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The pain decreasing effect of the alcoholic extract " of Trachyspermum ammi (L.) (Ajwain) in experimental animals .

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Abstract: In Iraq traditional medicine ,the decoction of *Trachyspermum ammi* is claimed to possess antinociceptive activity. The purpose of this research is to study the pain decreament of alcoholic extract " of *Trachyspermum ammi* in experimental animals , using three algesiometric methods and different doses.The results appears that extract intended to have an acceptable statistical values P<0.05)" as a pain decreasing agent The antinociceptive action of the extract was rapid in onset in action with prolong time of activity. The pain decreasing effect of the extract was inhibited by Metclopramide and Atropine ". Moreover, the extract has no sedative activity. The extract consist of many chemical componenets such as alkaloids , flavonoids , steroids , and polyphenols " that posses the pain decreament effect . The pain decreasing effect maybe due to "dopaminergic and cholinergic muscarinic activity" of the extract . The net effect of the extract shows effectiveness on neural and inflammed pain in the body . The effect obtained of this work wasa new aspects and determine that , *Trachyspermum ammi exert a long term high decreasing effect on different types of pain , which indicate its folkloric in uses as a pain dereament plant.*

Key word: *Trachyspermum ammi* (L.) (Ajwain) ,"*dopaminergic mechanisms, muscarinic mechanisms ", pain decreament ,toxic effect*

I. INTRODUCTION

Injuries to a living tissue usually lead to accumulation of plasma fluids and blood cell at the site of injury , which lead to inflammation as a" pathophysiological " response , the defense mechanism and the complicated aspects associated with pain and inflammation may cause and lead to different diseases [1] . Continuous studies on inflammation and the adverse effects of the of the available analgesic drugs exerts a big problem in clinical trial [2] . Therefore , exploring and finding a new and effective analgesic and pain decreasing chemical drug with less side effect is required *Trachyspermum ammi* (L.) ajwain " family is Umbelliferae and is used as spices in india and Pakistan . The plant fruit is small egged shaped ,grayish color fruit pods , the phytochemical investigations on the seeds of the ajwain plant shows the presence of many chemical components such as votatile aromatic essential oil , crystal substance , stearoptene " , thymol as essential oil also presents in the seeds of th plants. Alcoholic extracts of *Trachyspermum ammi fruit* contain a a watery absorbable hygroscopic saponin " crystal flavinoids and steroidal components [3- 8]Initial studies on the clinical therapeutic uses of the plant oil reveals that it had a parasympathetic " action , while the whole plant seems to exert an increase in diueresis process The using of small amount of the plant seeds as a flavoring agent and as a preservative , and to produce essential oil required for perfume manufacturing . Ajwain in Indian medical mainly used for any disorders of the stomach by crushing the fruits of the plantto make a paste and applied on the skin to relieve the pain associated with abdominal colic , also dry hot fermented fruits of the plant could be applied in asthmatic chest pain . Studies on medical uses of *T. ammi* plant elucidate that it shows an antispasmotic , stimulation of digestive process , protection of the liver and the gastric mucosa , galactogenisis . Ajwain plant also have a potential lowering in blood pressure , lipids , with diuretic and anti-platelets aggregation effects . Studies also reveals that the plant have antimicrobial , analgesic, inflammatory decrement and antitussive activities . The stimulant , carminative , tonic properties of Ajwain plant was studied and often recommended for cholera as it is a potent antimicrobial agent [4– 12] . However, as yet, its analgesic potential of alcoholic extracts of Ajwain has not been scientifically evaluated. The present study is an attempt to address this issue. The objective of this study was to scientifically investigate the effectiveness of the decoction made from leaves of this plant as an oral antinociceptive agent. In Iraq traditional medicine decoction made from Ajwain is recommended as an antinociceptive.

