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Research Article

Comparative Modulatory Assessment of the Sperm Quality and Testicular Function by Solvent Fractions of the Cannabis Extract in Rats

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ABSTRACT

Background: The bioactive ingredients present in medicinal plants have various pharmacological activities which have explored for therapeutic and management of disease conditions like infertility. **Objective:** This study evaluated the comparative modulations on semen quality and testicular function in the rat by solvents soluble portions of ethanolic leaf extract in varying polar solvents. **Methods**: Thirty Wistar rats were randomly placed into five groups (A, B, C, D, E and F) with five rats per group: A, control: 0.2 ml 2 % DMSO was administered, B (vitamin C): 90 mg/kg body weight, 4.4 mg/kg body weight of n-hexane, 3.65 mg/kg body weight of ethyl acetate, 0.52 mg/kg body weight of n-butanol and 0.18 mg/kg body weight of the residual portion of Cannabis leaf extract were given to groups C, D, E and F, respectively. The rats were sacrificed 24 hours after the last day of 60 days of treatment. **Results:** Sperm indices, testosterone, FSH, LH, and HDL showed significant increase ($p \leq 0.05$) across the treatment groups compared to normal control. Similarly, there was a significant increase in activity ($p \leq 0.05$) of ALP, ACP, SOD, and CAT across the treatment groups compared to normal control. In addition, there were significant increases in GSH, MDA, triglyceride, cholesterol, total protein concentration levels across the treatment groups compared to normal control. The n-butanol soluble portion showed normal histomorphology with typical seminiferous tubule with spermatogonia lying on the basement membrane. **Conclusion:** Testicular integrity and improved sperm quality were pronounced in rats administered n-butanol solvent portion.
Key words: Cannabis, Sperm, Fertility, Testis, Medicinal.

INTRODUCTION

In the current situation, approximately 8-12 per cent of individuals globally suffer from infertility[1]. Male infertility is mainly due to Hormonal imbalance and low sperm quality. Over 90 percent of male infertility issues are associated with low sperm levels[2]. The testis is prone to the use of harmful substances that may interfere with spermatogenesis, resulting in a decline in the quality of semen required for fertility[3]. Herbal treatments due to the abundant antioxidant phytochemical present in medicinal plants are currently being investigated as an effective option for infertile conditions in humans. Cannabis, an annual herbaceous plant of the genus cannabis, a cannabinaceae species, has been identified by researchers as a biologically active weed that can be pharmacologically potent for human fertility[4]. Cannabis was used experimentally to treat some disease conditions, including dementia, multiple sclerosis, Parkinson's disease, social anxiety disorder, depression, disorder, and neuropathic pain, etc., and some degree of efficacy was reported[5]. However, given its increasing prevalence in the medical and recreational sectors, substantial research is required to explain the potential negative and positive effects of marijuana. Changes in reproductive hormones, impaired semen requirements and decreased libido and sexual function have been associated with the use of cannabis, particularly in the field of male fertility[6]. The comparative potential of solvent portions of cannabis leaf extract for modulating testicular function and sperm quality in Wistar rats was therefore explored in this study.

MATERIALS AND METHODS

Procurement of Cannabis and Preparation of solvents portions of the extract

Dry cannabis leaves were obtained from the Ilorin National Drug Law Enforcement Agency (N.D.L.E.A). 20 g ethanolic leaf extract of cannabis was weighed and dissolved in 500 ml of a mixture of n-hexane and water (1/1 v/v) and left in an orbital shaker for homogenization. The mixture was placed and left overnight in a separate funnel. The n-hexane soluble portion was collected and kept, and then the remaining insoluble portion was added to the mixture of equivalent amounts of ethyl acetate and water (v/v). With the separating funnel, the homogeneous mixture produced was separated. A mixture of equivalent amounts of n-butanol and water was added to the remaining portion when the ethyl acetate soluble portion was extracted. The homogeneous mixture was separated with separating funnel. The n-butanol portion was gradually removed and the left water portion was stored as a residual portion. The extracted portions were separately concentrated using the rotary evaporator. Subsequently, they were dried at 40 ^{0}C in a water bath

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Chemicals and reagents used

The entire chemical used in this experiment was of analytical grade and were prepared according to the standard methods, and it consists of volumetric flask, measuring cylinder, distilled water, and reagents bottles. The reagents prepared were stored in air tight bottles at room temperature, while some were refrigerated.

Animal procurement and Experimental Design

Thirty Wistar rats weighing between 120-140 g were procured from the animal house of Department of Biochemistry, University of Ilorin. The rats were put in metal cages and were placed a well-ventilated space in the animal house of Landmark University and were allowed to acclimatize for seven days before the experiment commenced. The animals were allowed access to their feed and water *ad libitium*. Thirty Wistar rats were randomly placed into five groups (A, B, C, D, E and F) with five rats per group:

Group A (control): Rat were orally administered 0.2 ml 2 % DMSO for 60 days

Group B (vitamin C): Rats were orally administered 90 mg/kg body weight of vitamin C for 60 days

Group C: Rats were orally administered 4.4 mg/kg body weight of n-hexane portion of cannabis extract leaf extract for 60 days.

Group D: Rats were orally administered 3.65 mg/kg body weight of ethyl acetate portion of cannabis leaf extract for 60 days

Group E: Rats were orally administered 0.52 mg/kg body weight of n-butanol portion of cannabis leaf extract for 60 days

Group F: Rats were orally administered 0.18 mg/kg body weight of the residual portion of cannabis leaf extract for 60 days.

