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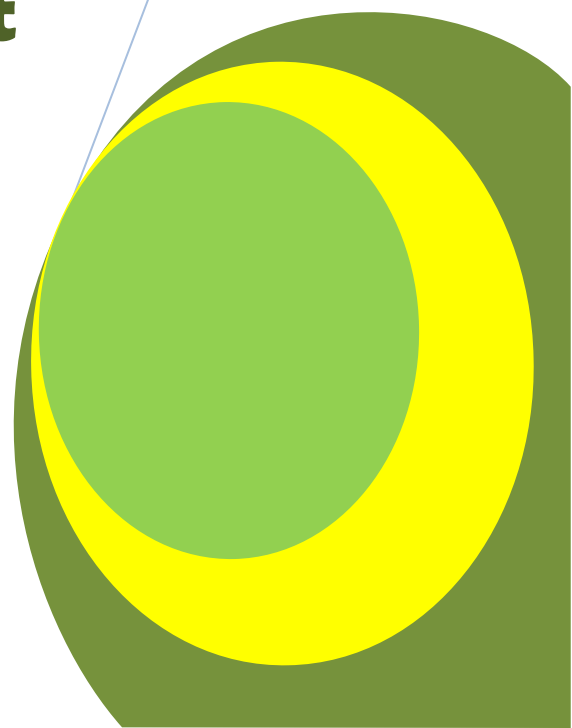
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Efficacious Effect of Cannabis Sativa (*Indian Hemp*) on Cutaneous Wound Healing and its Haemostatic and Chemical Profile in Adult Male Albino Wistar Rat

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Efficacious Effect of *Cannabis Sativa* (Indian Hemp) on Cutaneous Wound Healing and its Haemostatic and Chemical Profile in Adult Male Albino Wistar Rat

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ABSTRACT

Plants and plant extracts have been found very useful for purposes of treatment of disease among other benefits. *Cannabis sativa* is one of such plants whose effect on wound healing, haemostatic and biochemical profile was studied using albino wistar rats. *Canabis sativa* is an illicit drug with proven anti-inflammatory, analgesic and anti-diarrhea activities. This present study is aimed at ascertaining the impact of prolonged administration of *cannabis sativa* on haemostatic mechanism, cutaneous wound healing and biochemical profile of albino wistar rats. Forty (40) albino wistar rats weighing 180-200g were acclimatized in the animal house for 7 days. After this, they were divided into five groups respectively. Group A was the control male rats (6), Group B was the female control rats (6), Group C (10) was the male test rats and Group D (10) was the female test rats and Group E (8) for incision. Group C and D male rats were fed for 30 days with aqueous extract of *Canabis sativa* before 5.0ml of blood sample was collected from the animals for cardiac puncture and placed in haemogram bottles (EDTA and sodium citrate) for haemostatic analysis and in plain bottles for liver function tests while Group E rats were those with incision made in their skin that received topical treatment of aqueous extract of *cannabis sativa* and injection for 5 days.

Results from the study showed that the extract significantly increased the total and conjugated bilirubin, ($P < 0.05$), liver enzymes; Alkaline Phosphatase (ALP), Aspartate Transaminase (AST) and Alanine Transaminase (ALT), Haemoglobin concentrations, Packed cell volume, platelet count and white blood cell count. The bleeding and clotting times, Prothrombin time and Partial thromboplastin times were shortened compared to their corresponding controls. The incised wounds healed within shorter days than those in control rats. The alterations in the results are manifestation of mild hepatorenal toxicity and anti-hypercholesterolemic effect. These liver enzymes are highly affected by *cannabis sativa* in females than in males.

Key words: Wound healing, *cannabis sativa*, haemostatic, prothrombin time, bleeding time, liver enzymes.

INTRODUCTION

In the Eastern part of Nigeria, traditional medicine has been virtually used in preventing and curing diseases, thereby playing an important role in the health care services especially to the low socio-economic class. These herbs are mostly administered orally through the mouth or can be applied topically as ointment. Extract of roots, stem, bark, leaves sap of some medicinal plants have been known to be curative.

