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Effects of Ethanol Leaf Extracts of *Justicia carnea* on Reproductive Function of Male Wistar Albino Rats.

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Abstract:
Background*: Population explosion is the major cause of poverty and environmental degradation in developing countries. The geometric growth in human population has negatively affected the social, economic and technological development ofhuman race. There is need to solve this problem by looking in the direction of controlling the population size through effective, safe, and reversible contraceptives. The burden of contraceptives still lie on women, however there is a growing awareness that men are willing to share this burden. There is still yet no effective chemical contraceptives in use for males, hence the need to search the morass of medicinal plants for possible sources of male contraceptives.*
Materials and Methods*: Twenty male wistar albino rats divided equally into four groups were administered with ethanol leaf extract of Justicia carnea (ELEJC) by gavage at doses of 200, 400, 600 mg/kg BW two times daily (morning and evening) for 21 days.The control group was not treated with the extract. The sperm function parameters, antioxidant enzymes activities were assayed using standard methods. The histology of the testes were also carried out using standard procedures. The proximate compositions and phytochemical analyses were also carried out using standard methods.*
Results*: The phytoconstituents that are most abundant in ELEJC are phenols (13.8 ± 0.02 mgGAE/g) and flavonoid (12.12± 0.29 mg/g) others are oxalate (2.97 ± 0.03 mg/g), saponins (0.55 ± 0.09 %), phytate (0.01 ± 0.01 %), tannin (0.9 ±0.01 mg/g), and alkloids (0.28 ± 0.03 %). The proximate of J. carnea leaves include carbohydrate (63.45 ± 0.46%), protein (14.66 ± 0.46 %), ash (5.48 ± 0.06%), crude fibre (4.03 ± 0.02%), moisture (10.19 ± 0.01%), and fat (2.22 ± 0.14%)*
ELEJC caused a significant decrease ($P < 0.05$) in testes weight of rats that received 200 mg/kg BW. ELEJC induced the highest decrease in sperm count and testosterone levels in rats that received the 200 mg/kg BW of the extract. There was no statistical difference ($P < 0.05$) between the live – dead ratios, epididymis weight, and sperm motility of the rats that received 200, 400, and 600 mg/kg BW of ELEJC when compared to the control.The total defects (head + tail) observed in each of the experimental groups were lower than 12%.There was marked increase in malondialdehyde levels and decrease in antioxidant enzyme activities in rats that received 400, and 600 mg/kg BW of ELEJC. The group treated with 200 mg/kg BW of ELEJC was able to rein in oxidative stress as shown by the increased antioxidant enzyme activities, and with lower MDA level than other experimental groups. The histology of rats' testes showed ELEJC caused significant alteration in normal historachitecture of the testes of experimental group when compared to the control.
Conclusion: *In this study, ethanol leaf extracts of Justicia carnea has been shown be rich in pharmacologically bioactive compounds (flavonoids, and phenols) and nutrients. ELEJC has an antioxidant activities at low doses (200 mg/kg BW), but induces the opposite effect at higher doses (400 & 600 mg/kg BW) which could be due to its high iron content.. ELEJC causes a reduction in performance of most sperm dynamics and alteration of normal histoarchitecture of the testes of experimental rats, more so at 200 mg/kg BW. This suggests its antispermatogenic activities and its possible contaceptive potentials.*
Key Words*: (Justicia carnea, antispermatogenic, antioxidant enzymes, sperm count, sperm motility, live - dead ratio, testosterone, overpopulation, malondialdehyde, phytoconstituents, proximate)*

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I. Introduction

Overpopulation has created a serious setback in the growth of economy and human development in developing countries. Population explosion is the major cause of poverty and environmental degradation in developing countries. The geometric growth in human population has negatively affected the social, economic and technological development ofhuman race. Therefore to control our number, has to be the best approach to tackle imminent danger of population explosion[5]. Current population explosion demands an immediate discovery of new potential contraceptives. Though significant progress has been made in the search of highly

effective, safe and reversible methods of contraception among females, similar progress and possibilities on males are still slow and limited. With recent interest towards a better understanding of male reproductive physiology, there is a need to develop new contraceptive modalities for male[6]. Therefore in the developing nations such as Nigeria it is imperative to search among its abundant vegetation for plants that can serve as male contraceptives or sources for lead compounds for its synthesis.

Justicia carnea belongs to the family of Acanthaceae, which consists of about 600 species of shrubs, herbs, and tender perennial commonly found in the tropics and subtropics [1]. Its common names in Brazil and South America include: Brazilian plume, flamingo flower, jacobinia, pine-bur begonia, pink jacobinia, pink tongues, king's crown and cardinal's guard [2]. *Justicia carnea* is widely known as an ornamental plant [3]. Several species of *Justicia* are widely used in folk medicine for the treatment of inflammation, respiratory, and gastrointestinal disorder [1]. Today, there is a growing trend in the use of medicinal plants, or herbal preparations most especially in developing countries where these products are readily available. There are many herbs which are mainly used to treat numerous ailments. Because of their therapeutic effects, they can be used as drugs or supplements in the treatment and management of various diseases [4]. Herbal drugs or medicinal plants have shown a wide range of biological activities, and hence are used and will continue to be used as medicine or food supplements to alleviate various ailments and disorder