Animal sacrifice and Collection of the animal tissues

Following the experimental protocols for the handling of animals[7] , the animals in this study were sacrificed 24 hours after the last day of the 60-day experiment. The rats were anaesthetized with diethyl ether and the jugular vein was then cut open to pump the blood into the sterilized sample container. Blood samples were further centrifuged at 5000 rpm for 5 minutes using a refrigerated centrifuge to separate serum. The serum collected was transferred to fresh sterile sample bottles and placed in the freezer before biochemical tests. The testes were taken from rats, homogenized, frozen and used for biochemical assays. A small portion of the tissue from the representative testes was cut and put in 10 % of the formal saline solution for histological examination.

Preparation of organs for biochemical analysis

The testicular tissue was homogenized in an appropriate buffer for an assay. The homogenate was further centrifuged at 5000 x g for 10 minutes. The supernatants were used for the assay.

Biochemical assays

Glycogen concentration was estimated by the method described by Kemp *et al*.[8] while Nitric oxide (NO) concentration was evaluated by the procedure described by Ilavarasan *et al*[9]. Rao and Ramakrishnan[10] described the method for the evaluation of 3-hydroxy-3-methylglutaryl-CoA reductase activity in testis by measuring the ratio of 3-hydroxy-3 -methylglutaryl-CoA and mevalonate concentrations. Superoxide dismutase (SOD) was measured by the method described by Misra and Fridovich[11]. The GSH level in the testis was estimated according to the procedure described by Jollow *et al*[12]. Catalase activity in the testis was measured according to the method described by Aebi[13]. The principle of reaction between MDA and thiobarbituric acid (TBA) under acidic condition was described by Satoh[14] for the Malondialdehyde (MDA) assay. The total protein concentration estimated by the method described by Gornall *et al*.[15]. The methods described by Wright *et al* [16, 17](1972a &b) were used to estimate the activities of ACP and ALP. The LH FSH and testosterone were quantified in the serum on the procedure based on a solid phase enzyme-linked immunosorbent assay (ELISA).

Tissue lipid extraction and estimation of concentration

Lipids in the freshly harvested testis were extracted following the method described by Folch *et al*.[18] . Finally, the content of cholesterol and triglyceride were quantified spectrophotometrically using the kit. High-density lipoprotein (HDL-c) of the plasma was determined through the precipitation method using kit.

Histopathology

The testes were extracted and immediately fixed in 10 % of the formal-saline solution for histopathological analysis. The tissue samples were embedded in paraffin and then cut into a cross-section of 4–5 mm thickness and stained with hematoxylin-eosin. Histological sections were analyzed and photographed by Olympus BX50 (Japan) photo-microscope.

Data Analysis

The data were expressed as the mean of three ± SEM determinations; one-way variance analysis (ANOVA) followed by post hoc Tukey to compare mean and assess significant differences between variables at $p \leq 0.05$ between the variables. All statistical analyses were carried out using the Social Science Statistical Package, version 22 (SPSS Inc. Illinois, Chicago, USA).

RESULTS

There was an increase in the percentage weight change in the entire treatment group compared to the normal control (table 1). The increase in the sperm volume was not significant, contrary there was a significant increase in the following parameters of sperm: concentration count, motile count, total count and % motility in the rat treated with vitamins C and different solvents soluble portion the extract respectively (table 2). Similarly, for the progressive assessment, there was a significant increase in the numbers of fast sperms in the treated groups compared to the normal control, contrary the groups administered n-hexane, ethyl acetate, n-butanol and vitamin C showed a significant decrease in the slow sperm cells compared to the normal control (table 3). There was a significant increase in the number of normal sperm in the treated groups compared to the normal control (table 4). Also, there was a significant decrease in sperms with tail defect neck defect and head defect in the treated groups compared to the normal group (table 4). There was a significant increase in the serum levels of LH in rats in the treated groups compared to normal control (figure 1A). Similarly, the rats treated with n-hexane, ethyl acetate and n-butanol solvent portions respectively showed a significant increase in serum level of FSH compared to normal control (figure 1B). There was a significant increase in the testosterone serum level in the rats treated with n-butanol solvent portion and a significant decrease in levels in the groups administered n-hexane, ethyl acetate and the residual portion of cannabis extract (figure 1C). There was a significant decrease in the HMG-CoA/ mevalonate ratio in the testis of the n-hexane and n-butanol portions of the cannabis extract, respectively. There was also a substantial increase in the HMG-CoA/mevalonate ratio in rats administered vitamin C, the ethyl acetate portion of the extract and the residual portion of the extract relative to standard control (figure 2A). The concentration of testicular cholesterol showed a significant increase ($p \leq 0.05$) in the groups administered ethyl acetate and n-butanol portion of the

Table 1: Percentage weight change of testis of rats treated with solvents soluble portions of ethanolic leaf extract of cannabis for 60 days.

Group	Initial weight	Final weight	% Weight change
Normal control	212.62 ± 13.62	218.13 ± 9.81	8.03 ± 3.05
90 mg/kg BW Vitamin C	211.00 ± 9.86	237.49 ± 11.17	18.64 ± 5.99
4.4 mg/kg BW (n-hexane)	187.33 ± 8.41	217.71 ± 5.20	5.32 ± 2.14
3.65 mg/kg BW (ethyl acetate)	190.33 ± 12.99	219.97 ± 13.10	12.27 ± 5.19
0.52 mg/kg BW (n-butanol)	203.00 ± 3.00	238.95 ± 14.09	15.26 ± 6.03
0.18 mg/kg BW (residual)	198.33 ± 6.35	228.10 ± 3.91	18.20 ± 4.83

Table 2: Sperm parameters of rats treated with solvents soluble portions of ethanolic leaf extract of cannabis for 60 days.