Wound healing is a dynamic interactive process involving soluble mediators, blood cells, extra cellular matrix and parenchymal cells and has three phases; inflammation, tissue formation and tissue remodeling that overlap in time. The primary goals of the treatment of wounds are rapid wound closure and a functional and aesthetically satisfactory scar.

Recent advances in cellular and molecular biology have greatly shown the processes involved in wound healing repair and tissue regeneration (Clark, 1996).

Homeostasis is a process which arrest blood loss and whenever a vessel is damaged, haemostatic process is activated by successive mechanism involving vascular spasm, formation of platelet plug, blood coagulation and growth of fibrous tissues into the blood close the damaged vessel permanently (Arthur 2007).

The haemostatic activities of cannabis sativa, the biochemical profile and cutaneous wound healing in albino wistar rats were studied with the aims of determining the effect of the crude extract on the haemoglobin bleeding time, clotting time, partial thromboplastin time and liver function indices using albino wistar rats.

Cannabis sativa is a genus of flowering plants that includes a single species. Over the years, cannabis sativa remains the most widely used illicit drug worldwide due to its affordability and availability (Bauman and Phonsaven 1999). A review of the evidence surrounding the acute impact on memory concluded that cannabinoids impair all aspects of short-term memory, especially short-term episodic and working memory.

Cannabis has long been found to increase heart rate by 20-50%. This is the most immediate effect and occurs within a few minutes after cannabis intake. After cannabis usage, a sudden change of posture from lying down to standing up may produce orthostatic hypotension, a feeling of light-headedness and faintness that is often the earliest indication of intoxication in naïve users (Jones 2002). Other physical effects of Cannabis include reddening of the eyes due to congestion of the conjunctiva blood vessels, lowering of the body temperature, dry mouth, reduced intraocular blood pressure and relaxation of the muscles (Grothem-harmen, 2007).

Medicinal Uses of Cannabis

Clinical studies and survey data have found that Cannabis increases appetite (Techranipour and Ebrahnipour, 2009). The THC which is the main constituents of cannabis sativa has been shown to have effect on both the action and release of insulin (Friser et al 2010). This may explain why cannabis has been employed to self-medicate in diabetes. Cannabis sativa has also been reported to be used for treatment of specific human ailments such as allergies, burns, cuts and wounds, inflammation, leprosy, leucopenia, scabies, small pox and sexually transmitted diseases (Dilarand et al, 2000). The different preparation of Cannabis sativa has been used in Asian traditional medicine for treatment of variety of diseases including: inflammation, alopecia. It has also demonstrated a potent anti-inflammatory, analgesic, antipyretic anti-diarrhoal activities.

Chemical Constituents of Cannabis

Cannabis plants produce a group of chemicals called cannabinoids which produce mental and physical effects when consumed. Cannabinoids, terpenoids and other compounds are secreted by glandular trichomes that occur most abundantly on the floral calyxes and in the form of dried flower buds (marijuana) resin (harshish) of various extracts collectively known as harshish oil.

MATERIALS AND METHOD

Animals

The albino wistar rats were randomly selected and kept in a metal cage with iron netting in a laboratory environment. The study group is made up of male and female rats. The males were separated from the females and all were allowed to have free access to vital feeds (Guinea feed Benin) and water ad libitum. They were stabilized for two weeks before being used for the experiments.

Experimental Design

Forty (40) male and female albino wistar rats were placed into five groups namely: Groups A, B, C, D and E. groups A (6 male albino rats), Group B (6 females). Groups A and B were used as controls. Group C (10 males rats) Group D (10 female rats) were the test rats that was administered with the aqueous extract of cannabis sativa, finally group E (6 males) the group used for topical application of the cannabis sativa extract on lacerated wound in the animals. The test rats were fed normal rat pellets and water ad libitum in addition to oral administration of 5mg/g once daily of the leave extract of *cannabis sativa*.

