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Anti-inflammatory Activity of the Plant *Cannabis sativa* (L) Petrolium Ether Extract in Albino Rats

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In this study the plant *Cannabis sativa* seeds petroleum oil extract was investigated for anti- inflammatory activity on albino rats. The inflammation was firstly obtained by using *carrageenan* suspension 0.1 ml of 10% saline injected at the sub – plantar region of the left limb for inducing a local acute oedema. A decreased in oedema size was reported after 24 hours for the rats pretreated with carrageenan30 minutes before injection with suspension(4.56, 0.59 and 0.93 for control, 1ml/kg per day and 0.5ml/kg per day groups given C. sativa seed extracts respectively.), compared to Indomethacin standard anti-inflammatory drug which reported a decrease in oedema size diameter to 0.55mm, which indicated an increase inhibition percentages were reported for the different pretreated groups 0.00, 87.03, 79.56 and 87.91 including the comparative Indomethacin treated groups of rats respectively. On the other hand, the post-treated groups of rats (given *C. sativa* oil extract after 30 minutes of injection of suspension) showed a similar results for maximum concentration 1 ml/day of *C. sativa* oil extract in comparison to the standard drug. Hence, such results recommend the prospect focus for the preventive medication use of the extract. The study also highlights no significant changes for serum and protein of the blood taken from rats of the experiments. Although there were significant decrease in lymphocyte and neutrophil, but the changes were not significant. Indomethacin was given to the rats used for a comparative drug (10mg/*kg*). Moreover, the drug indomethacin used as a comparative parameter showed similar results in comparison to the extract, hence wise the reported results may be recommended for use as anti-inflammatory agent and should be explored more to formulate drug on basis of its activity.

Medicinal plants were known to man from prehistoric time. Sudan with an area of 1 million square miles is essentially a country of vast plains; many plants were used in the treatment of various disorders, these encourage students and researchers to investigate the effects and activities of plants. Sudan is very rich in medicinal plants beside varied vegetation because the largest land and various climates in different states give it good character.

Cannabis sativa is a member of the family *Cannabinaceae. Cannabis sativa* preparation is known by various names worldwide. It is called Marijuana, Bhang , Ganja , Charas , Kif and Dogga. But in Sudan, the most famous names of *Cannabis* preparations are bango and

hashish. The name bango in Sudan may be derived from the Indian name bhang. The description of *Cannabis sativa* is a mono specific plant. In some countries a type of these plants was found is both male and female blossoms, it is also, a shrub – type of plant with a strong fragrance and grows in different areas in the world. *Cannabinoids.* Only the female plant of *Cannabis sativa L.* have so far been thought to contain the active component Tetrahydrocannabinoal (THC) (Hanus and krejci, 1981).

At least 66 of Cannbinoids and all classes derived of cannabigerol-type compounds have been isolated from the *Cannabis* plant. Which differ mainly in the way this precursor is cyclized. Tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) are the most prevalent natural Cannabinoids and have received the most attentive study (Bums and Ineck, 2006).

The herb plant in many cultures and many countries was used therapeutically for Beriberi, constipation, Gout, Malaria and absent-madness (Marijuana).*Cannabis* was used in the twentieth century B.C in Egypt to treat sore eyes. In India, prior to tenth century B.C. bhanga, was used as an anesthetic and anti phlegmatic (Sachindra and Pradhan, 1977). The plant was used as an anesthetic in surgery in ancient China. Cannabis was also widely used in Indian medicine, in both the Hindu and Moslem systems of drugs. The plant was used as spasmolytic , hypnotic analgesic in mental conditions , and to increase resistance to severe physical stress (Mechoulam and Lander, 1980).

According to (Muruganandan *et al* , 2001), the ethanolic extract of the bark of *Sysygium cumin* (Myrtaceae) showed significant inhibition against kaolin- carrageenaan and formaldehyde induced paw edema in rats at doses of 100, 300 and 1000 mg/kg. The aqueous leaf extract of *Persea americana* at the dose 800 mg/kg, showed significant inhibition of edema produced by carrageenan in rats. This effect was similar to that produced by Indomethacin (Adeyemi *et al,* 2002). The methanolic extract of *Bryophyllum pinnatun* was found to produce significant anti-inflammatory effect against carrageenan induced inflammation in rats at doses of 100 mg/kg , 200 mg/kg and 300 mg/ kg, the highest dose of 300mg/kg showed significant inhibition higher than the phenylbutazone at 100mg/kg (Siddharthapal *et al* ,1990). Kumar and Busu, (1994), Dewan *et al*, (2000) and Kumar *et al*, (2001) have demonstrated a potent anti-inflammatory, analgesic, antipyretic and antidiarrheal activities of the latex of *C . procera* to rats.