Group/Parameter	Sperm volume (mL)	Concentration count (x 10^6 mL)	Motile count (10^6 mL)	Total count (x 10^6 mL)	Motility (%)
Normal control	1.59 ± 0.08^a	133.33 ± 6.67^a	56.67 ± 3.33^a	193.33 ± 9.28^a	50.00 ±0.00^a
90 mg/kg BW Vitamin C	1.71 ± 0.01^a	293.33 ± 6.67^b	246.67 ± 3.33^b	504.33 ± 8.67^b	85.00 ± 1.00^c
4.4 mg/kg BW (n-hexane)	1.60 ± 0.00^a	150.00 ± 11.55^{bc}	96.67 ± 3.33^{ac}	278.00 ± 2.00a^b	65.00 ± 3.33^b
3.65 mg/kg BW (ethyl acetate)	1.71 ± 0.07^a	220.00 ± 17.32^d	180.00 ± 20.00^c	440.00 ± 10.00^c	78.00 ± 2.00^c
0.52 mg/kg BW (n-butanol)	1.69 ± 0.02^a	230.00 ± 10.00^d	193.33 ± 6.67^d	397.00 ± 11.36^{aa}	80.67 ± 6.67 ^{c}
0.18 mg/kg BW (residual)	1.63 ± 0.02^a	186.67 ± 13.33^c	143.33± 6.67ad	322.00 ± 12.00^d	70.72 ± 3.12 ^{b}

The values are expressed as means of three replicates ± SEM. One way ANOVA was followed by Tukey's multiple comparison: abcd $P \leq 0.05$: significance differences compared with the normal control.

Table 3: Sperm progressive assessment of rats treated with solvents soluble portions of ethanolic leaf extract of cannabis for 60 days.

Group	fast	slow
Normal control	51.67± 3.33^a	50.00 ± 2.88^c
90 mg/kg BW Vitamin C	83.33 ± 0.88^c	17.67 ± 1.45^a
4.4 mg/kg BW (n-hexane)	64.33 ± 2.33^b	37.33 ± 2.67^b
3.65 mg/kg BW (ethyl acetate)	81.67 ± 1.67^c	17.67 ± 1.45^a
0.52 mg/kg BW (n-butanol)	81.67 ± 1.67^c	16.00 ± 1.00^a
0.18 mg/kg BW (residual)	68.00 ± 6.00^b	31.78 ± 3.78^d

The values are expressed as means of three replicates ± SEM. One way ANOVA was followed by Tukey's multiple comparison: ^{abcd} $P \leq 0.05$: significance differences compared with the normal control.

Table 4: Sperm morphology of rats treated with solvents soluble portions of ethanolic leaf extract of cannabis for 60 days.

Group	normal	Tail defect	Neck defect	Head defect
Normal control	51.00 ± 2.08^a	22.67 ± 1.45^c	11.00 ± 0.57^c	13.00 ± 2.08^b
90 mg/kg BW Vitamin C	82.00 ± 2.00^c	8.00 ± 0.57^a	4.33 ± 0.33^a	5.00 ± 1.73^a
4.4 mg/kg BW (n-hexane)	64.33 ± 2.33^b	21.33 ± 1.33^c	8.33 ± 0.88^c	5.67 ± 0.33^a
3.65 mg/kg BW (ethyl acetate)	80.00 ± 2.88^c	8.67 ± 0.67^a	7.00 ± 1.00^b	5.33 ± 0.88^a
0.52 mg/kg BW (n-butanol)	81.00 ± 1.00^c	8.00 ± 0.57^a	5.33 ± 0.33^b	5.0 ± 0.57^a
0.18 mg/kg BW (residual)	72.67 ± 1.45^b	13.33 ± 1.33^b	7.67 ± 0.33^b	9.67± 0.88^b

The values are expressed as means of three replicates ± SEM. One way ANOVA was followed by Tukey's multiple comparison: ^{abcd} $P \leq 0.05$: significance differences compared with the normal control.

cannabis extract, while the groups administered vitamin C, n-hexane and residual portion respectively showed a significant decrease in the concentration of testicular cholesterol (figure 2B). N-butanol and ethyl acetate solvent portion of cannabis leaf extract increased the concentration of testicular triglyceride significantly compared to normal control (Figure 2C), while vitamin C and n-hexane soluble portion of the extract significantly decreased testicular triglyceride. Also, the serum HDL concentration in rats administered soluble solvent portions decreased significantly compared to normal control but was significantly increased in the group administered vitamin C (figure 2D). There was a significant decrease in the organ-body ratio of testis in all the treated groups compared to the normal control (figure 3A). The rats treated with vitamin C, ethyl acetate portion, n-butanol portion and the residual portion of the extract showed a significant increase in the testicular total protein concentration compared to the normal control (figure 3B). Contrary, the ALP and ACP activity respectively in the testis of the treated groups significantly decreased compared to the normal control (figure 3 C&D). There SOD activity was not affected in the testis of rats treated vitamin C, n-butanol portion and the residual portion of cannabis extract compared with normal control whereas the activity of the enzyme was significantly increased in the testis of rats treated with n-hexane portion and ethyl acetate portion of cannabis extract (figure 4A). Similarly, there was an increase in the activity of catalase in the testis of the rats in the treated groups except the group treated with the n-butanol portion of the extract which was not affected compared to the normal control (figure 4B). The level of the reduced glutathione in the rats decreased significantly across the treated groups compared with normal control (figure 4C). The MDA level in the testis of rats in the treated groups showed a significant increase compared to the normal control (figure 5D). Rats treated with n-hexane portion and n-butanol portion respectively increased the serum glycogen concentration significantly compared to normal control (figure 5A). The concentration of serum glycogen in rats treated with vitamin C, the portion of ethyl acetate and the residual portion was not affected compared to normal. Similarly, there was a significant increase across the treated groups compared to the normal control

