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GC-MS Assay of Boiled Aqueous and Ethanol Extracts of Justicia Carnea Leaves and their Effects on the Male Reproductive Indices: Testicular Antioxidant System and Histoarchitecture of Male Wistar Albino Rats

[1]Stanley Chidi Udedi
Department of Applied Biochemistry,
Nnamdi Azikiwe University,
Awka, Nigeria.

[2]Victor Nwabunwanne Oguaka
Department of Human Biochemistry,
Nnamdi Azikiwe University,
Nnewi Campus, Nigeria

[3]Kingsley Ikechukwu Ubaoji
Department of Applied Biochemistry,
Nnamdi Azikiwe University,
Awka, Nigeria.

[4]Charles Chijioke Dike
Department of Human Biochemistry,
Nnamdi Azikiwe University,
Nnewi Campus, Nigeria.

[5]Kingsley Kelechi Asogwa
Department of Applied Biochemistry,
Nnamdi Azikiwe University,
Awka, Nigeria.

[6]Corresponding Author:
Victor Nwabunwanne Oguaka
Department of Human Biochemistry,
Nnamdi Azikiwe University,
Nnewi Campus, Nigeria.

Abstract:-

- ***Background:***

Justicia carnea has overwhelming evidence demonstrating its medicinal and nutritional benefits, some of its phytochemicals are antinutritional and can have negative consequences including impairment of the male reproductive functions when ingested. The adverse effects on male reproductive function by ethanol leaf extract of Justicia carnea has been reported including alteration of the antioxidant system of the testes, reduction in the weight of testes, reduced sperm count/motility and testosterone levels as well as distortion of the histoarchitecture of the testis in male wistar albino rats. Most agents that alter male fertility do so by altering the antioxidant system of the testes.

- ***Aims and Objectives*:**

The aim of this study is to determine the varieties of phytochemicals present in both boiled aqueous and ethanol extracts of the leaves of Justicia carnea, the impact of administration of these extracts on the testicular antioxidant system status (superoxide dismutase - SOD, catalase - CAT, malonaldehyde - MDA and reduced glutathione - GSH) and testicular/epididymal histology of male Wistar Albino rats.

- ***Methods:***

The GC-MS of both boiled aqueous extracts (AJC) and ethanol extracts (EJC) of the leaves of Justicia carnea were carried out to determine the phytochemical components of both extracts which were administered to the experimental male Wistar Albino rats. Forty-Two Albino Wistar rats (12-14 weeks of age) in seven groups (I-VII) with average weight of 180-200g were used for the study. The control was fed normal grower feed and water only. The test groups were in addition given 200, 400 and 600 mg/kg BW of either boiled aqueous or ethanolic-leaf extracts of Justicia carnea for a period of 21 days. The first batch of the experimental animals (drawn from each group) were sacrificed on day 21 and the second batch were fed normal grower feed and water only for further 52 days after discontinuation of the extracts before sacrificing them. Testicular tissue redox status was determined by assaying for superoxide dismutase (SOD) using Sun and Zigma method, catalase by Aebi method, Malondialdehyde (MDA) by Buege and Aust method, reduced glutathione (GSH) by Sedlak and Lindsay method. The histology of the testis/epididymis was also carried out. Statistical Package for the Social Sciences (SPSS) version 25 was used for analysis of the results.

ISSN No:-2456-2165

➢ ***Results:***

The GC-MS results show that the ethanol extract has six different known male antifertility compounds while the boiled aqueous extracts have two. There was no significant change (P < 0.05) in the level of testicular SOD after 21 days of administration of the extracts except for the group that received 400mg/kg BW of AJC which had a significantly higher level that was reversed 52 days after discontinuation. However, at 52 days after discontinuation, the group that received 400mg/kg BW of EJC had significantly higher SOD (P < 0.05) compared to the rest. Testicular catalase (CAT) was reduced but not significantly (P < 0.05) in all the experimental groups experimental groups at 21 days after administration of both extracts but exhibited variable changes at 52 days after withdrawal of administration of the extracts. While there was a non-significant reduction (P < 0.05) in the levels of CAT amongst most groups, there was a non-significant increase (P < 0.05) in the group that received 400mg/kg BW of AJC and a significant increase (P < 0.05) in the group that received 400mg/kg BW of EJC. The administration of 400mg/kg BW, 600mg/kg BW of EJC and 600mg/kg BW of AJC caused a significant decrease (P < 0.05) in the levels of reduced glutathione (GSH), while 400mg/kg BW of AJC caused a non-significant reduction (P < 0.05) after 21 days of administration. At lower doses, 200mg/kg BW of AJC caused a non-significant increase (P < 0.05) while 200mg/kg BW of EJC caused a significant increase (P < 0.05) in the levels of GSH after 21 days of administration. At 52 days after discontinuation, the group that received 200mg/kg BW of EJC still had a sustained significantly higher (P < 0.05) level of GSH, while the group that received 200mg/kg BW AJC still had no significant change (P < 0.05) in the level of GSH. However, the group that received 400mg/kg BW of AJC and 600mg/kg BW EJC still showed a significantly lower (P < 0.05) GSH while those that received 400mg/kg BW of EJC and 600mg/kg BW of AJC had a non-significant decrease (P < 0.05) in the GSH activity 52 days after withdrawal. Many of the treatment groups show significantly higher level (P < 0.05) of testicular malondialdehyde (MDA) after 21 days of treatment, except the groups that received 200mg/kg BW of AJC and 600mg/kg BW of EJC which showed non-significantly lower (P < 0.05) levels and unchanged levels respectively. The, group that received 400mg/kg BW of EJC had the least MDA levels at 21 days after administration which is significantly lower (P < 0.05) than the control. Most of the changes in the levels of MDA were in reversal at 52 days after discontinuation of the extracts as the 200mg/kg BW, 400mg/kg BW of EJC and 400mg/kg of AJC groups then had non-significantly elevated (P < 0.05) MDA levels. While the group that received 200mg/kg BW of AJC had significantly lower levels (P < 0.05) of MDA, the group that received 600mg/kg BW of EJC and 600mg/kg BW of AJC had non-significantly lower (P < 0.05) MDA levels. There was no obvious distortion of testicular histology or histoarchitecture in any of the experimental groups as the epididymis displayed normal ducts, while the testis showed seminiferous tubules with layers of germ cells at various stages of maturation.

➢ ***Conclusion***:

There are numerous male antifertility phytochemicals in both extracts of Justicia carnea. This study shows that boiled aqueous and ethanol extracts of Justicia carnea leaves can have a negative impact on the testicular antioxidant system and by extension the male fertility potentials but no recognizable effect on the histoarchitecture of the testes at the doses used in this study.

