p<0.01, ***p<0.001, ****p< 0.0001 when compared (One way ANOVA followed by t-test)

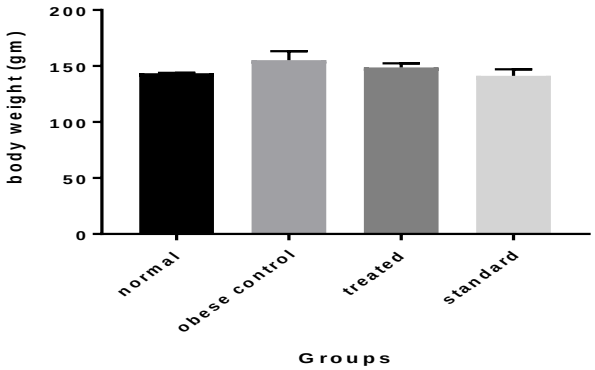

Figure 3: Changes in body weight

3.4 Effect on liver weight, fat pads and blood glucose: Weight of liver, fat pads and blood glucose of all the groups were illustrated in (table 3) and the graphical representation was depicted in (figure 4,5,6). Liver weight, fat mass and blood glucose of obese control rats were significantly increased as compared to normal rats. However standard and treated group rats liver weight and fat mass were significantly decreased as compared to

obese control rats. Blood glucose although reduced in treated rats as compared to control but it was insignificant.

Table 3: Assessment of liver weight, fat pads and blood glucose

Parameters	Normal	Obese control	Treated	Standard
Liver weight	6.783± 0.094	7.96± 0.128****	7.433± 0.152*	7.06±0.71***
Fat mass	4.56± 0.143	5.36± 0.133****	4.96± 0.331*	4.83± 0.021**
Blood Glucose	96.67±2.472	134.2± 4.729****	120± 3.787	107± 4.61***

All values are expressed in (mean ± SEM, n =6) *p<0.05, **p<0.01, ***p<0.001, ****p< 0.0001 when compared (One way ANOVA followed by t-test)

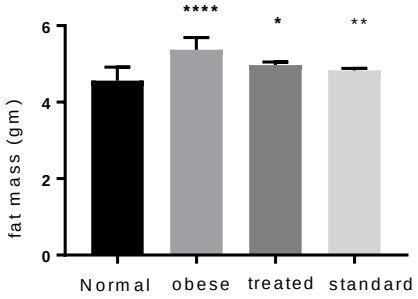

Fig.4

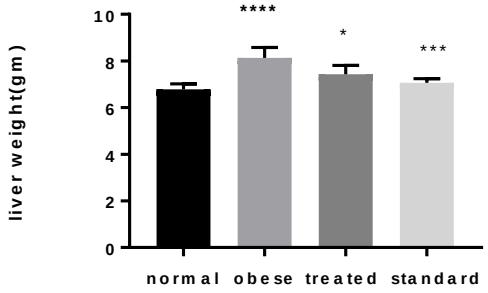

Fig.5

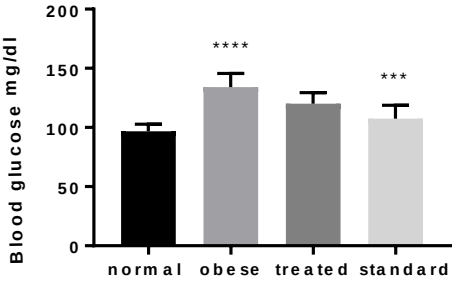

Fig.6

Fig.4 shows changes in fat mass, fig.5 shows changes in liver weight whereas fig.6 shows changes in blood glucose of all the group rats.

3.5 Effect on Lipid profile: High fat diet induced obese rats (Group II) exhibited significant (p<0.001) increase in the lipid profiles such as total cholesterol, triglycerides, LDL, and VLDL when compared to normal control (Group I rats). (Group III & IV) exhibited a significant (p<0.01) decrease in total cholesterol, triglyceride, LDL, and VLDL levels when compared to Group II animals. The Group II animals exhibited reduction in HDL when compared to Group I animals but it was little insignificant. Group III and IV animals exhibited significant increase in HDL level than Group II animals. Lipase enzyme was significantly reduced in group II compared to group I whereas in treated and standard (Group III & Group IV) it is increased. (Table 4).The graphical representation of lipid profile of all the groups are clearly depicted in (figure 7).

Table 4: Assessment of lipid profile

Lipid profile	Normal	Obese control	Treated	Standard
TC	107± 1.633	203.3± 3.575****	153.2± 5.108***	136.3± 2.231****
TGL	74.67 ± 2.155	155.8± 4.277****	129.7± 2.459****	99.5± 2.32****
HDL	47± 2.887	46.83 ± 1.276	54.83± 1.014*	64.17± 1.352***
LDL	38.07± 4.095	125.3 ± 4.342****	75.73 ± 4.403****	52.27± 1.069****
VLDL	14.93±0.431	31.17±0.855****	25.93± 0.491****	19.9 ± 0.464****
SERUM LIPASE	108.3± 4.014	92± 3.512*	98.33± 4.216	109.2± 2.386*

All values are expressed in (mean ± SEM, n =6) $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p< 0.0001$ when compared (Two way ANOVA followed by t-test)