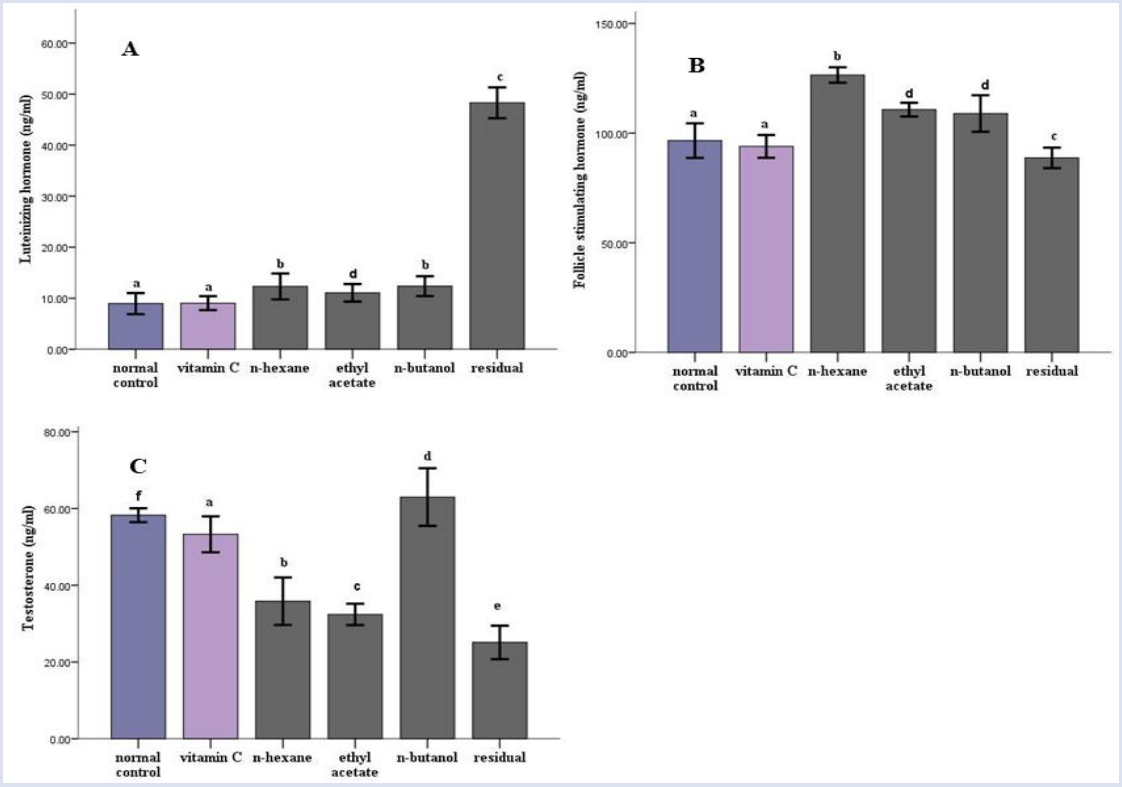

Figure 1: Serum levels of luteinizing hormone (A), follicle stimulating hormone (B), and testosterone (C) in rats treated with soluble portions of ethanolic leaf extract of cannabis for 60 days. The values are expressed as means of three replicates ± SEM. One way ANOVA was followed by Tukey's multiple comparison: [abcd] $P \leq 0.05$: significance differences compared with the normal control.