Materials and methods

Preparation of the plant extract

The seeds of *Cannabis sativa* were obtained from Niala, South Darfur, Sudan, cleaned and dried. The oil was extracted as follows: The powder of *Cannabis sativa* seeds obtained was successively extracted with Petroleum ether for 4 hr, using soxhelt apparatus. The extract was occasionally shaken during the first four hours and was then filtrated. The filtrate was evaporated under vacuum, and the residue is brownish in color. Pretreatment of carrageenan -induced paw oedema in rats with oil of *Cannabis sativa* seeds.

Animals, housing and management

Twenty four male and female white (Albino) rats weighing 100-130gm were obtained from the Medicinal and Aromatic Plants, Research Institute, National Center for Research, Khartoum, Sudan, where they were housed in cages and maintained in a room under standard environmental condition, controlled temperature (22±2c), relative humidity (60%) with free access to water and formula rat feed (2.5 Mcal and 20% crude protein). Animals were apparently healthy and they were identified by tail color marks. One week allowed as a preliminary adaptive period.

Administration and rats doses

Post adaptive period, rats were weight-distributed and divided randomly to 4 groups each of 6 rats. All groups individuals were injected subcutaneously with 0.1 ml w/v carrageenan suspension 0.1ml of a 10% saline (Sigma Chemical Co: St Louis, Mo, USA) in the sub-plantar region of the left hind limb as a local acute oedema inducer, 30 minutes subsequent to injection. Then *Cannabis sativa* oil was given orally to rats of group2 at 0.5ml/kg body wt. and at 1ml/kg body wt to rats of group 3. Rats in group 4 were treated with Indomethacin orally (Hikma pharmaceutical, Amman, Jordan) 10mg/kg body wt as a reference compound. Group 1 rats were the un-treated control and received only the carrageenan.

Parameters: Paw diameter was measured after 1, 2, 4, 6, and 24 hours post treatment using Hauptner Tuberculin Caliper (Hauptner , GmbH , Germany) to the nearest millimeter.

Post treatment of carrageenan -induced paw oedema in rats with oil of *Cannabis sativa* seeds.

Animals, housing and management

Fifteen, male and female white Albino rats weighing 90-140gm were obtained from the Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartoum, Sudan, where they were housed in cages and maintained in a room under standard environmental condition, controlled temperature (22±2c), relative humidity (60%) with free access to water and formula rat feed (2.5 Mcal and 20% crude protein). Animals were apparently healthy and they were identified by tail color marks. One week allowed as a preliminary adaptive period.

Administration and rats dose

Post adaptive period, rats were weight-distributed and divided randomly to 3groups each of 5rats. All individuals in each group were injected subcutaneously with 0.1 ml w/v *carrageenan* as suspension (0.1ml of a 10% Saline Sigma Chemical Co: St Louis, Mo, USA) in the sub-plantar region of the left hind limb as a local acute oedema inducer, 30 minutes later of the oil of the plants was given orally to rats of group2 at 1ml/kg body wt and rats of group 3 were treated with Indomethacin orally (Hikma Pharmaceutical, Amman, Jordan) 10mg/kg body wt. as a reference compound. Group1 rats were the un-treated control.

Parameters: Paw diameter was measured after 1, 2, 4, 6, and 24 hours post seed oil administration using Hauptner Tuberculin Caliper (Hauptner, GmbH, Germany) to the nearest millimeter.

Differential Leucocyte count

Thin blood film was made, fixed air dried in ethanol for 5 seconds, and stained in 10% Giemsa in buffer pH 6.8 for 30 minutes, and washed in tap water for 1 minute. Battlement method was used for counting the cells, and was carried out by surveying three fields horizontally, then two fields vertically, three fields horizontally, then a further two fields vertically to the edge of the film, counting until 100 cells have been identified on each side of the film. Giemsa stain was prepared by Giemsa powder 1.0g, Glycerol 66.0ml, Methanol 66.0ml according to Simon and Gundi (2001).