Preparation of Extract

Leaves of *cannabis sativa* were collected from botanical garden in the department of Biological Science Chukwuemeka Odumegwu Ojukwu University and it was identified by a botanist in the Botanical Department of the University. The crude extract was prepared according to the method described by Obiefuna et al (1998). The dried leaves of marijuana were oven dried at 45°C and 100g of dried leaf was pulverized and soaked for 72 hrs in 800mls of distilled water. It was then filtered with Whatman No.1 filter paper and residue discarded. The filtrate was subsequently evaporated to dryness in an aerated oven at 45°C. The resulting slurry was stored in closed glass bottles until used.

However 5mg/g of the slurry was prepared and used for oral administration of the animals in Groups C and D.

Phytochemical Analysis of Leaves

The leaves of the plant were screened for the presence or absence of various secondary metabolites using standard phytochemical screening procedures as described by Harbournes (1973), Trease and Evans (1996) respectively. The extracts were tested for glycosides, flavonoids, alkaloids, tannins, reducing sugars, calciums, saponins, acidic compounds, resins, fats and oil, carbohydrates and steroid.

Toxicity Study

The LD₅₀ of the extract in albino rats was determined using Lorke's method (1983).

Obtaining Of Blood Samples for Tests

At the end of 30 days administration of the crude extract, 0.02ml of Sodium Terpentiner were injected intraperitoneally into the rats so as to anaesthetize them. Bleeding time and clotting times were conducted on the animals. Later, blood samples (5ml) were collected by cardiac puncture and expressed into anticoagulant tubes (EDTA tube, sodium citrate tubes) and in plain tubes for haemostatic and biochemical analysis.

Haemoglobin Study

Haemoglobin estimation was determined by method described by Baker et al, 1998. The Packed cell volume estimation was estimated by method of Alexander et al, 1993. The total white blood cell count was also done by method described by Baker et al 1998. The platelet count was done as described by Brecher and Cronkite (1950) while the whole blood clotting time was determined using the method of Lee and White (1998), and the bleeding time was carried out as described by Dejana et al (1982). The Prothrombin time and the partial thromboplastin time. Kaolin tests were carried out as elaborated in Quick's one stage method (1998).

The biochemical test (LFT) was carried out by the method described by Baker et al, 1998.

Study on effects of the routes of administration on rats:

- a) Enteral: Oral administration of the aqueous extract of *Canabis sativa* to the rats for 3 days was carried out by intubation and the control rats were given normal rat diet during this period.
- b) Parenteral: This consists of intraperitoneal injection of the aqueous extracts of *Canabis sativa* to the rats. The dose of 5mg/kg of the extracts was used and it lasted for 5 days. This was used to compare the enteral administration.

Effect of *Canabis Sativa* on Clotting Time of Rats

In this study partial thromboplastin kaolin test and Prothrombin time (PT) were done to establish the possible pathway of the action of the extracts. The concentration of extract was 5mg/g. The weight of the control and test animals, their haematological and haemostatic parameters were done before the administration of the extract to the test animals

At the end of the 3 days of feeding their weight, full blood count (FBC), bleeding time, clotting time, platelet count, prothrombin time (PT) and partial thromboplastin time, kaolin (PTTK) tests and biochemical analysis (LFT test) was again carried out.

Statistical Analysis

The results obtained in the study were presented as Mean and Standard deviation (Mean \pm S.D) and ANNOVA was done using the Students' t- test, it was used to determine the level of significance.

RESULTS

Table 1: The phytochemical analysis of C. Sativa

Constituents in Extract of Cannabis Sativa							
	Alkaloids and flavenoids	Carbohydrate	Calcium	Sugar	Fats	Glycosides	Terpenoids, Resins, Acidic compounds, Tanins
Degree of concentration	++	+++	++	+	++	-	-

- Negative (absent),
- + Present in small concentrations.
- ++ Present in moderate high concentrations.
- +++ Present in very high concentration.