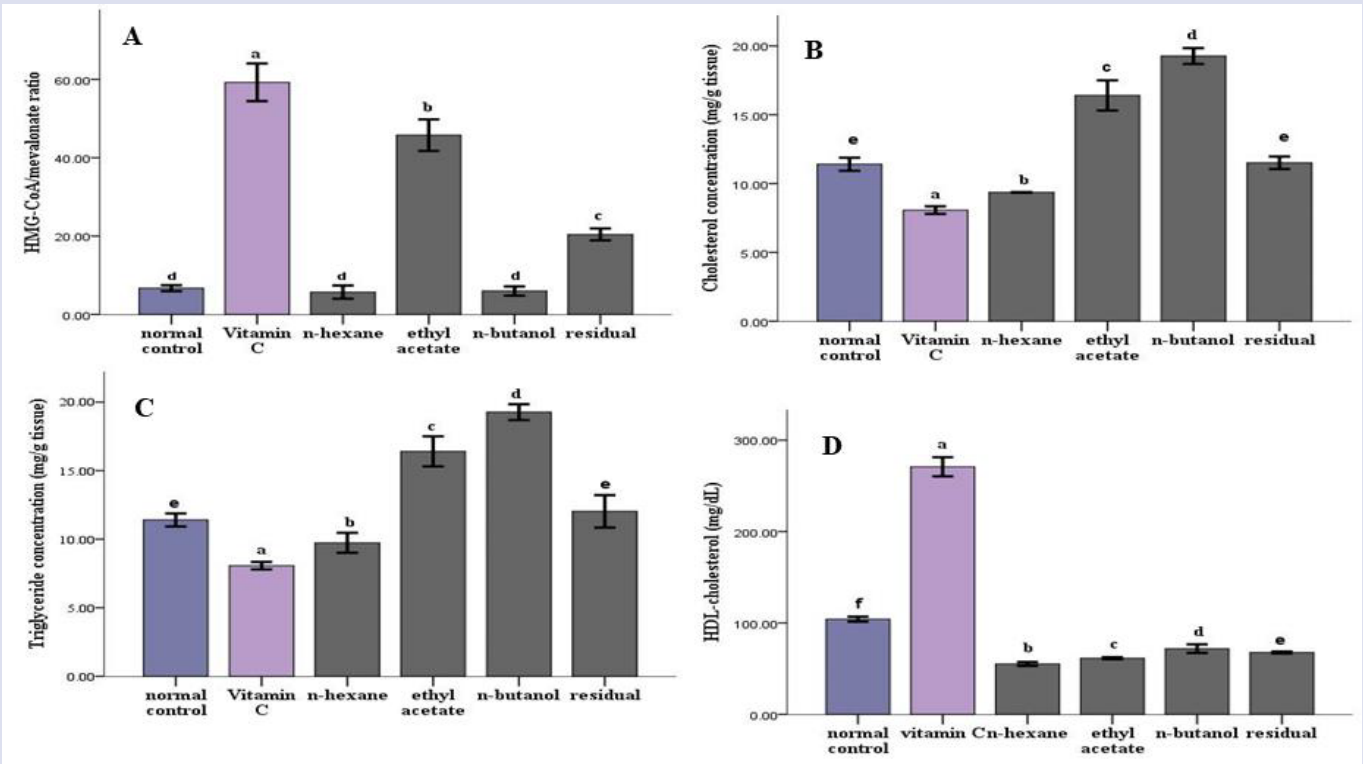

Figure 2: Testicular: HMG-CoA/Mevalonate ratio (A), cholesterol concentration (B), triglyceride concentration (C) and serum HDL concentration (D) in rats treated with soluble portions of ethanolic leaf extract of Cannabis for 60 days. The values are expressed as means of three replicates ± SEM. One way ANOVA was followed by Tukey's multiple comparison: [abcd] $P \leq 0.05$: significance differences compared with the normal control.

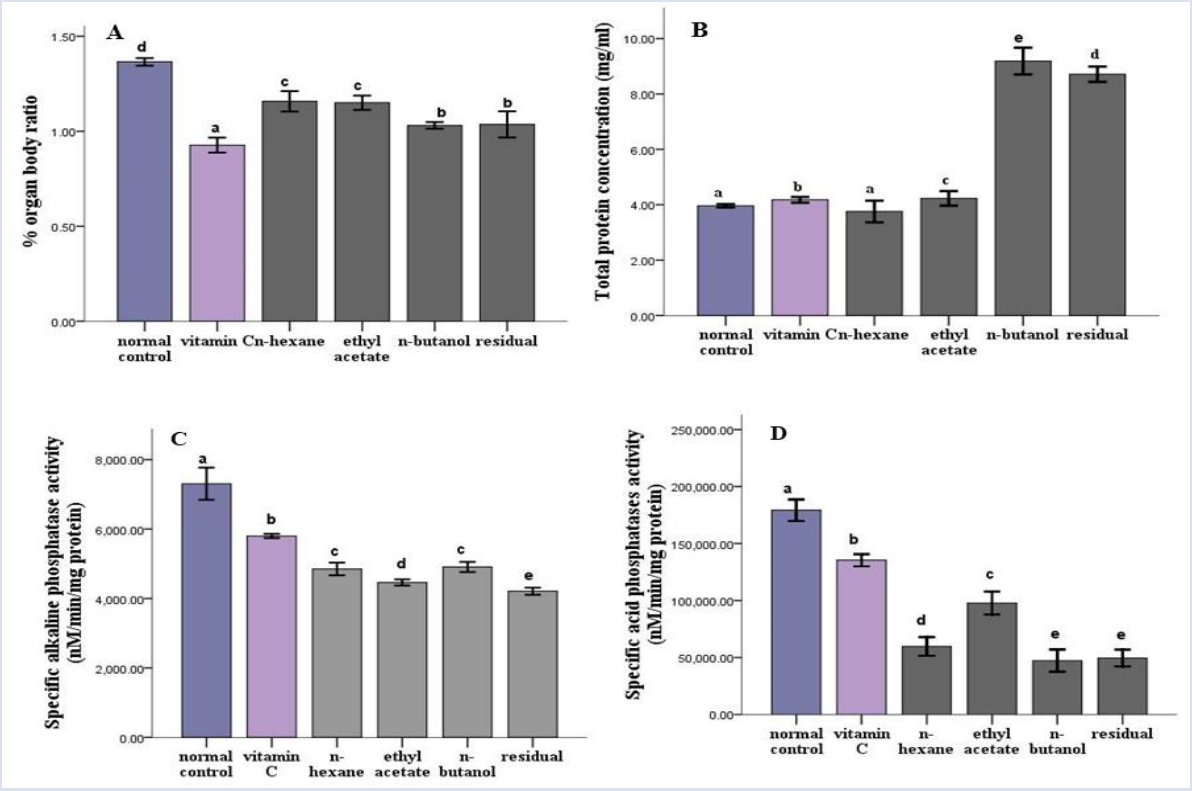

Figure 3: Testicular: organ body weights ratio (A), total protein concentration (B), specific acid phosphatase activity (C) and alkaline phosphatase activity (D) in rats treated with solvents portions of ethanolic leaf extract of Cannabis for 60 days. The values are expressed as means of three replicates ± SEM. One way ANOVA was followed by Tukey's multiple comparison: abcd $P \leq 0.05$: significance differences compared with the normal control.