Keywords:- *Justicia Carnea, Gc-Ms, Antifertility Compounds, Testicular Antioxidant System (Sod, Cat, Mda, Gsh), Male Fertility, Testicular Histology, Histoarchitecture.*

I. INTRODUCTION

Justicia carnea, commonly called Brazilian plume flower, Brazilian-plume, flamingo flower and jacobinia belongs to the largest genus (Justicia) of the family of Acanthaceae, which consists of about 600 species of shrubs, herbs, and tender perennial commonly found in the tropics and subtropics [1]. The plant can be brought indoors during the winter, placed in containers and can exhibit a variety of summertime hues, including white, pink, red, rose, magenta, orange, purple, and coral/apricot. The spike produces flowers that have a tube-like form and curl outward. While some of the smaller kinds only reach heights of 2 feet, others can reach heights of 6 feet and a width of 6 feet [2].

Fig 1 Justicia Carnea

Even though *Justicia carnea* has overwhelming evidence demonstrating its medicinal and nutritional benefits, various phytochemical studies have shown that *Justicia carnea* contains numerous phytochemicals with nutritive, ethnopharmacological and anti-nutritive properties [2], [3]. Some of these negative consequences can be harnessed by the nutraceuticals to the benefits of humans. The adverse effects on male reproductive function by ethanol leaf extract of *Justicia carnea* has been reported including alteration of the antioxidant system of the testes, reduction in the weight of testes, reduced sperm count/motility and testosterone levels as well as distortion of the histoarchitecture of the testis in male wistar albino rats [4]. Most agents that alter male fertility do so by altering the antioxidant system of the testes. Since both spermatogenesis and Leydig cell steroidogenesis are susceptible to oxidative stress, the testes' low oxygen tension may play a significant role in the methods by which they defend themselves from damage caused by free radicals [5].

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The testicular micro-environment is characterized by low oxygen tensions, but the availability of highly unsaturated fatty acids, especially 20:4 and 22:6, and the existence of potential ROS-generating mechanisms make this tissue susceptible to oxidative stress. The mitochondria and several enzymes, such as xanthine- and NADPH-oxidases [6], [7] and cytochrome P450s [8], can produce ROS. These enzymes either deliberately produce ROS or unintentionally create these harmful molecules because of their metabolic activity. The testes have created a complex array of antioxidant systems with both enzymatic and non-enzymatic components to reduce this risk. When oxidative stress is induced in the testes, it triggers a response that is characterized by the NF*k*B-mediated induction of mRNA species for the activities of glutathione peroxidase (GPx), glutathione-S-transferase (GST), and superoxide dismutase (SOD) [9]. Figure 1 summarizes the basic biochemistry of these antioxidant enzymes, which involves the quick conversion of superoxide anion (O_2-.) to hydrogen peroxide (H_2O_2) in the presence of SOD to stop the former from contributing to the formation of extremely harmful hydroxyl radicals. To stop the oxidative damage to lipids, proteins, and DNA from occurring, the H_2O_2 produced in this way must be quickly removed from the cell. Catalase or glutathione peroxidase, with the latter being more prevalent in the testes, are responsible for removing H_2O_2. [10], [11]. GST, on the other hand, consists of a sizable and intricate family of proteins that catalyze the conjugation of reduced glutathione via the sulfhydryl group to electrophilic centres on a variety of substrates in order to prepare for excretion from the cell. Both the metabolism of xenobiotics and the detoxification of peroxidized lipids depend on this activity.

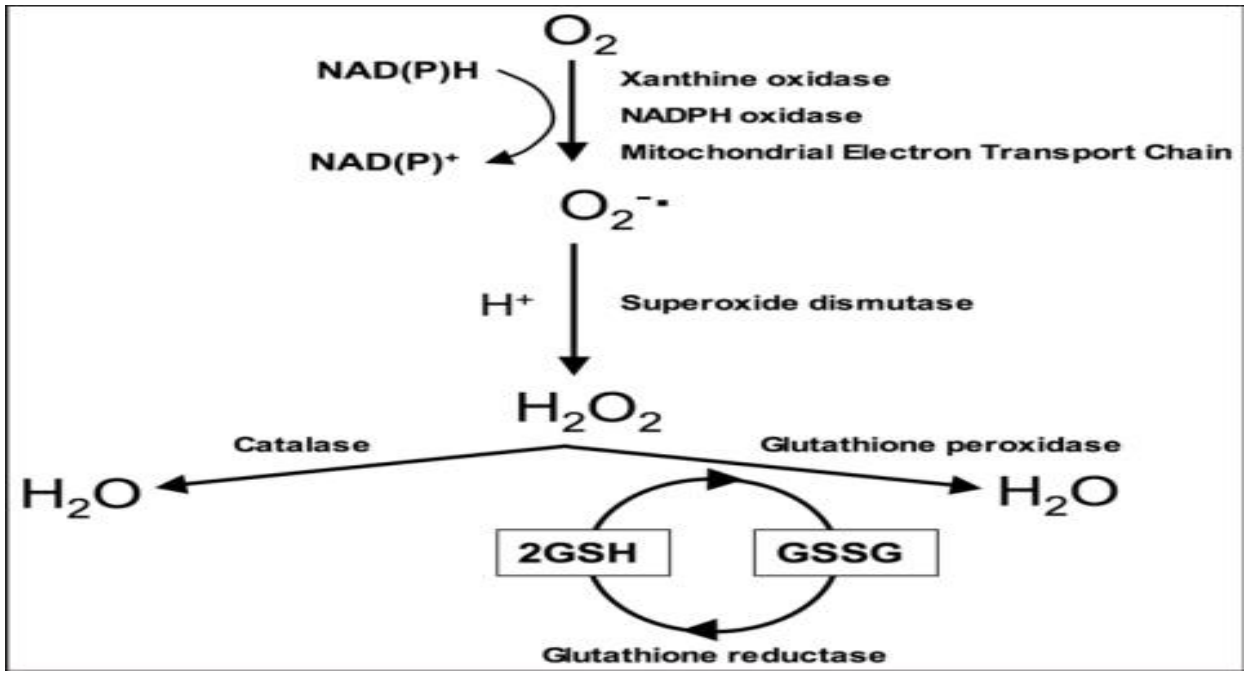

Fig 2 Basic Biochemistry of These Antioxidant Enzymes, Involving Quick Conversion of Superoxide Anion (O_2-.) to Hydrogen Peroxide (H_2O_2) in the Presence of SOD to Stop the Formation of Extremely Harmful Hydroxyl Radicals [5].