II. MATERIAL AND METHODS

Plant collection and identification

Plant material was collected from local market and authenticated at the National Herbarium of Iraq Botany Directorate in Abu-Ghraib . \

Preparation of the extract

Alcoholic extract of ajwain plant was made by maceration process by dissolving 250 g of dried shaded coarse powder in 600 ml ethanol (95%) . After 5 days ,the extract were filtered , concentrated , evaporated under vacuum (yield 15 g) , the yielded powder stored at 2-8 °C for the uses in further experiments (250, 500, 1000, and 2000 mg/kg) [9].

Animals used in the experiments :

Male healthy adult male rats (weight 200-250g) was applied in the research . The animals used were kept in a standard conditions in plastic cages in the animal house (humidity 50-55% , temperature 28-31 oC ,photo period 12 hours normal light , with free normal feeding pellet and drinking water except at the experimental time . Experiments were done according to the laboratory international standard aspects for animal care and uses .

Pain decreasing activity evaluation

Tail flick and hot plate experiment :

Thirty six male albino rats were randomly selected and fasted for 24 h before the experiment with free access to water and separated into six animal groups (6 animals in each group) and orally treated in as follows : Group 1: with 1mL of sterile water, Groups 2, 3, 4, and 5: with 1mL of 250, 500, 1000, 2000 mg / kg of freeze-dried aqueous extract, respectively, and Group 6: with 1mL of 15mg / kg of morphine sulphate (Pharmachemie B.V., Harlem, Netherlands), the reference drug of opioid receptor agonistas a positive control. One hour before treatment (pretreatment), then every hour for a period of 6 hours as post treatment , the experimental rats were evaluated for tail flick and hot plate taste [13]. The aqueous leaf extract treated rats were observed for elicitation of struab's tail reaction [14]. In the test of hot plate method ,every rat was subjected to hot plate with 50oC temperature and then record the reaction time which is time needed by the animal to lick the hind paw" or jump up . Experimental rats that needs a pre-treatment reaction time more than 15 seconds in the test of hotplate " method was not used in the experiment . A 20 seconds cut time was applied to avoid tissue damage [15]. In the test of tail flick , the reaction time was recorded which is the time needed for the tail to be flicked when immersed in a 55oC water bath 5-6 cm from the tail tip using a stopwatch . Experimental rats that exert more than 5 seconds as a reaction time for the test of tail flicking was not recorded . A 5 seconds cut time was applied to avoid tissue damage [15].

Formalin test

Twelve rats were randomly divided into two groups and treated orally in the following manner. Group 1: with 1mL of distilled water, Groups 2: with 1mL of 2000 mg / kg of freeze-dried alcoholic extract. After 3 hours of the extract treatment, every experimental rats were injected subcutaneously into hind left sub plantar paw surface with 2.5 % formalin solution (0.05 ml) obtained from BDH Chemical company, Poole, UK .Observation for 30 minutes of the experimental rats with recording of the numbers of lifting , licking ,shaking of hind paw "with determining the time spent in licking the animal paw at the injected site in two phases: first one range from 0-5 min and second ranges from 20-30 min [15].

Determination of pain decreasing mechanism of action

Evaluation of the dopamine receptor mediator " :

Two groups of randomly selected experimental rats (each of 6), group 1 were were intraperitoneally injected with 1.5 mg/kg body weight metoclopramide " (AivitaPharmaPvt Limited, Gujarat, India), as an ant dopaminergic drug , while group 2 were injected intraperitoneally with isotonic saline . After 10 minutes both groups of experimental rats were fed orally with freeze dried alcoholic extract (2000 mg/kg body weight).Later on these groups were examined by hot plate test 1 h before treatment and post treatment for 1-3 hours [15].

**Evaluation of cholinergic mediator for the muscarinic receptor : **

Two groups of randomly selected experimental rats (each of 6), group 1 were were intraperitoneally injected with 5 mg/kg body weight atropine sulphate (Harson Laboratories, Borada, India) , as an anti cholinergic muscarinic receptor drug , while group 2 were injected intraperitoneally with isotonic saline . After 10 minutes both groups of experimental rats were fed orally with freeze dried alcoholic extract (2000 mg/kg body weight). Later on these groups were examined by hot plate test 1 h before treatment and post treatment for 1-3 hours [15 ,16].

