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Antigiardial Activity and Toxicological Exploration of *Cannabis Sativa* Extracts

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***Abstract*— The present study has been attempt to elucidate antigiardial activity and explore the cytoxicity, investigation on liver Diagnostic Enzymes and Changes in serum constituents of *Cannabis Sativa* aerial parts and seeds, which were extracted by Petroleum ether and methanol. Aerial parts methanolic extract gave 63.6% mortality after 72 hours at concentration 1000 ppm (IC_{50} 0.13 ppm) comparing with metrondizole (IC_{50} 0.0125 ppm). While the other extracts found inactive as antigiardiasis after 72 hours. The slight increase in Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Alkaline phosphatase (ALP) liver enzyme and total protein, urea, albumin and calcium which indicate some degree hepatic nefropathy effect of such plant petroleum ether extract. Moreover, phytochemical examination was carried out firstly and revealed that, the petroleum ether extract of *Cannabis sativa* seed do not contain tetrahydrocannbinol (THC), cannabinol (CBN) and cannabidaiol (CBD). However, all extracts showed no significant cytotoxic activity against vero cell line.**

***Index Terms*— *Giardia lamblia*, Cytotoxicity, *Cannabis sativa*, Liver enzymes**

I. INTRODUCTION

Cannabis sativa is belonging to the family Cannabinaceae and known by various names worldwide. It is called Marijuana in America. Bhang , Ganja and Charas in India, Kif in North Africa, Dogga in South Africa, Takrori in Tunisia, Habak in Turkey, Hashish in Middle East, Djomba or liamba in Central Africa and Brazil, Sodom ,Tampl , Gum , Guage , Stuff , Kinshasha, Swala and Whiskt in Ghana, Grifa , in Mexico. Macohna in parts of South America [1], [2] 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.

Giardia lamblia is one of the most common intestinal pathogenic protozoan parasite [3]. It is becoming increasingly important among HIV/AIDS patients. There are reports that some cases of acute and chronic diarrhea in AIDS patients may be associated with giardial infection. However, Metronidazole, the common drug of choice, can cause mutagenicity in bacteria [4] and is carcinogenic in rodents [5]. It also possesses undesirable side effects and treatment failures have been reported [6].

The plants were selected to evaluate the activity of their petroleum ether and methanol crude extracts against *Giardia lamblia* trophozoites *in vitro*, evaluates the cytotoxicity and effect liver enzyme, total protein, urea, albumin and calcium.

II. MATERIALS AND METHODS

Preparation of the plant extract

The seeds and whole plants (aerial parts) of *Cannabis sativa* were obtained from Niala, South Darfur, Sudan were cleaned and dried. Then extracted as follows: The powder obtained was successively extracted with petroleum (oil) 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 obtained residue was brownish in color. The remaining extracts which was not soluble in petroleum ether was successively extracted by methanol using the same above technique.

In vitro antigiardial susceptibility assays

In vitro susceptibility assays used the sub- culture method of Cedilla *et al.*, [7]. This is highly stringent and sensitive method for assessing the anti-protozoal effects (gold standard) particularly in *Entamoba histolytica*, *Gairdia intestinalis* and *T. vaginalis* [8].

5 mg from each extract was dissolved in 50 µl of dimethyl sulfoxzide (DMSO) at eppendorf tube containing 950 µl D.W in order to reach concentration of 5 mg/ml (5000ppm). The concentrates were stored at -20 ℃ for further analysis. Sterile

96-well microtite plate was used for different plant extracts, positive control and negative control.

Twenty µl of complete RPMI medium (1640) was placed in the wells-except the first three wells C-1 (which 40 µl of an extract solution 5 mg/ml were added in the first three wells and the final concentrations were 1000 µg/ml). 20 µl of complete RPMI medium was placed in the wells in the following order such as C-2 was 500 µg/ml and C-3 which was 250 µg/ml. 80 µl of culture medium was complemented with parasite and then added to all wells. The final volume in the wells was 100 µl.