Table 2: Haematological indices of albino rats before the administration of extracts and thirty (30) days after the administration of Cannabis sativa

Extracts	Hbg/dl \pm S.D	PCV% \pm S.D	Platelet count $\times 10^9/l \pm$ S.D	Bleeding time (min) \pm S.D	Clotting Time min \pm S.D	Prothrombin time sec \pm S.D	Partial thromboplastin time (sec) \pm S.D
Control rat Grp A; Males n=6 (Extract free).	13.6 \pm 0.72	40.2 \pm 1.5	186.5 \pm 24	2.5 \pm 0.3	5.2 \pm 0.2	11.5 \pm 0.4	22 \pm 2.7
Control rat Grp B; Females n=6 (extract free).	13.5 \pm 0.8	40.1 \pm 1.2	188.4 \pm 20	2.4 \pm 0.7	5.0 \pm 0.4	11.0 \pm 1.2	21 \pm 2.2
Day 1 before C. sativa Group C males n=10.	13.9 \pm 0.2	4.0 \pm 0.5	190 \pm 56	2.7 \pm 0.3	5.0 \pm 0.6	10.4 \pm 1.2	21 \pm 0.7
Grp D Females n=10	13.8 \pm 0.3	40.0 \pm 0.2	186 \pm 32	2.5 \pm 0.4	5.4 \pm 0.4	10.4 \pm 1.2	21 \pm 0.7
30 dys after C. sativa Gp C Males n=10	15.9 \pm 0.3	46 \pm 0.5	210 \pm 28	1.8 \pm .2	3.0 \pm 0.2	8.2 \pm 0.7	18 \pm 0.5
Grp D females n=10	15.0 \pm 1.7	45 \pm 0.8	220 \pm 30	1.6 \pm 0.5	3.2 \pm 0.2	10.6 \pm 1.0	18 \pm 0.3
Grp E males n=6 (Extract free)	13.5 \pm 1.2	40 \pm 0.8	194 \pm 45	2.7 \pm 0.2	5.2 \pm 0.6	10.6 \pm 1.0	21 \pm 0.8
Level of significance	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

There was a significant increase ($P < 0.05$) in haemoglobin concentration, packed cell volume, platelet count, shortening of the bleeding time, clotting time, prothrombin time and partial thromboplastin time kaolin in the test rats group C and D compared with their corresponding controls A & B respectively.

Table 3: Bleeding and clotting times in albino rats following intraperitoneal administration of extracts for 5 days

Extracts	Dose of Extract	Bleeding time (min) Mean \pm S.D	Clotting time Mean \pm S.D	Observation	Level of significance
Control (placebo)	5mg/kg	2.5 \pm 8.3	5.2 \pm 0.2	NIL	$P > 0.05$
C. sativa	5mg/kg	1.3 \pm 0.2	3.28 \pm 0.6	Reduced	$P < 0.05$

Table 4: Liver function profile of albino wistar rats on extract feed of cannabis sativa

Extracts	Total Bilirubin mg/dl	Conj. Bilirubin mg/dl \pm S.D	Alk. Phosphataseiu/l \pm S.D	ASP Transaminaseiu/l \pm S.D	Alanin Transaminase iu/l \pm S.D
Control rats Male Grp A n=6 (Extract Free)	0.10 \pm 0.00	0.2 \pm 0.00	42 \pm 0.5	8.0 \pm 0.3	7.2 \pm 0.5
Control rats Females Grp B n=6 (Extract free)	0.010 \pm 0.00	0.2 \pm 0.00	42 \pm 0.5	8.0 \pm 0.3	7.2 \pm 0.5
Day 1 before c. sativa Grp C Males n=10	0.010 \pm 0.00	0.2 \pm 0.06	41 \pm 0.06	8.0 \pm 0.3	7.0 \pm 0.5
Grp D Females n=10	0.010 \pm 0.00	0.2 \pm 0.06	40 \pm 0.05	7.0 \pm 0.2	7.0 \pm 0.5
30 days after C. sativa Grp C males n=10	0.40 \pm 0.05	0.2 \pm 0.00	293.6 \pm 1.41	93.00 \pm 1.34	795.9 \pm 1.14
Grp D, Females n=10	0.60 \pm 0.2	0.2 \pm 0.04	408.6 \pm 0.92	8.71 \pm 0.3	797.6 \pm 1.43
Level of significance	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

There was a significant effect of Cannabis sativa in the liver profile of the test rats. Result indicates gradual increase in the liver enzymes (alkaline phosphatase, alanin transaminase and aspartate transaminase) compared with their corresponding controls.