Evaluation of mediators for opoid receptor ":

Two groups of randomly selected experimental rats (each of 6), group 1 were were intraperitoneally injected with 1.5 mg/ kg body weight(1 ml) naloxone hydrochloride(Samarth Life Sciences Pvt. Ltd, Mumbai, India), as an anti opoid receptor drug , while group 2 were injected intraperitoneally with 1 ml isotonic saline . After 45. minutes both groups of experimental rats were fed orally with freeze dried alcoholic extract (2000 mg/kg body weight). Later on these groups were examined by hot plate test 1 h before treatment and post treatment for 1-3 hours [15,16].

Investigation **for the strength of muscle and muscular coordination :**

Two groups of randomly selected experimental rats (each of 6), group 1 were were oraly fed with 1 ml freeze dried alcoholic extract (2000 mg/kg body weight). , while group 2 were orally fed with 1 ml isotonic saline . After 3h, these rats were subjected to the bar holding test (to evaluate muscle strength) and Bridge test and righting reflex test[17] , the latency of muscles response were recorded and expressed in seconds .

Investigsation of sedative activity :

Two groups of randomly selected experimental rats (each of 6), group 1 were were oraly fed with 1 ml freeze dried alcoholic extract of T.ammi (2000 mg/kg body weight) , while group 2 were orally fed with 1 ml isotonic saline . After 3 hours , both group experimental rats were examined by rat hold board test" for the sedation effect [18] . Every experimental rat examined individually by measuring number of crossing , number of head dipping ,and number of rearing for 7.5 minutes by placing the animals on a standard rat hole board instrument " , and the time cumulative needed for head dipping was measured .

Statistical analysis of data

The data were expressed as the mean ± SEM. Statistical comparisons were made by one-way analysis of variance (ANOVA). Significant values was expressed by $P \leq 0.05$ ".

III. RESULTS

The test of tail flicking and hot plate :

Table – 1 shows the results recorded that 250 mg/kg body weight of alcoholic extract of T.ammi reveal longer reaction time in the test of hot plate model (signifiancy of $p \leq 0.05$)for the 1st – 6th hours of the test when compared to the control group (1st hour 70% , 2nd hour 78%, 3rd hour by 66%, forth hour by 49%, fifth hour by 42% and sixth hour by 28%) and the first and second hour compare with the own pre-treatment value (first hour by 59%, second hour by 61%)%). A 2000 mg/kg body weight of T.ammi alcoholic extract when examined in the test of hot plate model shows a longer reaction time ($p \leq 0.05$ significance) started in the 1st hour to the fifth hours when compared with the control (1st hour 88%, 2nd hour 61%, 3rd hour 80%, forth hour 55% and fifth hour by 37%) and from the first hour to the forth hour compare with the own pre-treatment value (first hour by 93%, second hour by 59%, third hour by 66%, forth hour by 34%). A longer reaction time was significantly evident with 500 mg/kg body weight (with control: second hour by 62%, third hour by 68%, forth hour by 34% and sixth hour by 29%, with own pre-treatment: second hour by 50%, third hour by 45%) and 1000 mg/kg (with control: first hour by 42%, second hour by 49%, forth hour by 66%, fifth hour by 47% and sixth hour by 41%, with own pre-treatment: forth hour by 43% and sixth hour by 42%). Morphine causes a highly increases in the reaction time ($p<0.05$ significancy) until the fifth hour after treatment in comparism with the control (1st hour 102%, 2nd hour 152%, 3rd hour 106%, forth hour 64% and fifth hour 19%) and to forth hour post treatment compared with its own pre-treatment (1st hour 85%, 2nd hour by 121%, 3rd hour by 69% and forth hour 26%). On the other hand , in the test for tail flicking model there was no prolongation in reaction time in experimental rats treated with different doses of T.ammi alcoholic extract compared to control experimental rats group ($p>0.05$). Furthermore, none of the alcoholic extract *T.ammi* of treated rats exhibited characteristic straab's tail reaction.