Each test included metronidazole pure compound [(1-(2-hydroxyethl)-2-methyl-5 nitroimidazole]. A antigiardial was used as positive control in concentration 312.5 µg/ml, whereas untreated cells used as a negative controls (culture medium plus trophozoites). Samples were taken for counting at 0, 24, 48, 72 and 96 interval hours. In all well 6 x 10^3 of trophozoits was added.

For counting the samples were mixed with Trypan blue in equal volume. The final numbers of cells were determined with haemocytometer in triplicate.

The mortality % of vero cell for each extracts activity was carried out according to the following formula:

$$\text{Mortality of cells (\%)} = \frac{\text{(Control negative – tested sample with extract)}}{\text{Control negative}} \times 100\%$$

Only 100% inhibition of the parasite was considered, when there was no motile parasite observed.

MTT (3-(4,5 dimethylthiozol-2-yl)-2,5-diphenyl-tetrazolium bromide cytotoxicity) test

Serial dilutions of extracts were prepared in a 96 well flat bottomed plate (Nalge Nunc, Inter). except the last row 6 middle wells (B - G), which are used for the negative control receiving 200 µL of culture medium and 2 µL of sterile 0.5% Triton X to the rest of the plate, 40 µL/wells (CRPMI) were added to second column wells (B–G) that were used as first extract dilution wells. To the first dilution wells in the row, 80 µl of extract suspension was added to the 40 µl. extracts then serially diluted by twofold dilution from well B3 and continoued till B11 by transferring 40 µL to the next well after proper mixing. From the last dilution wells (B-11), 40 µL were discarded. Each extract was tested in triplicate. Cell suspension in a complete culture medium containing 7 × 10^6/ml was properly mixed, and 160 µL of it was transferred into each well of the plate. The final numbers of cells in each well was 1.2 x 10^3 cells. The plate was covered and placed in 5% CO_2 incubator at 37 °C for 72 hours. On the third day, the supernatant was removed from each well without detaching the cells. MTT stock (5 mg/mL) was prepared earlier in 100 mL PBS. The clear suspension was filter sterilized with 0.2 µm Millipore filter and stored at 4 ℃ or –20 ℃ until use. MTT was diluted (1:3.5) in a culture medium and brought to room temperature. To each well of the 96 well plates, 50 µL of diluted MTT was added. The plate was incubated further at 37 ℃ for 3 hours in CO_2 incubator. MTT was removed carefully without detaching cells, and 200 µl of DMSO was added to each well. The plate was agitated at room temperature for 15 min then read at 540 nm using microtitre plate reader.

Toxicity of oil of seeds of Cannabis sativa petroleum ether extract against Albino rats

Forty, Wister white (albino) rats of different sex weighting 100-150gm were obtained from the Animal House of 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 conditions. The animals were weight- distributed and allotted randomly to four groups, each of ten rats. Rats in Group 1 were the untreated control *Cannabis sativa* oil, was given orally in daily doses at 0.01ml /kg body wt/rat to group 2. Rats in group3 received 0.1ml /kg body wt/rat/day, while rats in group4 received 1ml /kg body wt./rat/day. Dosing continued for four weeks. Clinical signs were recorded. Blood samples were obtained from the ocular vein before the start of the experimental dosing and then subjected to hematological investigations followed by statistical analysis Statistical methods.

Mean values of data was analyzed by the one way (ANOVA), SPSS statistical program. More over, the total reading of the data obtained on the first week, second week and the forth week was analyzed by the origin statistical program for the comparison of the means. The efficacies were obtained by calculating the differences between the edema size in the treated and the control and weeks data) two population analyzes. Control and the values were transformed into percentage using mean index according to the formula: (A-b)/a*100 = efficacy
Where a mean of the edema is size in the control and is the mean of edema size in the treated rats

III. RESULTS AND DISCUSSION

The prepared sample (oil of *Cannabis sativa* extracted with petroleum ether), did not show any types of cannabinoids (THC, CBD or CBN), spots on the silica gel plates when compare with THC reference control.The compounds that appear in (TLC) was detected by (GCMS), none of them showed the cannabinoids **figure 2**.The big peaks were compared to the instrument library reference were give a good similarity search.