Statistical methods - Mean values of data was analyzed by the one way (ANOVA). The efficacies were obtained by calculating the differences between the edema size in the treated

and the control and the values were transformed into percentage using mean index according to the formula:

$$(A-b)/a*100 = \text{efficacy}$$

Results

Pretreatment of edema induced by carrageenan with *Cannabis sativa* oil in rats

Effects of *Cannabis sativa* oil on oedema

The anti-inflammatory effect of petroleum ether extract of *Cannabis sativa* seeds on rats is shown in table (1) and the effect on edema size is shown in fig (1) and on the inhibition parentages is shown in fig (2). Rats in group 2 (1ml/kg) showed significant ($p<0.05$) decreased in edema size in the , second , fourth , six and twenty fourth hours and inhibition percentage 6.15, 2.36, 34.17, 57.30 and 87.03 at the first, second, fourth , six and twenty fourth hours respectively.

Rats of group 3 (0.05ml/kg) showed decreased on the edema size when compared to the control (carrageenan group at first, second, fourth, six and twenty fourth hours respectively.

Rats in group 4 (indomethacine) showed high decreased ($p<0.05$) in edema size when compared to the (untreated group) at the first , second , fourth , six and twenty fourth hours and inhibition percentage of 17.32, 30.17, 78.99, 86.24 and 87.91 respectively.

Table (1) Average (mean ± S.E) values of paw Oedema of the rats pretreated (given the extact before the injection of suspension) with *Cannabis sativa* oil (before inducing Oedema in albino rat).

Groups	1 hr	2 hr	4 hr	6 hr	24 hr					
\doses	Oedema Size (mm)	Inhibi-tion (%)	Oedema Size (mm)	Inhibi-tion (%)	Edema Size(mm)	Inhibi-tion (%)	Oedema Size (mm)	Inhibi-tion (%)	Oedema Size (mm)	Inhibi-tion (%)
G1	1.90±0.11d	00.00	2.54±0.07c	00.00	3.57±0.05b	00.00	3.56±0.17b	00.00	4.55±0.14a	00.00
G2	1.79±0.30bc	6.15	2.48±0.09a	2.36	2.35±0.02ab	34.17	1.52±0.05c	57.30	0.59±0.09d	87.03
G3	1.12±0.17b	37.43	2.34±0.11a	7.87	2.03±0.12a	43.14	1.08±0.04b	69.66	0.93±0.06b	79.56
G4	1.48±0.44a	17.32	1.76±0.02a	30.71	0.75±0.03d	78.99	0.49±0.17b	86.24	0.55±0.14b	87.91

G1= (control = Carrageenan); G2= (1 ml/kg/day *Cannabis sativa* oil + Carrageenan); G3= (0.5 ml/kg/day *Cannabis sativa* oil + Carrageenan) G4= (10mg/kg Indomethacine + Carrageenan).
Means in the same column with the same letter are not significantly different ($P>0.05$).

Changes in leukocytes values

Table (2) is summarizing the changes in neutrophil, oesinophil, lymphocyte, and monocyte percentages of rats treated with *Cannabis sativa* oil. After twenty four hours of treatment, group 2 and 3 showed significant decrease in neutrophil and lymphocytes percentages and no change in oesinophil and monocyte percentages. Indomethacine group showed significant decreased in neutrophil and lymphocytes percentages and no change in oesinophil, monocyte percentages

Post treatment of oedema induced by carrageenan with *Cannabis sativa* oil in rats.

Effects of *Cannabis sativa* oil on edema

As shown in table (3) and the effect on edema size in fig (3) and on the inhibition percentage. the anti-inflammatory effect of petroleum ether extract of *Cannabis sativa* seeds on rats in this study, rats of group 2 (1ml/kg) showed significant decrease on oedema size, the level of

significant in this group showed at first, fourth and twenty fourth hours and inhibition percentage of 58.79, 55.73, 46.52, 57.22 and 92.32 at the first , second , fourth , six and twenty fourth hours,respectively.