Formalin test

The results of the formalin test shown in Table 2 indicate that oral administration of 2000mg/kg dose of the alcoholic extract of *T.ammi* significantly ($p<0.05$) impaired the number of licking (first phase by 27% and second phase by 23%), licking time (first phase by 46% and second phase by 69%), cumulative time spent per licking (first phase by 25% and second phase by 60%) and total cumulative time spent on licking (by 54%). However number of flicking, number of lifting were not significantly ($p<0.05$) impaired by highest dose of the alcoholic extract of *T.ammi* .

Impairment of licking hind paw time

Table 2 express the impairment of licking hind paw time (p<0.05 of significant) by the experimental rats when orally fed with 2000 mg/kg dose of the alcoholic extract of *T.ammi* (first phase by 46% and second phase by 69%), cumulative time spent per licking (1st phase 25% and 2nd phase by 60%) and total cumulative time spent on licking (by 54%). However number of flicking, number of lifting were not significantly (p<0.05) impaired by highest dose of the alcoholic extract of *T.ammi* .

The mediator for dopaminergic receptor

Table 3 demonstrate the mediator for dopaminergic receptor , when using the hot plate model technique . Metochlopramide were injected intraperitoneally , and it exert inhibition of the enlongation reaction time (p ≤ 0.05) shown by 2000 mg/kg of the alcoholic extract of *T.ammi* in first and third hour compared with control.

Muscarinic mediators at the receptor site Table 4 investigate the muscarinic mediators at the receptor site by using the model of hot plate " , intraperitoneal injection with atropine did not impair elongation of time reaction (p>0.05) produced by 2000 mg/kg body weight of the alcoohlic extract of *T.ammi* at 1st hour. However , atropine injected intraperitoneal decreases the reaction time revealed by using 2000 mg/kg body weight of alcoholic extract of *T.ammi* at 3rd hour (p<0.05).

Table- 1 :- Activity of different doses of alcoholic extractof T.ammi given orally on reation time " in hot plate model .

Dose mg/kg	Reaction Time "(Sec) Means " ± SEM						
	P-T	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**	**6 hr**
Control	**6.73 ±0.67**	**67.8 ± 0.4**	**6.6 ± 0.5**	**6.18±0.93**	**5.8±0.8**	**5.9±1.2**	**6.2±0.9**
250	**7.32 ± 1.35**	**11.63±1.6 #***	**11.78±2.6 #***	**10.2±3.1#**	**8.7±1.7 #**	**8.4±1.7 #**	**7.9±1.1#**
500	**7.13 ± 1.10**	**7.28 ±2.1**	**10.7±11.9 #***	**10.3±1.2#***	**7.7±1.3 #**	**7.4±2.2**	**8 ±1.6 #**
1000	**6.75 ± 1.12**	**9.73 ±3.1#**	**9.83±3.2#**	**9.6±3.9**	**9.6±2.7 #***	**8.7±1.9 #**	**8.8±1.6 #***
2000	**6.7 ± 1.29**	**12.92±1.6 #***	**10.52±1.9 #***	**11.1±2# ***	**8.9±0.9 #***	**8.1±1.8 #**	**7.8±1.4**
Morphine	**7.52 ± 1.27**	**13.9 ±0.95#***	**16.62±1.2 #***	**12.7±1.8#***	**9.5±1.1 #***	**7.1±1.6**	**6.7±0.9**

 # = compared to control P<0.05 ,*= compared to paracetamol P<0.05PT= pretreatment

Table 2:- Activity of alcoholic extract of *T.ammi fedding oraly in the test of formalin*.