To protect the testes from oxidative stress and preserve their dual spermatogenic and steroidogenic capabilities, they habour a complex array of antioxidant enzymes and free radical scavengers. These antioxidant defence mechanisms are vital because peroxidative damage is currently thought to be the sole factor contributing to impaired testicular function, which underlies the pathological effects of a variety of conditions, including testicular torsion, diabetes, and xenobiotic exposure [5].

Numerous studies have revealed that several commonly used medicinal plants can impair male fertility potentials. Saalu and co-workers have listed up to 40 medical herbs that have a variety of antifertility properties [12]. In the current search for the creation of the ideal male contraceptive, we can benefit from the apparent negative effect of *Justicia carnea* extracts on the male reproductive function.

II. MATERIALS AND METHODS

A. Materials

Leaves of *Justicia carnea* were collected at Ahucol Phase 2, Udoka Housing Estate, Awka, and identified by the botanist at the Department of Botany, Nnamdi Azikiwe University, Awka. The College of Health Sciences at Nnamdi Azikiwe University, Nnewi, Animal Facility Unit provided the albino rats that were used for the study.

The analytical grade chemicals utilized in this investigation were all purchased from British Drug House Ltd. in England through a sales representative in Ikeja, Lagos State, Nigeria. Prof Udedi's research lab unit of the Department of Human Biochemistry, Nnamdi Azikiwe University, Nigeria, provided the distilled water that was used. Filter papers (Whatman number 1), an oral canula, a lyophilizer, a centrifuge machine (Model 800, China), a rotary evaporator (Model: TT-52, USA), a water bath

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(Model: H-H-W470, China), and a shaker (Model: 073185, Denley, England) are some of the additional tools used.

B. Methods

➢ *The Study Location*

Much of the bench work was done in Prof. S.C. Udedi's Research and Development Laboratory at NAU in Awka, Anambra State's Department of Applied Biochemistry.

➢ *Preparation of Plant Extract*

The leaves of *Justicia carnea* were hand-picked and air-dried at room temperature in the shade. The leaves were then manually milled into a fine powder. The powder was soaked in 70 % ethanol for 48 h with occasional shaking and then filtered using Whatman No. 1 filter paper and concentrated in a water bath at 40 ^{O}C [13], to obtain the ethanol extract. The boiled-aqueous extract used was obtained by boiling the powder in distilled water for 10 minutes, filtered through muslin cloth and then filter paper. The resultant fluid was then heated in a water bath to obtain a concentrated liquid which was used for GC-MS assay and for administration to the experimental animals.

➢ *Phytochemical Analysis by Gas Chromatography-Mass Spectrometry:*

The phytochemical investigation of ethanol and aqueous extract was performed on a GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II according to the method of Kanthal and co-workers [14]. Experimental conditions of GC-MS system were as follows: TR 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. Flow rate of mobile phase (carrier gas: Helium) was set at 1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 40°C raised to 250°C at 5°C/min and injection volume was 1µl. Samples dissolved in chloroform were run fully at a range of 50-650 m/z and the results were compared by using Wiley Spectral library search programme.

➢ *Animal Handling:*

The rats were fed a commercial pellet diet - Vital Growers obtained from Gland Cereals Ltd. in Jos, Plateau State, Nigeria, through her sales representative in Awka, Anambra State. Animal care and handling followed the standards specified by the Nnamdi Azikiwe University, Awka, Ethical Committee, and WHO recommendation [15]. The rats were housed in metal cages and allowed unlimited access to food and water. They were housed under the identical conditions—the same temperature, humidity, and 12-hour light/dark cycle—for two weeks to help them acclimatize.

➢ *Study Duration:*

Sample collection took up to 4 months. The extracts were administered for a period of 21 days to the experimental groups. The first set of experimental animals was sacrificed 24 hours after the last treatment to assess the effects on the parameters to be measured.

The time interval of 21 days was chosen because it takes about 13 days to achieve degeneration of the germ cell epithelium of the Wistar rats as 13 days is the life cycle. A degenerative change (reduced spermatid count) will become obvious after this period. Any degenerative changes that occurs becomes much more pronounced by the 21st day. Also, any inhibition of plasma testosterone levels becomes evident by the 21st day as such changes were not detected after a time interval of 13 days [16].

The second set of the experimental animals were fed normal diet only for another 52 days before they were sacrificed and the parameters reassessed. The essence of waiting for 52 days was to allow for possible reversal of derangements (if any) in the fertility functions that may have resulted from administration of the extracts. This is because spermatogenesis in rats takes about 52 days [17].

➢ *Administration of the Extracts to the Subjects:*

Forty-two adult male Wistar albino rats (12-14 weeks) weighing 180-200 grams were randomly divided into seven groups of six rats each.

- Group I (Control) (A1-A6): Rats were fed normal diet and administered with 0.5ml of water.
- Group II (B1-B6): Rats were administered 0.5ml (200mg/kg BW) of the ethanol leaf extract.
- Group III (C1-C6): Rats were administered 0.5ml (400mg/kg BW) of the ethanol leaf extract.
- Group IV (D1-D6): Rats were administered 0.5ml (600mg/kg BW) of the ethanol leaf extract.
- Group V (E1-E6): Rats were administered 0.5ml (200mg/kg BW) of the boiled aqueous leaf extract.
- Group VI (F1-F6): Rats were administered 0.5ml (400mg/kg BW) of the boiled aqueous leaf extract.
- Group VII (G1-G6): Rats were administered 0.5ml (600mg/kg BW) of the boiled aqueous leaf extract.

The extracts were administered to the rats orally with gavage tube two times daily (morning and evening) for 21 days.

➢ *Sacrifice and Sample Collection:*

The first set of animals were sacrificed under anaesthesia with diethyl ether, twenty-four hours after the last treatment for 21 days. This first set was constituted by half (three) of the animals in each group.

The testis and epididymis of the sacrificed rats were harvested into a clean specimen container and were processed for tissue redox status determination and the remaining fixed in formalin and sent for histological assays.

The second set of rats constituted by half (three) from each of the groups were sacrificed 52 days after discontinuation of the extracts. Specimens were taken and processed as previously done for the first set of the control and experimental animals.

ISSN No:-2456-2165

III. RESULTS

The results of the GC-MS of the aqueous and ethanol extracts of the *Justicia carnea* leaves used for the study shows varieties of phytochemicals. The most abundant phytochemicals in the AJC include 2,4-Di-tert-butylphenol (9.67%) with RT of 22.130 minutes, Decyl prop-1-en-2-yl ester (9.26%) with RT of 9.430 minutes, 9,12-Octadecdienal (9.20%) with RT of 5.238 minutes, Nonyl prop-1-en-2-yl ester (8.64%) with RT of 9.815 minutes, 5-Methyl undecane (8.0%) with RT of 10.159 minutes, 17-Pentatricontene (3.48%) with RT of 8.878 minutes and 1-Bromo undecane (3.25%) with RT of 8.985 minutes.