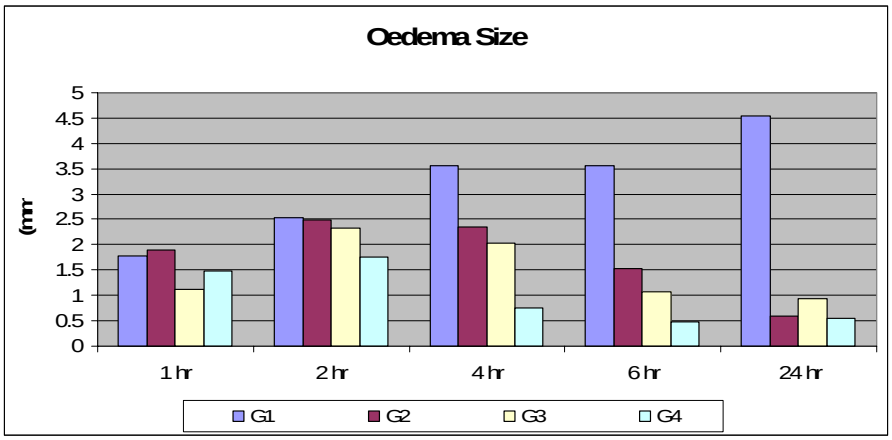

Figure (1): Comparison of size of Oedema in rats dosed with *C. sativa* oil (pre-carraganan injection). G1= (control + Carrageenan); G2= (1 ml *Cannabis sativa* oil + Carrageenan); G3= (0.5 ml *Cannabis sativa* oil + Carrageenan); G4= (10mg/kg Indomethacine + Carrageenan

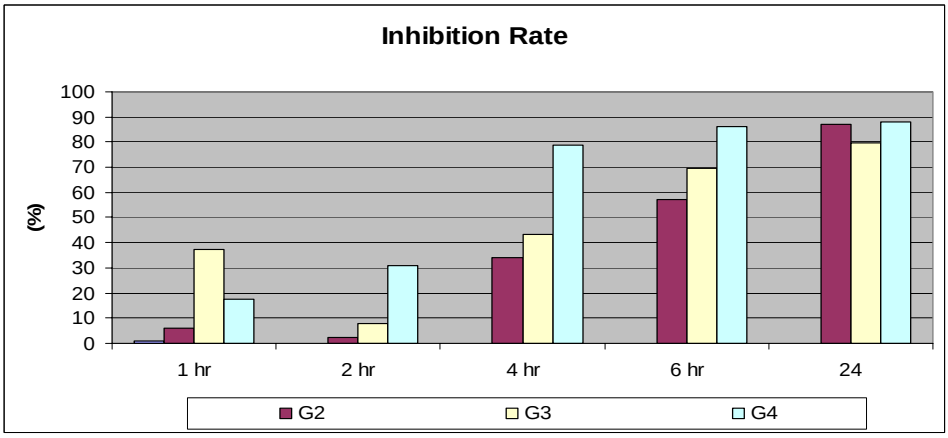

Figure (2) Comparison of inhibition percentage of oedema in rats dosed with *C. sativa* oil pre-carraganan injection. G2= (1 ml *Cannabis sativa* oil + Carrageenan); G3= (0.5 ml *Cannabis sativa* oil + Carrageenan); G4= (10mg/kg Indomethacine + Carrageenan).

Table (2) Average (mean ±SE) values of Leucocytes of rats treated with petroleum ether extract of *Cannabis sativa* oil 24 Hours.

Groups\ doses	Neutrophil (%)	Lymphocytes (%)	Monocytes (%)	Eiosinophil (%
G1	57.00±2.52a	65.00±2.31a	1.67±0.33a	0.67±0.33b
G2	35.67±0.67c	61.67±0.33a	1.40±0.00a	1.67±0.33ab
G3	31.67±2.60c	51.67±0.33b	1.33±0.33a	1.00±0.58a
G4	46.00±0.58b	41.33±2.33c	1.33±0.33a	1.33±0.33ab

G1= (control + caraganann); G2= (0.5 ml *cannabis sativa* oil+ caraganann); G3= (1 ml *cannabis sativa* + oil caraganann); G4= (10mg/kg/indomethacine + caraganann). Means in the same column with the same letter are not significantly different (P>0.05).