Treat-ment	First phase			Second phase			Total Cumu-lative Time Per Licki-ing	No. Of flick-ing	No. Of Lift-ing
	No. of lick-ing	Lick-ing time (s)	Cumul-ative time per lick-ing	No. of lick-ing	Lick-ing time (s)	Cumul-ative time per lick-ing			
Control	**14.0± 1.79**	**109.6 ±14.6**	**7.84 ±0.71**	**49± 9.01**	**532 ±88.6**	**10.9 ±0.81**	**10.21 ±0.68**	**6.83 ±2.79**	**5.33 ±2.73**
2000 mg/kg	**10.17± 1.84***	***59.0*± 9.38***	**5.87 ±0.71***	**37.5 ±7.42***	**163.6 ±28.8***	**4.41 ±0.42***	**4.6 9±0.29***	**8.00 ±2.97**	**7.83 ±3.66**

***= P,0.05 compared to control.**

Table3:- Activity of IP injection of Metclopromide on time of reaction in the model of hot plate when given with 2000mg/kg alcoholic extract of *T.ammi*

Treatment	reaction time "(S) Means ± SEM

	0 hr	1st hour	3rd hour
Saline + extract " (n=6)	**6.97± 1.15**	**13.9±1.88**	**11.82±3.3**
Metclopromide	**7.03±1.65**	**7.73±1.43 ***	**8.15±1.17***

*** = P<0.05**

Table4:- Activity of IP injection of atropine on time of reaction in the model of hot plate when given with 2000mg/kg alcoholic extract of *T.ammi* .

Treatment	**reaction time (S) Means ± SEM**		
	Pretreatment	**1st hour**	**3rd hour**
Saline + extract" (n=6)	**6.97 ± 1.15**	**13.9 ± 1.88**	**11.82 ± 3.3**
Atropine	**6.57 ± 1.34**	**12.97 ± 3.63 ***	**6.73 ± 1.10 ***

- **= P<0.05**

Investigation of mediators for opioid receptor"

Ingection of naloxone intraperitonealy didn't inhibit the reaction time elongation (p>0.05) produced by 2000mg/kg of alcoholic extract of *T.ammi* (naloxone + ALE vs. saline + ALE at 1st hour 14.27±3.71 sec vs. 14.40±4.14sec, at 2nd hour 16.20±1.27sec vs. 13.77±2.97sec).

Coordination of the tone of the muscle :

The latency of muscle tone strength was not significantle affected (p>0.05) by 2000 mg/kg body weight of alcoholic extract of *T.ammi* (control vs. treatment: bar-hold test, 6.94±5.47 sec vs. 4.88±2.88 sec; Bridge test, 5.52±3.32 sec vs. 7.80±2.86 sec; righting reflex test, 1.15±0.17 sec vs. 1.11±0.14 sec).

Test for hole board " sedation activity :

\ All the parameters tested didn't affected (p>0.05) by giving 2000 mg /kg body weight of alcoholic extract of *T.ammi* : crossing number (67±5.24 vs 9.50±2.74), time of dipping (42±3.92 sec vs 4.41±2.80 sec), rears number (4.83±2.64 vs 6.17±2.64) , head dipping number 5.33±3.67 vs 2.67±1.21) , dipping time 1.29 ±0.25 sec vs 1.67±0.55 sec).