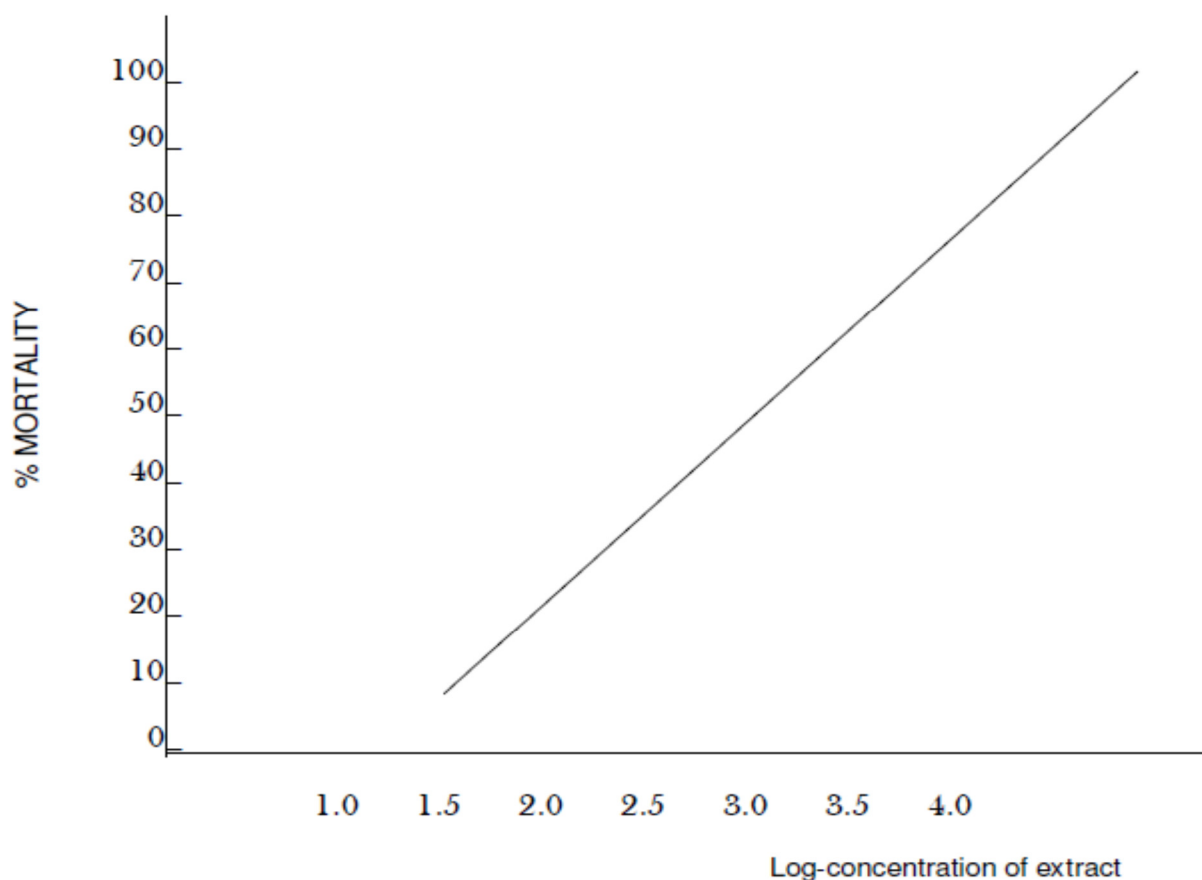


Figure 1: indicates lethality dose (LD_{50}) of *Canabis sativa*

DISCUSSION

The primary goals of the treatment of cutaneous wounds are rapid wound closure and a functional and aesthetically satisfactory scar (Clark 1996). The efficacious effect of *Cannabis sativa* (Indian hemp) on cutaneous wound healing and its haemostatic and biochemical profile in adult male albino wistar rat has been studied. Crude extracts of plants have been found very useful in ethnomedical practice as part of the process for the treatment of diseases (IWU 1982).

In this study, it was shown from the phytochemical studies that there is presence of typical plant constituents (Okoli 2007) such as carbohydrate, calcium, alkaloids, reducing sugar, flavonoids, saponins and glycoside, fats and oils, while other constituents such as tanins, resins, terpenoids, acidic compounds, steroids were absent. The acute toxicity study showed that the *Canabis sativa* extract was non-toxic as shown by the LD_{50} (fig 1) of 3000mg/kg. The dose used in this study (5mg/kg) was hence lower than the lethal dose and so was considered safe for use throughout the study. In the study, to compare the effect of route of administration, it was observed that intraperitoneal administration of the extract demonstrates rapid onset of action than the oral route. This could be because in oral route, the extract underwent first pass effect and a reduction in bioavailability while the intraperitoneal route has a higher bioavailability.

It takes shorter period for the clotting and bleeding times to occur via this route. The calcium and alkaloids (protein precipitants) contained in this plant extract are known to be involved in the precipitation of coagulation factors (Taofeeq et al, 2005) which promotes clotting in rats.

The mean value of Packed cell volume (L/L) hemoglobin concentration (g/100cationsml) Platelet count (count $\times 10^9/l$) were observed to be increased in the test, male and female rats on the extract feed compared to their corresponding controls, thus suggesting possible promotion on erythropoiesis. This can also indicate that consumption of the leaves of *Canabis sativa* can result to weight gain in the users.