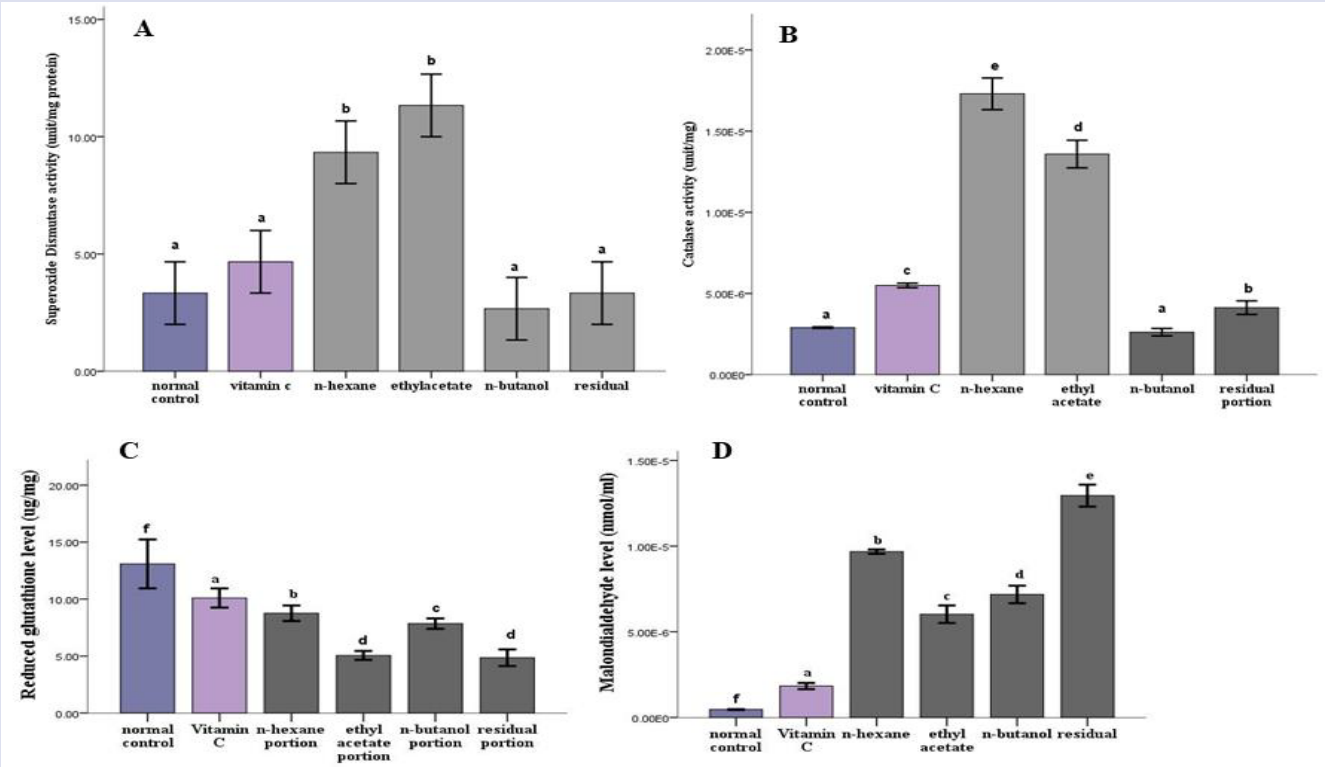

Figure 4: Testicular: SOD activity (A), catalase activity (B), reduced glutathione level (C) and malondialdehyde level (D) in rats treated with solvents portions of ethanolic leaf extract of Cannabis for 60 days. The values are expressed as means of three replicates ± SEM. One way ANOVA was followed by Tukey's multiple comparison: [abcd] $P \leq 0.05$: significance differences compared with the normal control.