The ethanol extracts of *Justicia carnea* leaf used for the study also has numerous phytochemicals. The most abundant phytochemical in the EJC is (E)-Beta-famesene (15.21%) at 2 different RTs of 19.364 (4.34%) and 20.718 (10.87%) minutes respectively. Other abundant phytochemicals present are 1-Piperonyl-3,5-diamino-1,2,4-triazole (12.02%) at 2 different RTs of 36.885 (3.70%) and 34.884 (8.32%) minutes respectively. Cyclohexane has an abundance of 7.59% with RT of 21.089 minutes. Aromandendrene has an abundance of 6.27% with RT of 18.364 minutes, while 1H-cyclopenta (1,3)-cyclopropa (1,2)-benzene has an abundance of 3.74% with RT of 19.982 minutes. Other phytochemicals present are 1,4 dichloro benzene (0.28%) and RT of 6.484 minutes and Naphthalene (2.07%) with RT of 20.114 minutes.

Several phytochemicals are present in the GC-MS study of the *Justicia carnea* extracts with documented antifertility potentials in this study include Erucic acid which is present in the boiled aqueous extract, 2,4-ditert-butylphenol present in both boiled aqueous and ethanol extracts. Others like Naphthalene, 1,4-dichlorobenzene, Dibutyl phthalate, 1H-cyclopenta (1,3)-cyclopropa (1,2)-benzene, (E)-5-(benzo[d]-1,3-dioxol-5-yl)-N-isobutylpent-2-enamide are present in the ethanol extract. The EJC contains six different antifertility compounds as against the two in the AJC.

Table 1 shows the effects of the administration of both extracts on testicular SOD and CAT after 21 days of administration of both extracts while figs. 3 and 4 show the levels of the two enzymes 52 days after discontinuation.

Table 1 Effects of Aqueous and Ethanolic Leaf Extract of *Justicia Carnea* on Testicular SOD and CAT Levels after 21 Days of Administration

Parameter	Groups	Mean	±Std	P-Value	F-Value
SOD ($x10^{-6}$U/mg)	Group A (control)	0.74	±0.17		
	Group B (200mg/kg EJC)	1.49	±0.12	0.089 [a]	
	Group C (400mg/kg EJC)	1.21	±0.64	0.282 [a]	
	Group D (600mg/kg EJC)	1.17	±0.26	0.320 [a]	1.813
	Group E (200mg/kg AJC)	0.70	±0.18	0.918 [a]	
	Group F (400mg/kg AJC)	1.68	±1.09	0.038*	
	Group G (600mg/kg AJC)	1.58	±0.06	0.061 [a]	
CAT ($x10^{-6}$U/mg)	Group A (control)	7.34	±1.66		
	Group B (200mg/kg EJC)	4.80	±0.40	0.109 [a]	
	Group C (400mg/kg EJC)	3.88	±3.88	0.457 [a]	2.161
	Group D (600mg/kg EJC)	4.47	±0.41	0.074 [a]	
	Group E (200mg/kg AJC)	6.87	±1.44	0.756 [a]	
	Group F (400mg/kg AJC)	5.03	±1.68	0.141 [a]	
	Group G (600mg/kg AJC)	5.07	±0.21	0.148 [a]	

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p<0.05$. AJC: aqueous leaf extract of *Justicia carnea*; EJC: ethanolic leaf extract of *Justicia carnea* (*: significant; a: not significant).

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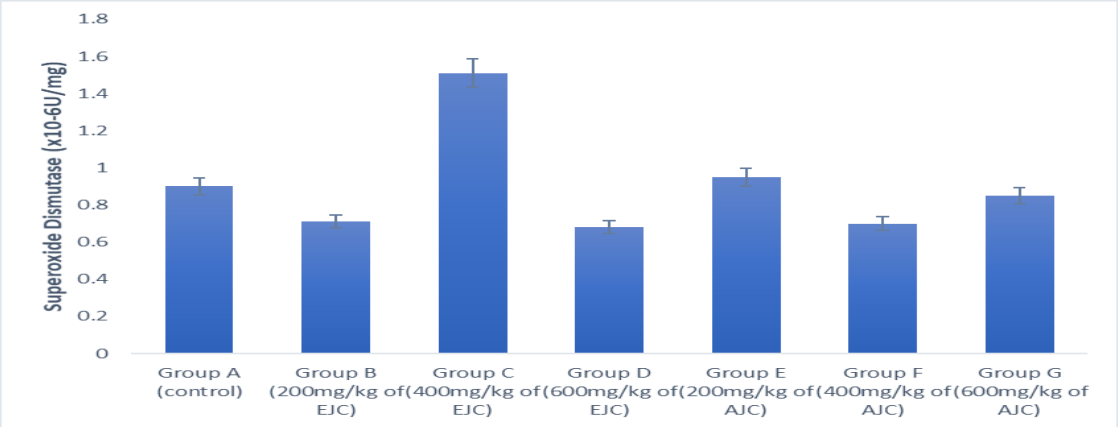


Fig 3 Effects of Aqueous and Ethanolic Leaf Extract of *Justicia Carnea* SOD Level after 52 Days

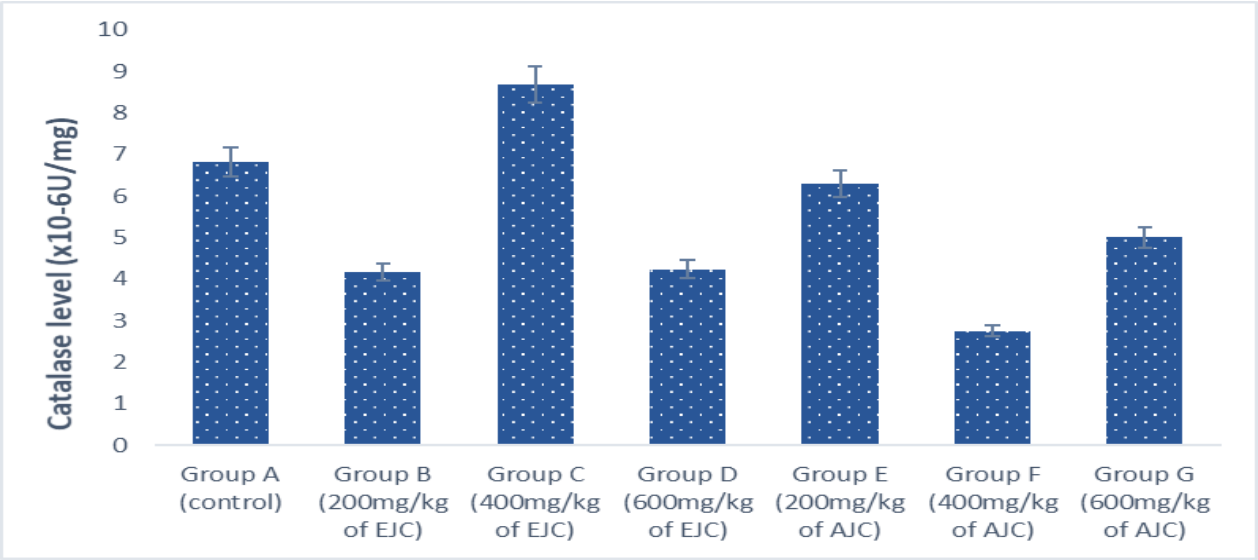


Fig 4 Effects of Aqueous and Ethanolic Leaf Extract of *Justicia Carnea* CAT Level after 52 Days