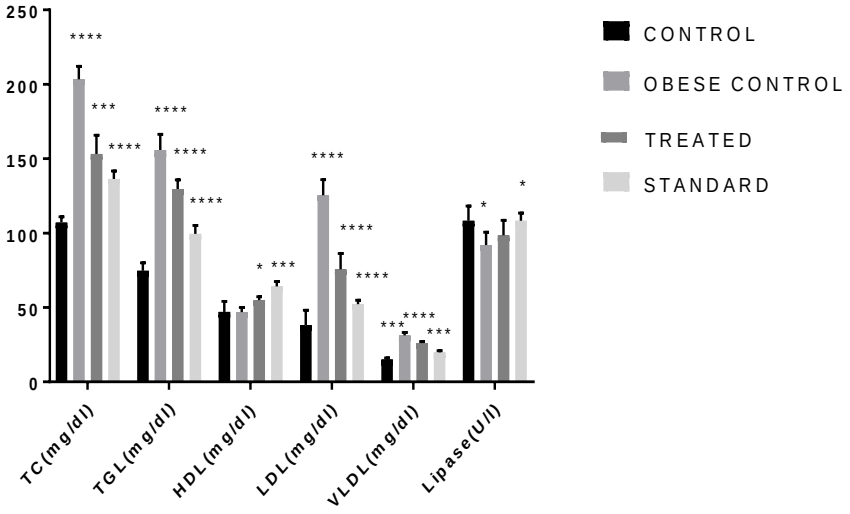

Figure7: Lipid profile of all group rats

3.6 Histopathological studies: Histopathology of all four groups rats was performed and shown in (Figure 8,9,10,11)

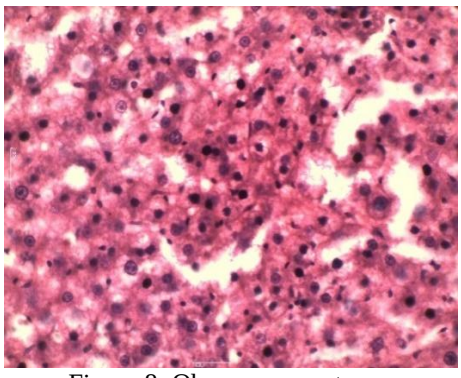

Figure 8: Obese group rat

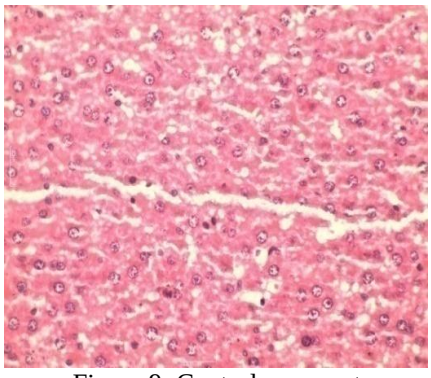

Figure 9: Control group rat

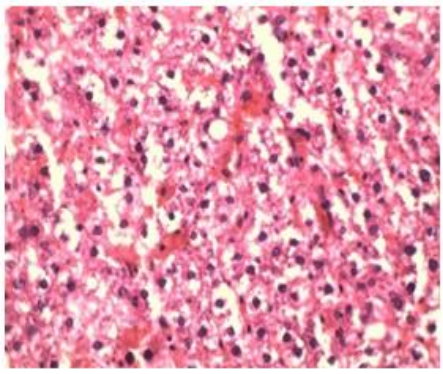

Figure 10: Treated group rat

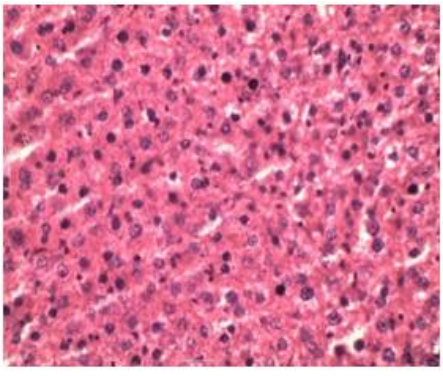

Figure 11: Standard group rat

From the above figures it is concluded that changes in cell size due to deposition of large amount of adipose tissues in obese rat liver as compared to others.

IV. DISCUSSION AND CONCLUSION

Obesity continues to be a growing problem worldwide with no suitable treatment options available till now without side effects. Obesity can be easily induced by high fat diet model in laboratory. This shows that animals fed with HFD have higher energy intake than the normal diet group, and the HFD contribute to the onset of obesity development of profound metabolic alterations [22]. Similar results are reported that consumption of cafeteria diet and atherogenic diet promotes obesity and fat accumulation in several animal species, including rats, mice, and pigs [23]. The rats were fed with high fat diet throughout the study which resulted in further increase in weight. A treatment should be considered successful if further weight gain is controlled, and reduces up to 5–10 % weight from initial body weight, and allows long-term maintenance of the weight [24]. The standard drug Orlistat was found to prevent from increase in the bodyweight of rats and induced more than 10% weight loss from the initial weight. The periepidydimal adipose tissue of test drug weights were found to decrease significantly when compared with obese control group however the decrease in fat mass was more prominent in standard group. The hypoglycemic effect of test groups showed decrease in blood glucose level and there was also significant decreased in triglyceride of test group and standard group when compared with obese control group this might be due to presences of active constituents like chebulic acid, neochebulic acid and Gallic acid which was claimed to reduce triglycerides, total cholesterol and LDL.

Fatty liver was seen in obese rats as compared to treated and the standard group due to the accumulation of fats whereas the histological sections of liver and adipose tissue were observed for different animal groups. The size of adipocytes and their number was found to be higher in case of obese animal. The treated drug has shown significant decrease in the adipocytes size and number as observed from the histological section but was less significant than standard drug. From above study it was found that test drug have a potential to reduce the obesity effects through different biochemical and body parameters as compared to obese group so further more research could be carried for longer periods including molecular mechanistic studies to explore it's anti obese potential.

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