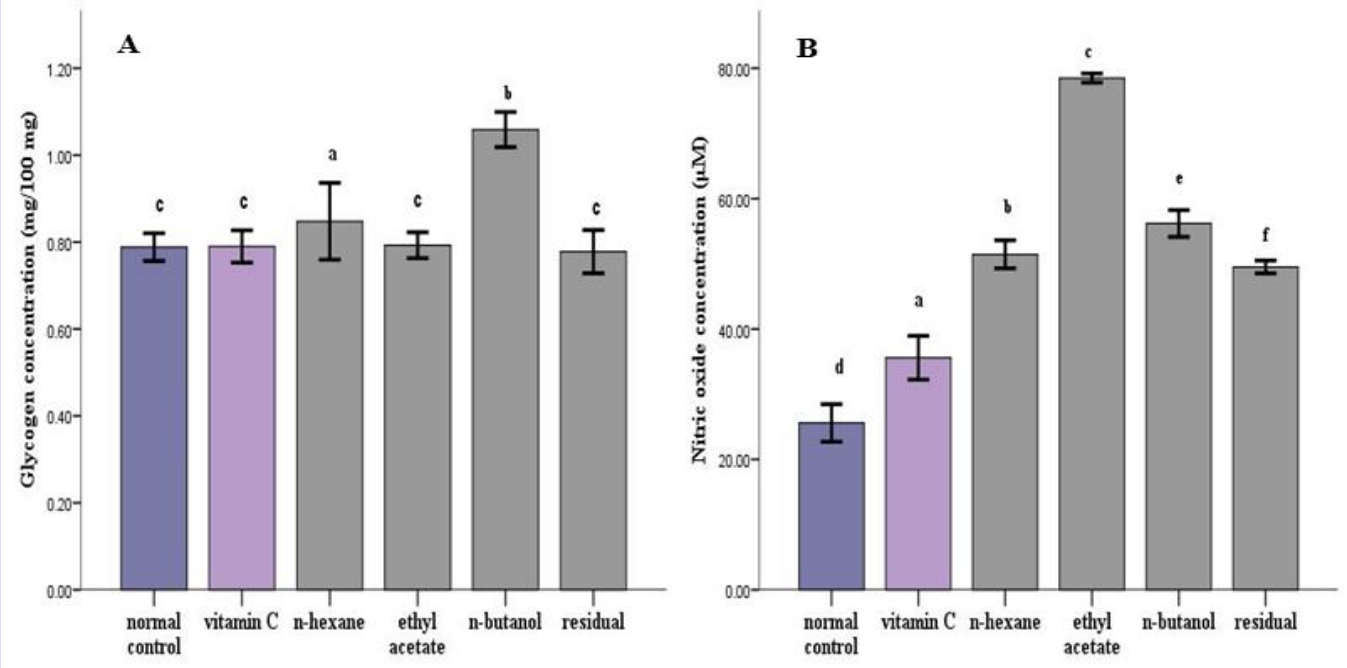

Figure 5: Testicular: glycogen concentration (A) and nitric oxide concentration (B) concentration of rats treated with soluble portions of ethanolic leaf extract of Cannabis for 60 days. The values are expressed as means of three replicates ± SEM. One way ANOVA was followed by Tukey's multiple comparison: [abcd] $P \leq 0.05$: significance differences compared with the normal control.

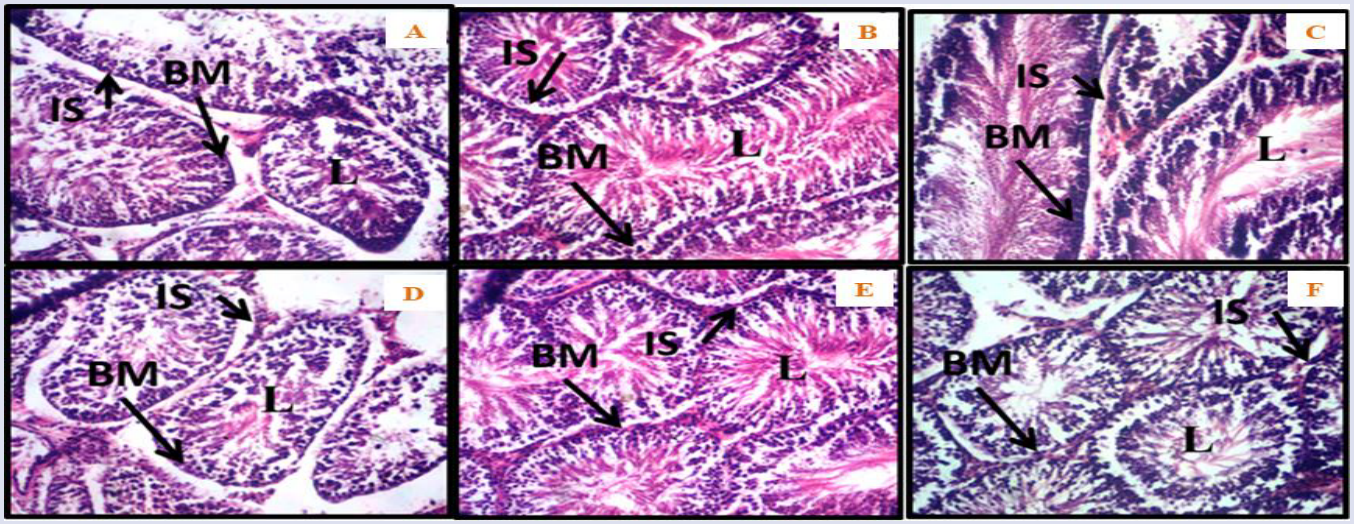

Figure 6: (H and E X100) Photomicrograph of the transverse section of testis experimental animal showing: **A (normal control)**; degeneration of interstitial cells and increased intracellular spaces of the seminiferous tubules; **B (vitamin C)**; showing normal histomorphology with typical seminiferous tubule containing different types of germ cells; spermatogonia lying on basement membrane with other cells proliferating in a centripetal direction; **C (n-hexane sub-fraction)**,showing degeneration of interstitial cells and increased intracellular spaces of the seminiferous tubules; **D** (**Ethyl acetate sub-fraction)**,showing abnormal widening of interstitial spaces (IS) with degeneration of interstitial cells. Increased intracellular spaces of the seminiferous tubules were also observed; **E (n-butanol sub-fraction)**, showing normal histomorphology with typical seminiferous tubule containing different types of germ cells; spermatogonia lying on basement membrane with other cells proliferating in a centripetal direction. No apparent histopathological alteration; **F (residual fraction)**, showing abnormal widening of interstitial spaces (IS) with degeneration of interstitial cells. (**BM**: Basement membrane, **IS**: interstitial space, **L**: lumen).

(5B). Testis histopathology revealed standard histomorphology with a typical seminiferous tubule containing various forms of germ cells with spermatogonia lying with other cells on the basement membrane (figure 6A).