Table 2 shows the effects of EJC and AJC on the testicular MDA and GSH levels after 21 days of administration while figs. 5 and 6 show their levels 52 days after discontinuation.

Table 2 Effects of Aqueous and Ethanolic Leaf Extract of *Justicia Carnea* on Testicular MDA and GSH Levels after 21 Days of Administration

Parameter	Groups	Mean	±Std	P-Value	F-Value
MDA ($x10^{-6}$µmol/mg)	Group A (control)	4.13	±1.60		
	Group B (200mg/kg EJC)	7.31	±1.47	0.001*	
	Group C (400mg/kg EJC)	1.85	±0.12	0.013*	
	Group D (600mg/kg EJC)	4.13	±0.24	1.000 [a]	11.961
	Group E (400mg/kg AJC)	3.70	±1.23	0.599 [a]	
	Group F (400mg/kg AJC)	6.48	±0.24	0.010*	
	Group G (600mg/kg AJC)	6.48	±0.49	0.010*	
GSH ($x10^{-4}$µmol/mg)	Group A (control)	2.57	±0.01		
	Group B (200mg/kg EJC)	2.88	±0.07	0.006*	

ISSN No:-2456-2165

	Group C (400mg/kg EJC)	1.79	±0.14	0.000*	44.629
	Group D (600mg/kg EJC)	1.63	±0.13	0.000*	
	Group E (200mg/kg AJC)	2.61	±0.16	0.710 [a]	
	Group F (400mg/kg AJC)	2.47	±0.15	0.335[a]	
	Group G (600mg/kg AJC)	2.34	±0.04	0.027*	

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p<0.05$. AJC: aqueous leaf extract of *Justicia carnea*; EJC: ethanolic leaf extract of *Justicia carnea* (*: significant; a: not significant).

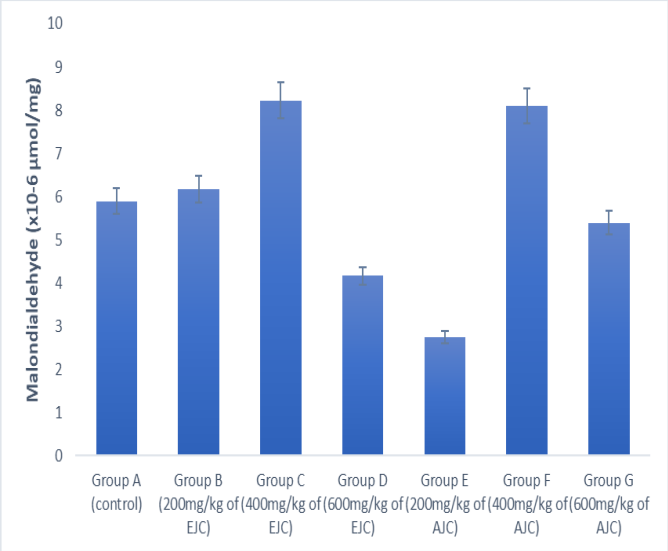


Fig 5 Effects of Aqueous and Ethanolic Leaf Extract of *Justicia Carnea* MDA Level after 52 Days

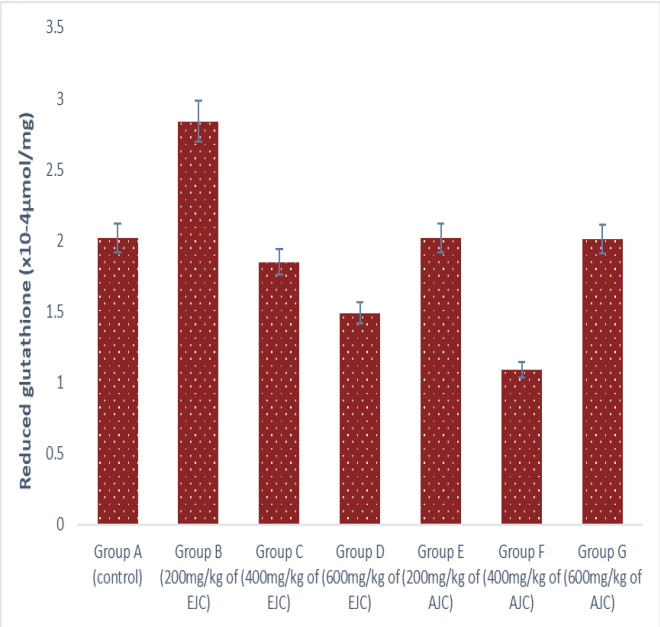
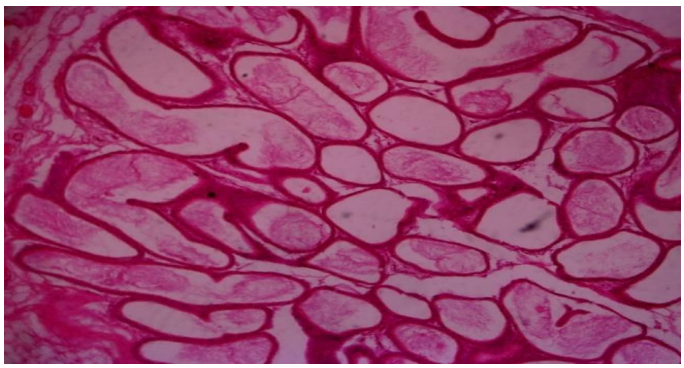


Fig 6 Effects of Aqueous and Ethanolic Leaf Extract of *Justicia Carnea* GSH Level after 52 Days

➢ *Histology Results:*

The photomicrographs show the histologic patterns of the epididymis and the testes of the control, after 21 days of administration of both extracts and 52 days after withdrawal of the extracts. The histology shows ssections of the epididymis which displays normal ducts, while the testis shows seminiferous tubules have layers of germ cells at various stages of maturation. There is no evidence of distortion of the histoarchitecture in any of the experimental groups (Fig. 7-11).