The evaluation of the hemostatic effects in albino wistar rats showed that it reduced bleeding and clotting times, prothrombin and partial thromboplastin kaolin times, hence demonstrating haemostatic activity.

The increase in platelet count in the test rats within the normal range is advantageous to wound healing. Platelets are vital in some biochemical reactions particularly haemostasis where they are involved in formation of platelet lugs aimed at closing any ruptured blood vessel to prevent undue haemorrhage. When any blood vessel is ruptured, the sub-endothelium and collagen are exposed and this stimulates the platelets to undergo viscous metamorphosis, which culminates in aggregation, adhesion and secretion. The biochemical reaction is further propagated against by ADP, adrenaline, serotonin, prostaglandins and some other vasoactive substance (Kroll, 1998). Calcium is one of the important cations necessary for many biochemical reactions of haemostasis. It occurs in animal and plant tissues which serve as dietary sources. The ionic form of calcium (Ca^{2+}) is involved in clotting mechanism, bone formation, muscle contraction, enzyme and complement activation.

The observed significant ($P < 0.05$) increase in the total bilirubin concentration implies that the albumin, globulin and total protein increased as well. Thus, this implies that the extract produced an increase in protein synthesis and mobilization. The observed increase in globulin level may indicate the efficiency of the plant extract to produce antibody or due to the presence of bioactive constituents like flavonoids and alkaloids (Puri et al 1993). Significant increase in the serum level of both total bilirubin and conjugated bilirubin is an indication that the drug might induce injury to the hepatic tissue or cause conjugated hepatobiliary injury on the wistar rats. Serum alanine amino transferase (ALT) is known to increase when there is liver disease and it has been used as a tool for measuring hepatic necrosis (Bush, 1991). Hence, the observed increase in serum ALT suggests that the extracts may not be safe to the hepatic tissue at 1, 2 and 3mg/kg body weight. The research study shows that liver enzymes are highly affected by *canabis sativa* in females than in male rats.

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