DISCUSSION

A healthy testicular function and reproductive hormonal balance are necessary for optimal sperm production. Medicinal plants have been reported to possess multiple bioactive components which can interact with some receptor in the body to trigger a cascade of reactions that will influence the biochemical reactions such as increased hormone production or antioxidant activity. In the process of isolating and further characterization of the target compound, the crude extract was preliminarily concentrated through solvent partitioning. The bioactive components of the cannabis extract were partitioned in this study using solvents with ranging polarities. The portion with the most active modulatory effect may imply a high concentration of the target component. Testicular size is expected to be proportional to the weight

of the experimental animal which may also be indicative of healthy testis. Testicular cell death: Sertoli, Leydig, and spermatogonia contribute to a decrease in testis weight[19]. The increased percentage of organ weight reported in this study may correlate to intact testicular cells. This may imply a protective effect of the extract which was reported by previous work to be rich in antioxidants. Conversely, the decrease in organ body weight recorded in the experiment was attributed to the disproportion in the weight of the animals within the group. Semen analysis involves descriptive sperm cell and seminal fluid parameter measurements which are indicative of semen quality [20]. Similarly, the quality of semen could be a marker to fertility assessment[21]. The assessed sperm volume, concentration, motile count, total count, per cent motility, progressive sperm assessment, and morphology of sperm were evaluated for sperm quality in this study. The enhanced sperm parameters reported may be suggestive of the effect of the extract on the spermatogenesis process. This altered spermatogenesis may be as a result of modulation of the hormone production process by the extract [22]. The observed increase in the reproductive hormone levels (LH, testosterone and FSH) observed also corroborated the sperm parameters reported. The increase in FSH recorded is reflective of mature and viable sperm cell[23]. Sperm quality in this study is also attributed to the antioxidant activity by the extract. Likewise, previous studies have reported a high flavonoid concentration[24,25]. The reduced level of HMG-CoA/ mevalonate ratio also indicates the activity of HMG-CoA reductase activity, the rate-limiting step in the de-novo cholesterol biosynthesis pathway, the precursor molecule for testosterone synthesis[26]. The consequent increase in cholesterol levels was therefore indicative of the extract's steroidogenic impact. Similarly, the increased amount of triacylglycerides identified as an energy source for spermatogenesis was influenced by this extract[27]. In previous studies, HDL was reported to a source of cholesterol for steroidogenesis[28], but the decrease in HDL in this study gave credence to testicular *de-novo* cholesterol synthesis reported in this present study[29]. Testicular protein concentration is responsible for the maturation of spermatozoa[30]. The reported protein concentration increase was indicative of the effect of the extract on the testicular protein synthesis apparatus and this also may be indicative of the sperm quality recorded in this present study. The key source of energy required for the energy-requiring biochemical task of FSH in the Sertoli cells is glycogen storage in the testis[31, 32]. The increased concentration of glycogen in the current study also verified the increased level of FSH mentioned earlier. Similarly, the increased NO reported in this study is also reflective of the effect of the extract on testicular function. NO was implicated in the role of spermatogenesis and germ cell degeneration[33]. The increased NO concentration recorded in this study could be due to the extract. Lysosomal enzyme ACP is a biomarker of spermatogenesis steps mediated by androgenic influence[34]. Likewise, Alp is correlated with plasma membrane permeability and germ cell biomarkers in the cell[35]. The compromised plasma membrane integrity due to lipid peroxidation may have caused leakage of the enzyme resulting in the reduced activity of the enzymes. Also, in this study, the increase in the MDA level was due to oxidative stress occasioned by the long use of the extract[36]. Enzymatic cellular antioxidants that defend against oxidative stress are SOD and catalase. SOD proffers the first line of defence against the oxy-radical deleterious effect on the cell[37].

In this research, the increase in SOD and catalase activity was indicative of the neutralizing action of the oxidative radicals involved in damage to the membrane. Besides, reduced glutathione is an antioxidant peptide molecule known to protect cells from lipid peroxidation by scavenging superoxide ions and inhibiting oxygen radical formation[38]. The reduced amount of GSH in the testis in this study is also reflective of the antioxidant activity. Histopathological examination of testis revealed normal histomorphology with typical seminiferous tubule containing different types of germ cells. However, the normal histology was reported in the n-butanol fraction, which could be as a result of the antioxidant protective effect of the testicular cells by the extract.

CONCLUSION

The n-butanol portion of the extract appeared to be the most active in improving the sperm quality and preserve the testicular function reported for the crude extract. The solvent may have precipitated the target compound implicated for the function.

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GRAPHICAL ABSTRACT

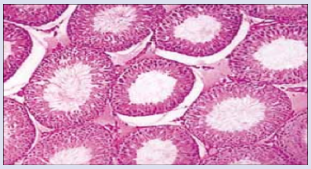
Cannabis leaf

Ethanolic leaf extract of cannabis

Solvent fractions of the extract

n-hexane → ethylacetate → n-butanol → Residual

Rat treated with portions of the cannabis extract for 60 days



Testicular Photomicrograph of rat

ANTIOXIDANT EFFECTS

MODULATION OF TESTICULAR FUNCTION AND SPERM QUALITY

Conclusion: The antioxidant activity, sperm quality and testicular functions modulation by the solvent portions of the extract was observed in this study.

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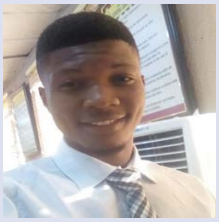

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