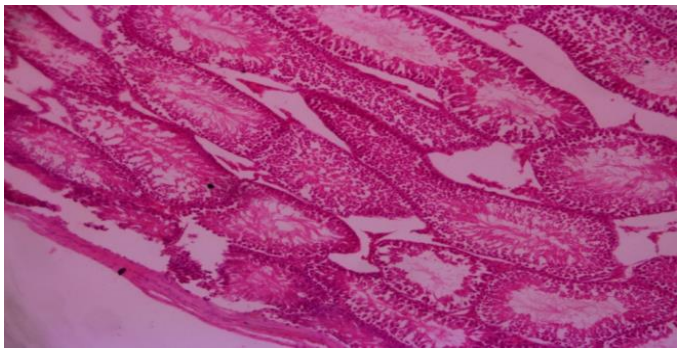


Fig 7 Sections of the Epididymis (Above) and the Testis (Below) of the Representative of the Control Group Showing Epididymis Which Displays Normal Ducts, while the Testis Shows Seminiferous Tubules with Layers of Germ Cells at Various Stages of Maturation.

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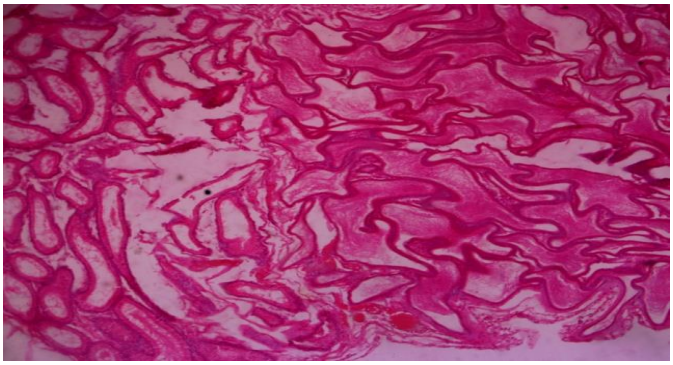


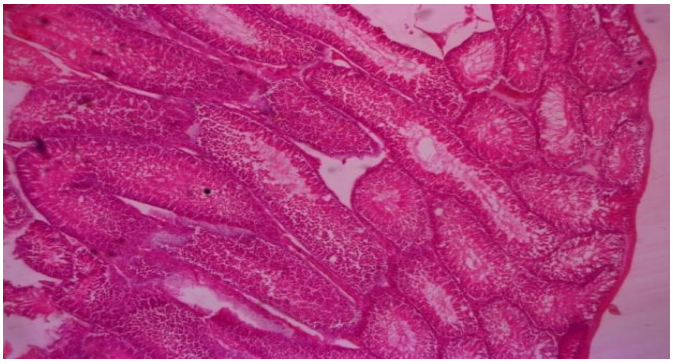
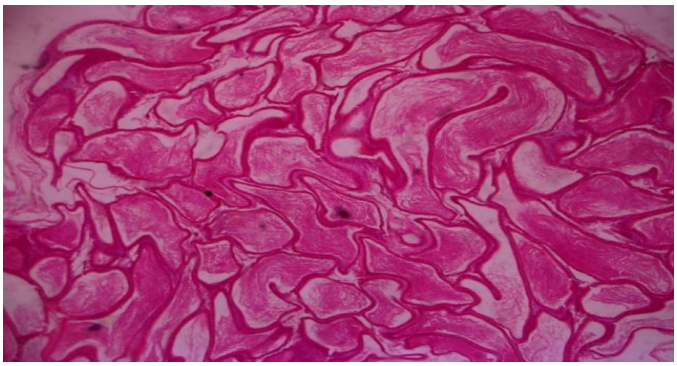


Fig 8 Sections of The Epididymis (Above) and the Testis (Below) of the Group That Received 600mg/Kg BW of Boiled Aqueous Extract 21 Days after Administration Showing Epididymis which Displays Normal Ducts, while the Testis Shows Seminiferous Tubules with Layers of Germ Cells at Various Stages of Maturation.



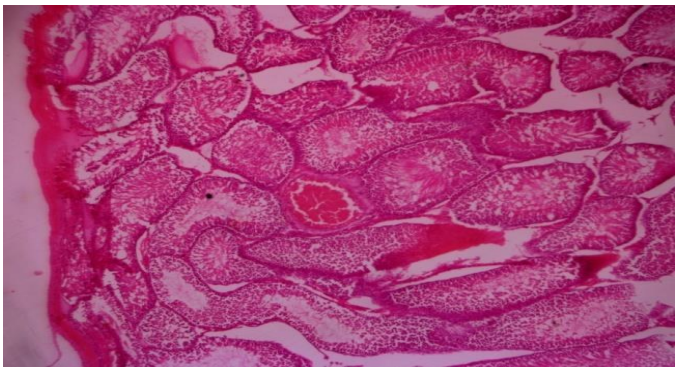
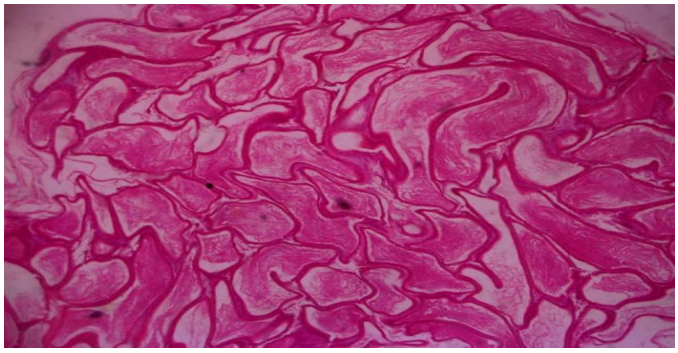


Fig 9 Sections of the Epididymis (Above) and the Testis (Below) of the Group That Received 600mg/Kg BW of Ethanol Extract 21 Days after Administration Showing Epididymis which Displays Normal Ducts, while the Testis Shows Seminiferous Tubules with Layers of Germ Cells at Various Stages of Maturation.