All extracts from *Cannabis sativa* reported in the experiment for their no cytotoxicity activity against vero cells by using MTT assay Fig3. The results of cytotoxicity evaluation of extract was ranging from (1000 to 250) ppm as shown in **Fig. 3 and 4.**

Fig. 4 indicates that the mortality of *C.sativa* seed methanolic extract showed 10.9% mortality for the all tested concentrations (1000 ppm) with IC_{50} 8.9 ppm, while whole plant gave 63.6% mortality at the same concentrations of IC_{50} 0.13 ppm. On the other hand, whole plant and seed of *C.sativa* extracted by petroleum ether gave mortality 21.2 %

with lC_{50} 1.9 ppm and 9.8% with IC_{50} 1.8 ppm respectively in comparison with metrondizole powder gave 70.9% mortality at 156 ppm at the same time.

Clinical signs

Rats in groups 2 (0.01ml/kg), 3 (0.1ml/kg) and 4 (1ml/kg), showed rough coat and long hair during the experimental period (4weeks). No mortalities were recorded .No abnormal behaviors were recorded in the un-dosed control rats (group1) and also no mortalities recorded.

Change in serum constituents

Table 1. shows the changes in serum constituents of rats treated with *Cannabis sativa* oil for 4 weeks. At the first week (1), the activities of AST, ALP and ALT, Showed no significant changes ($p>0.05$) for all groups, normal values were recorded from the control group. Further more, all groups showed no significant changes in the concentration of urea, albumin, total protein and calcium.

At week two there was significant increase in ALT activity in group 3 (0.1ml/kg), and in AST activity in group 3 (0.1ml/kg), and group 4 (1ml/kg), and in ALP activity in group 2 (0.01ml/kg), and group 4 (1ml/kg), increase in the concentration of Albumin in group 3 (0.1ml/kg),in urea in group 2 (0.01ml/kg) and in calcium in group 3 (0.1ml/kg), when compared to the control.

At week four of the experiment there was significant increase in the activities of ALT and AST in groups 2,3 and 4, and of ALP in groups 3 and 4 while no changes in total protein, Albumin and urea concentration when compared to the control.