IV. DISCUSSION

The results convincingly revealed that , alcoholic extract of *T.ammi plant shows a pain decreasing activity* in rats, when given orally (in doses acceptable in rat models), and evaluated in the hot plate (in terms of prolongation of reaction time) and the formalin (in terms of shortening of measured parameters) algesiometric tests. However, antinociceptive action was not evident when assessed on the tail flick test: these tests are scientifically validated widely used standardized methods employed in the evaluation of potential antinociceptive agents. Compared to morphine, alcoholic extract of *T.ammi* was less efficacious in eliciting the antinociceptive action. Further, alcoholic extract of T. ammi neither induced motor deficits (as reflected from bar test and unimpaired locomotory activity the test for hole board in experimental rats , nor incardination of the nervous system (as judged by the reflex tests of righting and bridge models) Thus, all results obtained are reliable, valid and meaningfully interpreted. The results obtained from the test of hot plate model assure that alcoholic extract of *T.ammi* is effective against transient phasic pain which is centrally mediated at the supra spinal level: hot plate technique predominately measures supra spinal reflexes [19].On the other hand, impairment of different parameters, namely, number of licking, licking duration, additional time spent on licking (in the test two phases) suggest that alcoholic extract of T.ammi is effective against periphcial pain of both neurogenic and inflammatory origins [20]. This may result from included inhibition of mediators for inflammation such as cytokines, bradykinin ", serotonin prostaglandins ", or histamines[21], possibly via phenolic and steroidal phytoconstituents present in the extract. Continuous pain always is due to the change in the injured tissues pathologically and this will cause inflammation with constant practical persistent" pain which affect the life style qualitatively [22].Conversely, a lack of an effect of alcoholic extract of *T.ammi* on tail flick test suggests that spinal mechanisms are not involved in its antinociceptive action [19]. The antinociceptive

activity of alcoholic extract of *T.ammi* exert a rapid action (onset of action 1st hour)"had and a prolonged duration of action (six hour). This is presumably due to fast absorption of the active phytoconstituent/s and its/their quick transport to the final site/s of action. Having a rapid onset of action of antinociceptive action is a much soughted featureof a pain killer. Food restriction imparts antinociception in rats [23], but this explaination cannot be undertaken in this study as a mechanism of action since food was available in the period of the study and there was no evidence for hypophagia. Stress usually lead to antinociception [24].But, this mechanism of antinociceptive can be ruled out, in this study, as expothalamia was not noticed , for erection ", diarrhea or aggressive behaviors. Sedation is implicated with antinociception [25], and several sedatives have shown to possess marked antinociceptive activity [26].Albeit, such mode of action was not recommended in this research since neither of the parameters " tested (crossing number, dippings number , rears number count, time of dipping and time per dip) was impaired. Naloxone, the universal opioid receptor antagonist, failed to block alcoholic extract of *T.ammi* induced antinociception. This indicates that opioid mode of action was not predicted in this research. This notion is further reinforced by the fact that alcoholic extract of T.ammi failed to elicit characteristic Straub's tail reaction which is characteristic of opioid receptor mediated drugs [14]. On this context, it is worth noting that alcoholic extract of *T.ammi* contained alkaloids and several plant alkaloids which are known to induce antinoceciptive*via* opioid mechanisms [26,27]. Although it was not the case in this study. This discrepancy may be attributed structural differences between alkaloids. Dopamine is now recognized to play an important role in pain modulation and dopamine receptor blockers and known to suppress pain [27]. In this study, alcoholic extract of *T.ammi* induced antinociception was inhibited (both at 1st and 3rd hours) by metaclopramide, a dopamine recapture (D2 type) antagonist. This is indicative of dopamine D2 receptor mediation in alcoholic extract of *T.ammi* induced antinociception. Cholinergic mechanisms are also now linked with pain [29]. In this study, alcoholic extract of *T.ammi* induced antinociception was blocked by atropine, a well-known muscarinic cholinergic receptor antagonist at 3rd hour but not at 1st hours. This suggests the involvement of muscarinic cholinergic mechanisms, at least, at the 3rd hour (mid period) of alcoholic extract induced antinociception. However, an absence of a synergetic antinociceptive action at 3rd hour, compared to the 1st hour, argues against this mode of action. Interestingly, even with daily sub chronic treatment with a big dose" of alcoholic extract of *T.ammi* , there was no morbidity, motility or sign symptoms of toxic effect (in term of salivation ", diarrhea, excessive urination, fur losses, postural abnormalities, behavioral change", intake food and water impairment").

V. CONCLUSION "

In this research , the alcoholic extract of *Trachyspermum ammi* can act as a natural safe, orally active, moderately strong antinociceptive. The results also justify the therapeutic claim in I*raq* traditional medicine that alcoholic extract of *Trachyspermum ammi* has painkilling activity.

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