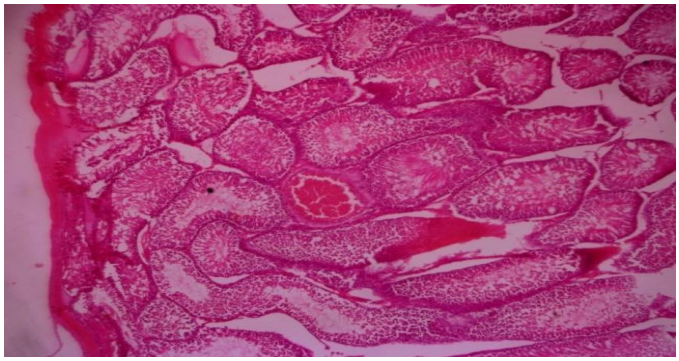
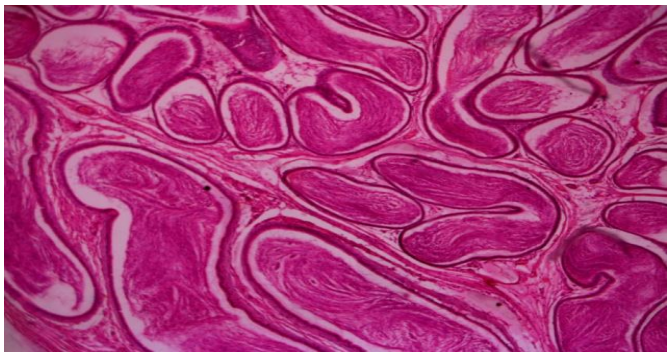


Fig 10 Sections of The Epididymis (Above) and the Testis (Below) of the Group That Received 600mg/Kg BW of Boiled Aqueous Extract 52 Days after Withdrawal Showing Epididymis which Displays Normal Ducts, while the Testis Shows Seminiferous Tubules with Layers of Germ Cells at Various Stages of Maturation.



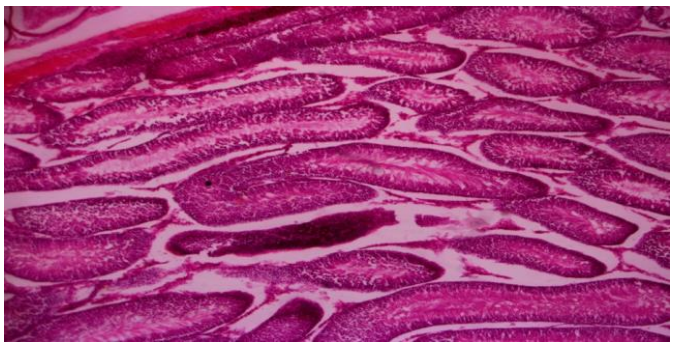


Fig 11 Sections of the Epididymis (Above) and the Testis (Below) of the Group That Received 600mg/Kg BW of Ethanol Extract 52 Days after Withdrawal Showing Epididymis which Displays Normal Ducts, while the Testis Shows Seminiferous Tubules with Layers of Germ Cells at Various Stages of Maturation.

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IV. DISCUSSION

Various phytochemical studies have shown that *Justicia carnea* contains numerous phytochemicals with nutritive, ethnopharmacological and anti-nutritive properties [2], [3] . The GC-MS of both ethanolic and boiled aqueous extracts in this study showed scores of phytochemical components with varying male antifertility potentials. Several phytochemicals present in the GC-MS study of the *Justicia carnea* extracts with documented antifertility potentials in this study include Erucic acid [18] which is present in the boiled aqueous extract, 2,4-ditert-butylphenol [19] present in both boiled aqueous and ethanol extracts. Others like Naphthalene [20], 1,4-dichlorobenzene [21], Dibutyl phthalate [22]–[24], 1H-cyclopenta (1,3)-cyclopropa (1,2)-benzene [25], (E)-5-(benzo[d]-1,3-dioxol-5-yl)-N-isobutylpent-2-enamide [26] are present in the ethanol extract.

The ethanol extract contains six different antifertility compounds as against the two recorded in the boiled aqueous extract. Of these two in boiled aqueous, only erucic acid is not found in the ethanol extract. Evidently, ethanol extract contains many more well-known potent male antifertility factors (phthalates, naphthalene and dichlorobenzene compounds) than the boiled aqueous extract [20]–[22].

There was a non-significant increase in the level of testicular SOD (Superoxide Dismutase) after 21 days of administration of the extracts except for the group that received 400mg/kg BW of boiled aqueous extracts of *Justicia carnea* (AJC) which had a significantly higher level that was reversed 52 days of withdrawal. This finding is similar to the earlier work by [4] but at variance with that by Ukpabi and co-workers [27] who found a decrease in the level of SOD among male Wistar albino rats administered with methanol extracts of *Justicia carnea*. The group that received 400mg/kg BW of ethanol extracts of *Justicia carnea* (EJC) had significantly higher SOD compared to the rest after 52 days of withdrawal. This is in tandem with the work by Chuemere and other researchers who found significantly higher levels of SOD with administration of low doses 200mg/kg BW of hydromethanol extracts of *Justicia carnea* to male Wistar albino rats [3]. Superoxide dismutase converts superoxide into hydrogen peroxide, that is, it is one of the primary antioxidants. The ability to maintain the physiological concentration of superoxide radicals in tissues, made possible by the presence of superoxide dismutase in the human body, enables the survival of the human body in the oxygen environment and the use of oxygen as the final electron acceptor [28]. With myocardial infarction, this enzyme protects the heart muscle from the action of free radicals formed during ischemia (the activity of superoxide dismutase in the blood with myocardial infarction is high). The activity of superoxide dismutase is reduced in patients with weakened immune system, which makes such patients more sensitive to respiratory infections with the development of pneumonia [28]. In other words, the level of tissue superoxide dismutase is directly proportional to the level of oxidative challenge faced by the tissue. However, certain tissues under certain conditions may not be able to mount such a counter-challenge like in weakened immunity and therefore become overwhelmed by oxidative stress.

The observations that decreased seminal plasma scavenger antioxidant capacity, particularly in the form of low SOD activity, can be the cause of male infertility seem to be confirmed by significantly lower SOD activity in seminal plasma of infertile patients compared to healthy sperm donors as well as a positive correlation and positive impact of SOD activity on human semen quality parameters [29].

The variation in the time of manifestation of significant rise in the level of SOD between the group administered with 400mg/kg BW of AJC and 400mg/kg BW of EJC (21 days of administration and 52 days after withdrawal respectively) may be due to the varying pharmacokinetic/ pharmacodynamic properties of the offending agent.