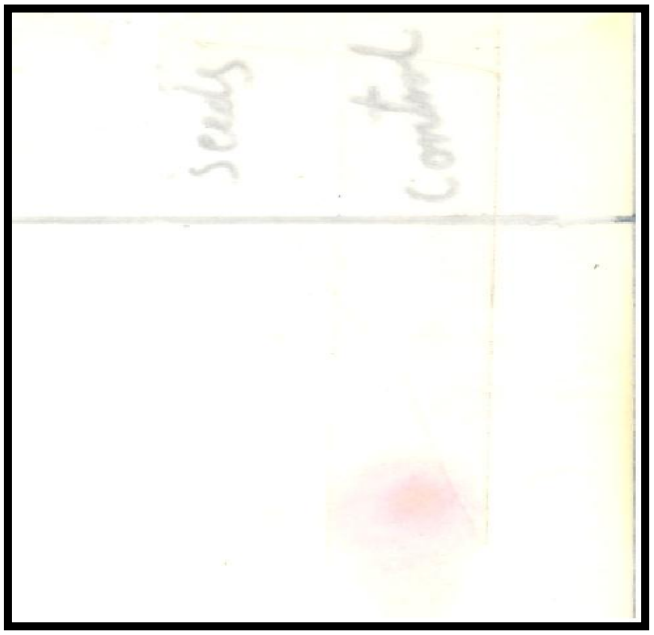

Figure 1. Thin Layer Chromatography (TLC) of *Cannabis sativa* seed

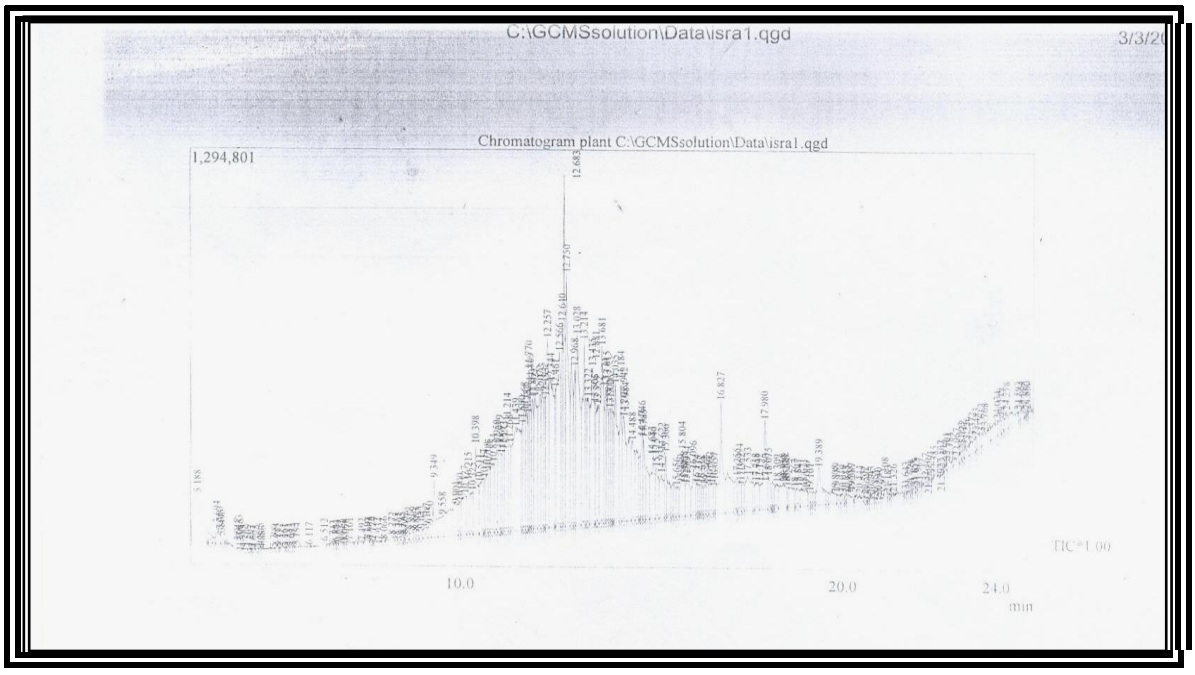

Figure 2. Gas Chromatography Mass Spectrometer (GC.MS)