Rats in group 3 (indomethacine) showed decrease in edema size at first, second , fourth and twenty fourth hours and inhibition percentage at 2.20, 37.55, 11,42, 51.39 and 86.40 at the first , second , fourth , six and twenty fourth hours respectively.

Changes in leukocytes values: Table (4) is summarizing the changes in neutrophil, oesinophil, lymphocyte, and monocyte percentages of rats treated with *Cannabis sativa* oil. After twenty four hours in groups 2 there were significant decrease in lymphocyte and lneutrophil, but the changes is not significant in monocyte, oesinophil percentages. Indomethazine10mg/kg there were significant decrease in lymphocyte and neutrophil, but the changes is not significant in monocyte, oesinophil percentages.

Table (3). Average (mean±S.E) values of paw edema of rats treated with *Cannabis sativa* oil after inducing edema in albino rats(inducing oedema in albino rat).

Group \dose	1 hr	2 hr	4 hr	6 hr	24 hr					
	Edema Size (mm)	Inhibi-tion (%)	Oedema Size (mm)	Inhibi-tion (%)	Edema Size (mm)	Inhibi-tion (%)	Edema Size (mm)	Inhibi-tion (%)	Oedema Size (mm)	Inhibi-tion (%)
G1	1.82±0.08d	00.00	2.53±0.05c	00.00	3.59±0.04b	00.00	3.60±0.13b	00.00	4.56±0.10a	00.00
G2	0.75±0.22c	58.79	1.12±0.20bc	55.73	1.87±0.28a	46.52	1.54±0.16ab	57.22	0.35±0.14c	92.32
G3	0.87±0.09b	48.20	1.58±0.01b	37.55	1.92±0.37a	40.42	1.75±0.37b	51.39	0.62±0.15c	86.40

G1= (control = Carrageenan); G2= (1 ml/kg/day *Cannabis sativa* oil + Carrageenan); G3= (10mg/kg Indomethacine + Carrageenan); Means in the same column with the same letter are not significantly different (P>0.05).

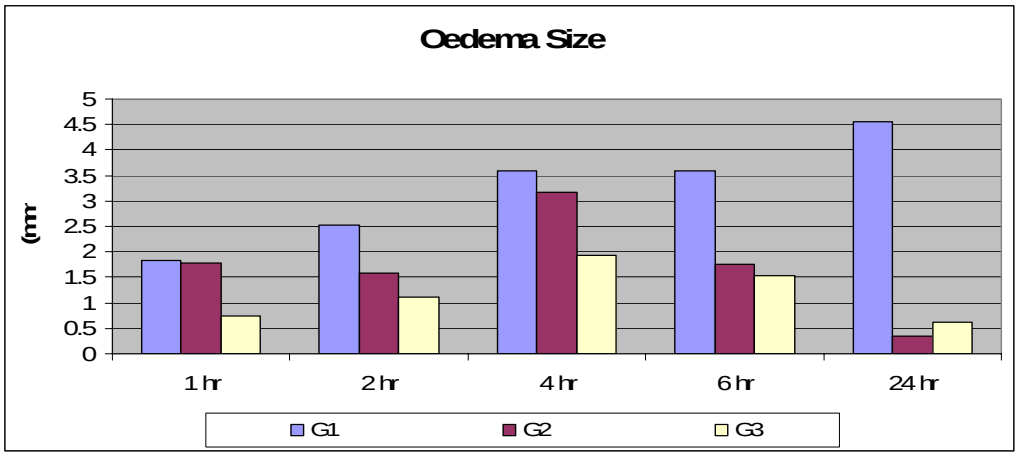

Figure (3): Comparison of size of oedema in rats dosed with *C.sativa* oil (post-carraganan injection). G1= (control = Carrageenan); G2= (10mg/kg Indomethacine + Carrageenan); G3= (1 ml/kg/day *Cannabis sativa* oil + Carrageenan)

Table (4). Average (mean ±SE) values of Leucocytes of rats treated with petroleum ether extract of *Cannabis sativa* oil (24 Hours)