Testicular catalase (CAT) was reduced but not significantly in all the experimental groups experimental groups at 21 days after administration of both extracts. This was equally in agreement with a similar studies [4], [27] where a non-significant reduction in the level of catalase was found. However, this is at variance with another work by [3] where an increase was found. Noteworthy is the fact that Chuemere and co-workers used a very high dose of 1000mg/kg body weight of methanol extract. At 52 days after withdrawal of administration of the extracts, variable changes were noted in the levels of CAT. While there was a non-significant reduction in the levels of SOD amongst most groups, there was a non-significant increase in the group that received 400mg/kg BW of AJC and a significant increase in the group that received 400mg/kg BW of EJC. In humans, catalase breaks down hydrogen peroxide in the tissues, which is necessary for specific cell functions but can also harm DNA. By speeding up the conversion of hydrogen peroxide into oxygen and water, catalase reduces damage. Catalase levels are regulated by a variety of variables, including pH levels, regulatory molecules, cofactors, and compartmentalization [30], [31]. However, rise in the levels of tissue catalase is a response to oxidative insult from a high level of hydrogen peroxide. Different cell types are shielded from the harmful effects of hydrogen peroxide in part by the enzyme catalase (CAT). Idiopathic male infertility is one of the many physiological and pathological problems in humans for which CAT is implicated. The volume, motility, concentration, and morphology of the semen were found to have positive relationships with CAT activity, although sperm DNA integrity did not [32].

Therefore, the significant rise in the level of catalase in the group that received 400mg/kg BW of EJC after 52 days of withdrawal of the extract and not in the other groups is a pointer that the rise in SOD found predominantly in this group may be related to oxidative damage that occasioned the rise in catalase levels. The persistent of this alteration even 52 days after withdrawal is an indication that the damage is long-lasting beyond one life cycle of the rats' spermatozoa.

The administration of the extracts at 400mg/kg BW, 600mg/kg BW of EJC and 600mg/kg BW of AJC caused a significant decrease in the levels of reduced glutathione (GSH), while the administration of 400mg/kg BW of AJC caused a non-significant reduction of GSH after 21 days of administration. These findings are similar to the earlier findings [3], [4], [27] who also found lower levels of GSH compared to the control. At lower doses of the extracts, 200mg/kg BW of AJC caused a non-significant increase while 200mg/kg BW of EJC caused a significant increase in the levels of GSH over the same time frame.

There appears to be a dose-dependent effect in the pattern of oxidative damage that resulted after 21 days of administration of the extracts. While the lower doses of 200mg/kg BW of the extracts appears to improve the levels of GSH significantly with EJC and non-significantly with the AJC, the higher doses impact negatively on the level of GSH. This negative impact is significant with the 400mg/kg BW, 600mg/kg BW of EJC and 600mg/kg BW of AJC but not significant with 400mg/kg BW of AJC.

At 52 days following the withdrawal, the group that received 200mg/kg BW of EJC still had a sustained significantly higher level of GSH, while the group that received 200mg/kg BW AJC still had no significant change in the level of GSH. However, the group that received 400mg/kg BW of AJC and 600mg/kg BW EJC still showed a significantly lower GSH while those that received 400mg/kg BW of EJC and 600mg/kg BW of AJC had a non-significant decrease in the GSH activity 52 days after withdrawal. This is suggestive of a long-lasting impact on the tissue antioxidant system by both extracts of *Justicia carnea*.

Studies on the glutathione (GSH) enzymatic system have revealed its connection to oxidative stress in the ejaculate. Men who are infertile have changed intracellular sperm GSH system components, and these changes appear to be related to sperm morphology [33].

Many of the treatment groups show a significantly higher level of testicular malondialdehyde (MDA) after 21 days of treatment, except the groups that received 200mg/kg BW of AJC and 600mg/kg BW of EJC which showed non-significantly lower levels and unchanged levels respectively. This finding is essentially in agreement with the work done earlier by two independent researchers [4], [34] but at variance with others [3], [27], who found lower levels of MDA amongst male Wistar albino rats treated with 100 to 200mg/kg BW of hydromethanolic and 1000mg/kg BW of methanolic extracts respectively. Incidentally the, group that received 400mg/kg BW of EJC had the least MDA levels at 21 days after administration which is significantly lower than the control and is in keeping with the work by [3], [27].

Most of the changes in the levels of MDA were in reversal at 52 days following withdrawal of the extracts as the 200mg/kg BW, 400mg/kg BW of EJC and 400mg/kg of AJC groups then had non-significantly elevated MDA levels. While the group that received 200mg/kg BW of AJC now had significantly lower levels of MDA, the group that received 600mg/kg BW of EJC and 600mg/kg BW of AJC had non-significantly lower MDA levels. The reason for these variable trends is unknown but may not be unconnected to the different pharmacokinetics/pharmacodynamic properties of the active ingredients of the various extracts.

Studies have shown a significant correlation between seminal MDA levels and abnormal sperm morphology, decreased semen total antioxidant capacity, and weak sperm motility. This suggests that free radicals harm the integrity of sperm membranes. Research has also shown a negative correlation between seminal MDA levels and normal sperm motility and morphology [35].

The overall histology results showed no abnormal findings in any of the groups of either the experimental or the control. This is not in agreement with the findings by [4], who found various degrees of distortion of the histoarchitecture of the testes/epididymis. However, other researchers found that aqueous extracts of *Justicea carnea* ameliorated testicular histological distortion occasioned by chloramphenicol-induced lymphoma [34]. Akintimehin and co-workers also found normal histological features in the liver and kidneys of male Wistar rats administered with ethanol extracts of Justicia carnea in doses of 500mg/kg BW and below. However, they noted variable histological distortions at doses as high as 1,200mg/kg BW of the extracts [36]. Distortion of the histo-architecture of tissues by different extracts of plants is well documented [37]–[39]. The ability of phytochemical extracts to cause distortion of the histological pattern of tissues/testis is largely dependent on the type of phytochemicals present, the dose administered and the duration of exposure. The mechanism of distortion will include the alteration of the anti-oxidant system of the tissues and resultant oxidative injury. It will not be out of place to anticipate that given the tendency to cause oxidative damage by the extracts used in this study, that if the subjects are exposed to higher doses and/or for longer duration, there will be recognizable histological changes.

V. CONCLUSION

Evidence from this and other studies show that boiled aqueous and ethanol extracts of *Justicia carnea* leaves can have a negative impact on the testicular antioxidant system and by extension the male fertility potentials. This is due to the presence of numerous male antifertility phytochemicals in both extracts of *Justicia carnea*, like Erucic acid, 2,4-ditert-butylphenol, Naphthalene, 1,4-dichlorobenzene, Dibutyl phthalate, 1H-cyclopenta (1,3)-cyclopropa (1,2)-benzene, and (E)-5-(benzo[d]-1,3-dioxol-5-yl)-N-isobutylpent-2-enamide. There is need to extract, isolate, characterize and purify these phytochemicals for further *in vitro* and *in vivo* studies. This may help the pharmaceutical industries in the quest for the discovery of an “ideal” male contraceptive. This study may also help to educate those who use these extracts as local herbs for the treatment of illnesses to exercise caution especially if they have need to preserve their fertility.

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