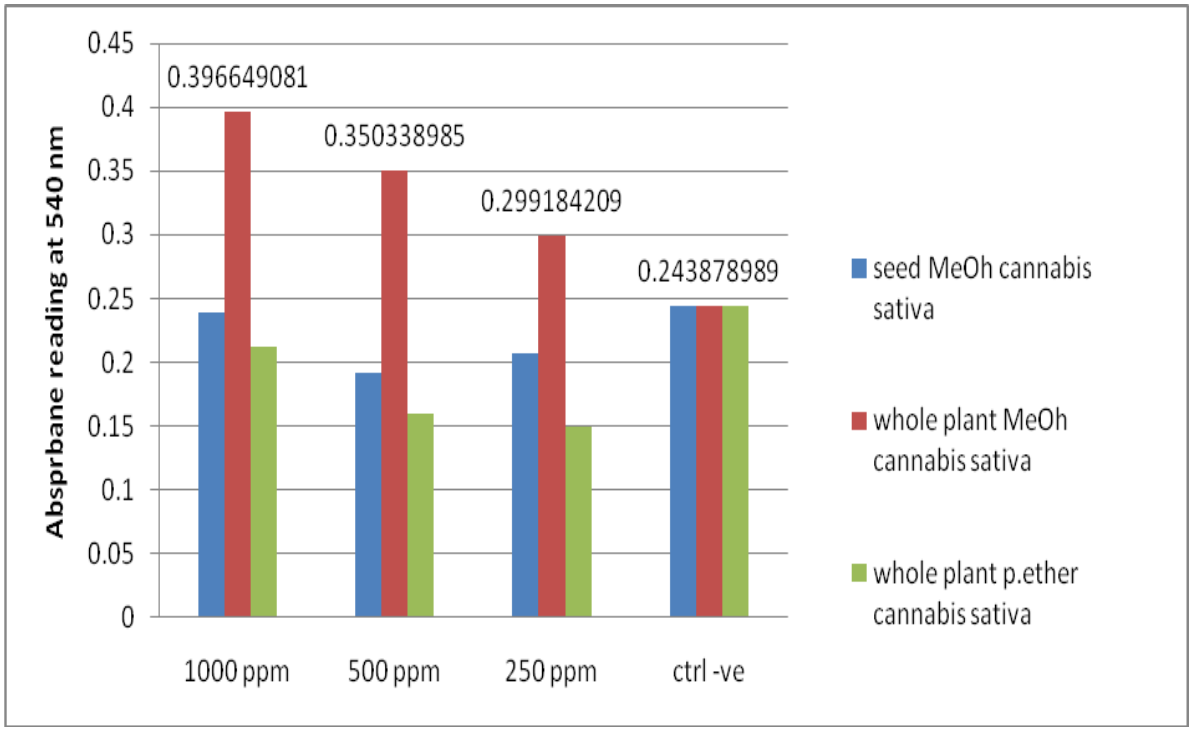

Figure 3 . MTT reduction cytotoxic assay for evaluation of plant extract

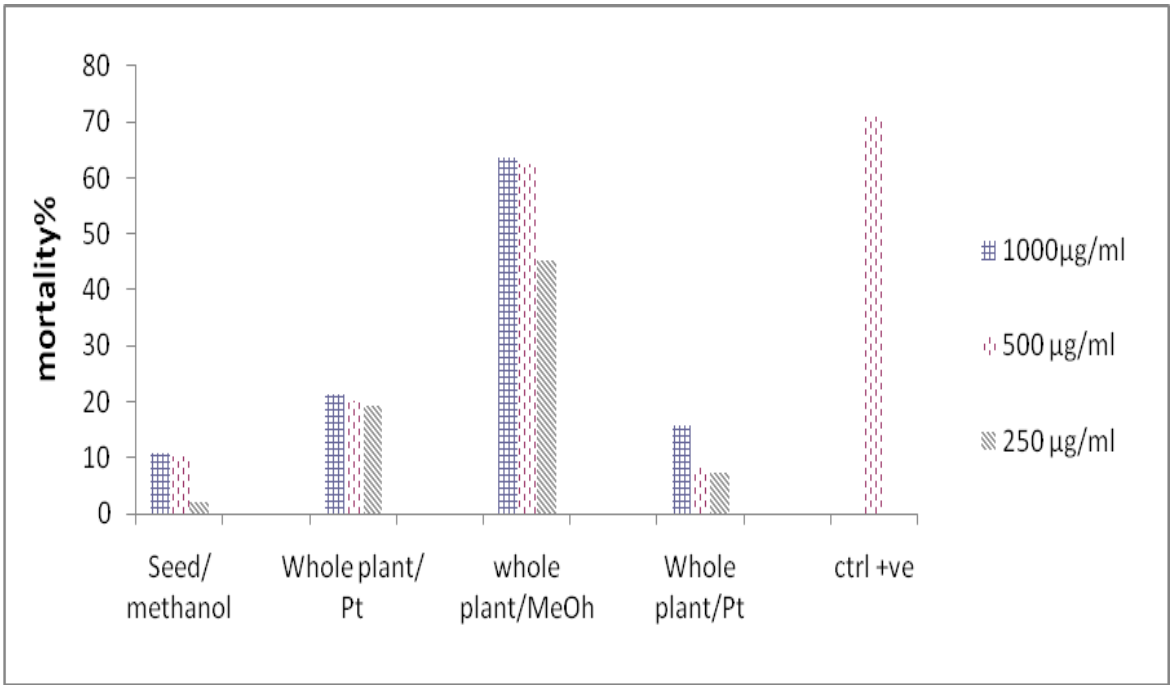

Figure 4.The mortality of *Cannabis sativa* against *Giardia lambelia* with different solvents after 72 hours

IC_{50}: seed/methanol=8.9, whole plant/methanol=0.13, whole plant pt=1.9 and seed /pt=1.8

Key: Pt: petroleum ether ,MeOH: Methanol

Table 1

Average (mean ± S.E) values of serum constituents of rats treated with *Cannabis sativa* seed's oil for 4 weeks