Groups\doses	Neutrophil (%)	Lymphocytes (%)	Monocytes (%)	Eiosinophil (%)
G1	77.00±2.52a	61.33±2.33b	0.67±0.33a	0.67±0.33b
G2	42.00±6.43b	45.33±6.36a	1.33±0.33a	0.57±0.58a
G3	69.67±0.33a	28.67±0.67c	1.67±0.67a	0.47±0.02a

G1= (control = Carrageenan); G2= (1 ml/kg/day *Cannabis sativa* oil + Carrageenan); G3= (10mg/kg Indomethacine + Carrageenan). **Means in the same column with the same letter are not significantly different (P>0.05).**

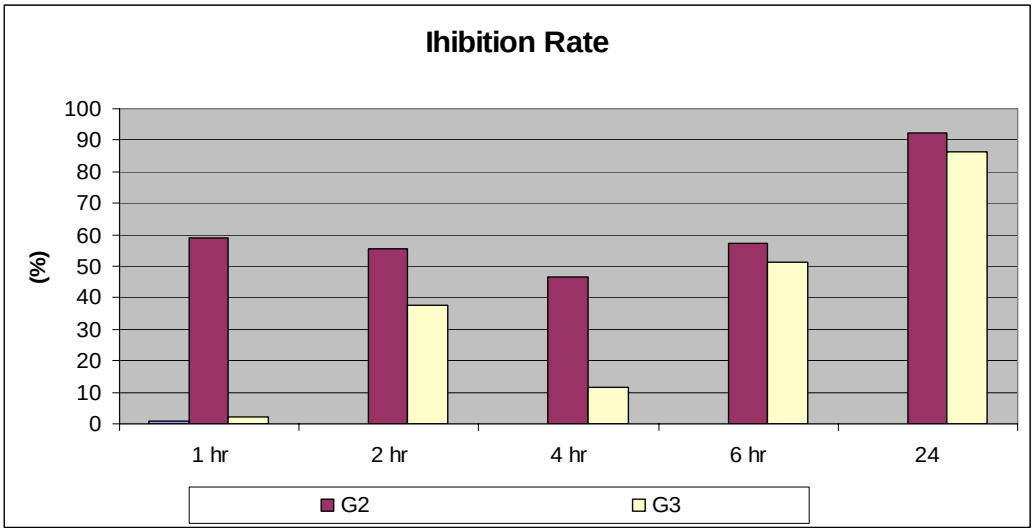

Figure (4): comparison of inhibition percentage of oedema in rats dosed with *C. sativa* oil (post-carraganan injection). G1= (control = Carrageenan); G2= (10mg/kg Indomethacine + Carrageenan); G3= (1 ml/kg/day *Cannabis sativa* oil + Carrageenan)

Discussion

In this study the result of anti-inflammatory experiment induced by *carrageenan* demonstrated an inhibition effects in the oedema size in pretreatment with *Cannabis sativa* oil. These observations were reported also by Twadu (1998) in her study showed that the ethanolic extract of *Leptadenia arborea* demonstrates an inhibitory effects in the oedema size. Also our results of anti-inflammatory demonstrates high inhibition effects in the oedema size in post treatment of *carrageenan* induced paw oedema in rats with oil of *Cannabis sativa* this may be due to the constituents of the oil (Indol, ethane, octanoic acid and nono). The ethanolic extract of *Syzgium aromaticum* showed high inhibition at the dose 250-500mg/kg, reported by (Badilla, *et al* 2006), and these results due to the constituents of the oil too. The results of this study are agreed with the earlier studies of anti-inflammatory activities of some medicinal plants against rat paw oedema (Speroni *et al.,* 2005 Khairalla, 2002; Penna *et al.,* 2003 and Osman, 2005.; Chattopadhayay, 1998; Maulik *et al* 1997 and Yanpallewat *et al*., 2002; 2005). These findings obtained in the rats paw oedema indicate the anti-inflammatory potential of the plant. The authors in this study conclude that the oil of *Cannabis sativa* posses marked anti-inflammatory activities verified by high percentage inhibitory effect of the oedema size in both the pre and post treatment stud. However, more investigation on toxicity of *C. sativa* oil by using higher doses are much needed and recommended.

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