Weeks	Groups	ALT (i.u/I)	AST (i.u/I)	ALP (i.u/I)	T.protein(mg / dL)	Albumin (g/ dL)	Urea (mg/ dL)	Calcium (mg/ dl)
One	G1	54.25 ±2.06a	228.75 ±14.33a	270.00 ±24.79a	7.73 ±0.36a	3.38 ±0.11a	42.25 ±2.29a	10.15 ±0.13a
	G2	57.50 ±3.57a	241.75 ±16.11a	328.25 ±17.90a	7.45 ±0.25a	3.30 ±0.09a	39.25 ±2.1.4a	9.20 ±0.30a
	G3	58.75 ±5.66a	227.75 ±25.17a	338.75 ±23.58a	7.58 ±0.19a	3.58 ±0.08a	37.50 ±2.55a	9.95 ±0.52a
	G4	65.50 ±4.17a	273.25 ±22.42a	332.50 ±43.99a	7.83 ±0.46a	3.70 ±0.20a	29.75 ±7.95a	9.935 ±0.5a
Two	G1	42.00 ±5.70b	167.50 ±18.32c	296.50 ±28.01c	7.73 ±0.36a	3.38 ±0.11b	42.25 ±2.29b	10.15 ±0.13b
	G2	47.25 ±4.64ab	184.50 ±11.59bc	451.50 ±52.53ab	27.13 ±0.19a	3.48 ±0.13b	50.75 ±2.10a	10.28 ±0.31b
	G3	65.75 ±8.46a	226.25 ±16.10ab	350.75 ±39.58bc	7.75 ±0.19a	3.90 ±0.08a	46.25 ±2.84ab	11.00 ±0.09a
	G4	58.75 ±3.20ab	257.50 ±12.10a	508.50 ±46.39a	7.45 ±0.10a	3.68 ±0.08ab	39.50 ±1.55b	10.83 ±0.26ab
Three	G1	38.50 ±0.87c	119.00 ±7.95c	307.50 ±16.83c	7.03 ±0.31a	3.88 ±0.26a	55.00 ±1.78a	11.15 ±0.57a
	G2	47.00 ±2.45b	135.00 ±1.68b	373.50 ±30.43bc	7.45 ±0.41a	3.60 ±0.11a	47.00 ±3.42a	9.28 ±0.49b
	G3	51.00 ±2.04ab	145.75 ±1.80ab	408.25 ±27.82b	7.75 ±0.29a	3.68 ±0.17a	48.50 ±2.72a	10.65 ±0.49ab
	G4	55.25 ±2.17a	157.50 ±3.23a	533.75 ±11.71a	7.83 ±0.22a	3.88 ±0.06a	49.50 ±2.84a	9.95 ±0.52ab

G1= Group 1 (UN – dosed control)
G2 = Group 2 (0.01 ml/kg/day *cannabis sativa*)
G3= Group 3 (0.1 ml/kg/day *cannabis sativa*)
G4= Group 4 (1 ml/kg/day *cannabis sativa*)
Means in the same column with the same letter are not significantly different (P>0.05).

This data showed that the extract possessed the lowest cytotoxicity activity against the vero cell line. The present study highlights the efficiency of *Canabis sativa* extracts acquisition alternative natural and chemical treatment for giardiasis.In this study the daily doses of *Cannabis sativa* oil administered orally to rats did not caused death .However, the oil as reported by Twadu [9] may cause some changes in the liver histopathology.

The slight increase in AST, ALT and ALP activities and in total protein, urea, albumin and calcium of *cannabis sativa* oil, and this results correlated with the results obtained in the toxicity of some plants namely *Aristolochia brachteta* on goats [10], [11] [12]. Also intoxication with *Capparis tementosa* [13].The assessment of the activities of enzymes AST and ALT in the serum and concentration of urea, total protein and albumin used routinely for evaluating the functional status of the liver and of the renal toxicity, [14]. Although in this study some of the data reported showed no significant difference, this may highlight and recommend more histopathological exploration study on both liver and kidneys.

IV. CONCLUSION

This study concludes that the oil of *Cannabis sativa* contains no cannabinoids although the extract showed signs of toxicity less than death to rats so anthor toxic substances may present hence further investigation is recommended.

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