Fig 1 : picture of justicia carnea

GC – MS analysis of ethanol leaf extracts of *J. carnea* showed the presence of six phytocompounds – isonicotinic acid N- oxide (2.55 %), phosphoinodithioc acid, diphenyl (1.73 %), hexadecanoic acid (10.5 %), 2,2,3,3,5,5,5 – nonafuoro – pentanoic acid methyl ester (73.19 %), 9,12,15 – octadecatrien- 1 – ol (9.33%), and 7H – purine, 7 – benzyl – 2,6 dichloro (2.45 %)[23]. Isonicotinic acid is known to inhibit nitric oxide synthase and down-regulate androgen [7]. Nitric oxide (NO) helps to enhance sexual performance[51]. Hexadecanoic acid, 2,2,3,3,5,5,5 – nonafuoro – pentanoic acid methyl ester, and phosphoinodithioc acid, diphenyl are known as inhibitors of arachidonic acid synthesis [7]. Arachidonic acid liberation has been shown to reverse cortisol – mediated suppression of GnRH – induced secretion of luteinizing hormone[8]. Sertoli cell produces prostaglandin in response to follicle stimulating hormone; this implicates a potent role of arachidonic acid in spermatogenesis [9]. Arachidonic acid is a regulator of steroidogenesis in the leydig cells and it is a component of sperm plasma membrane among other PUFAs [10, 11]. Prostaglandin receptors have been shown to be present in leydig cells, sertoli cells and seminiferous tubule wall . Dp prostanoid receptors have been found in germ cells of fetal mouse testis, where as functional PPARγ and PGE receptors have been found in sperm[52]. Arachidonic acid (AA), a compound secreted by sertoli cells in FSH - dependent manner is able to induce the release of Ca from internal stores in round spermatids and pachytene spermatocytes . It has been reported that AA acts by interacting with a fatty acid G protein coupled receptor, initiating a G protein signalling cascade that may involve PLA2 &ERK activation which in turn opens intacellular ryanodine - sensitive channels and also NAADP - sensitive channels in acidic intracellular Ca stores in rat [52] . Aristolochic acid, an inhibitor of PLA2 significantly decreased the AA - induced changes in [$ca2^{+}$]i in round spermatids. Thus the effects of AA could involve a positive feed back loop, releasing AA or other PUFAs in round spermatids via the activation of PLA2. It has been reported that Orai1 deficient male mice are sterile and have defects in spermatogenesis in mid - to late - stage elongating spermatids development. The none - store - operated subunit of Orai1 has been shown to be regulated by AA. This channel is activated downstream of phospholipase C, suggesting a signalling mechanism involving G-protein coupled receptors [55]

7H – purine, 7 – benzyl – 2, 6 dichloro (found in ELEJC) is known to inhibit the activities of 11β–HSD, 17β – HSD, and 5 –HT [7]. The up-regulation of 11β –HSD expression, is suggested in positive feedback control of 11β – Ketotestosterone production and modulation of cortisol level to protect testes from excess circulating cortisol [12]. Also inhibitors of 11β – HSD 1 & 2, cause elevated glucocorticoids, and hence decrease in testosterone in testis [50]. Inhibitor of 17β- HSD causes decrease in testosterone levels in the testes [13]. Whereas

5- HT, at normal levels has been shown to improve sexual function,sperm motility and morphology, therefore its inhibitor causes a negative effect on fertility [14].

The prostanoid receptors DP and FP have been described in both hamster and human leydig cells. While PGD has stimulatory effect on basal testosterone production in hamster leydig cells, PGF exerts an inhibitory effect on the expression of StAR protein and 17 β - hydroxysteroid dehydeodenase enzyme, as well as on the synthesis of testosterone induced by LH/hCG.It stands to reason that at least in hamster leydig cells, there exists a regulating loop in which testosterone induces COX2 expression and PGF production, in turn PGF inhibits StAR and HSD 17β expression and consequently, testosterone production, thereby setting a brake on testicular steroidogenesis. These cross talks between between testosterone biosynthetic pathway and COX - 2 - initiated pathway could be exploited as molecular targets of male contraceptives [56] .

It is interesting to state that 7H – purine, 7 – benzyl – 2, 6 dichloro has some similarities with adjudin, with both sharing same dichloro phenyl functional group. Adjudin is being explored as possible non - hormonal male contraceptives; it has been shown that it induced 100% infertility in male mice, and acts through the disruption of apical ectoplasmic specialisation in the sertoli cells thereby inducing premature spermiation [57].

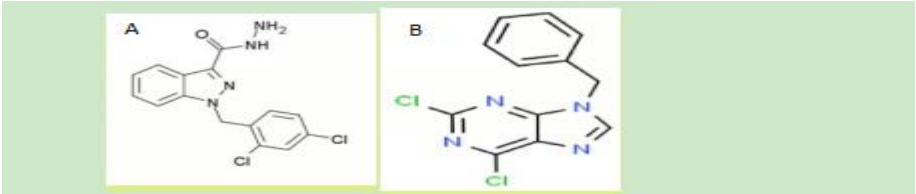


Fig 2 : Structures of adjudin (A) and 7H – purine, 7 – benzyl – 2, 6 dichloro (B)

This similarity between 7H – purine, 7 – benzyl – 2, 6 dichloro (found in ELEJC) and adjudin, coupled with the preponderance of arachidonic acid inhibitors, and appreciable amounts of testosterone synthesis inhibitors in ELEJC point to its potential fertility regulatory effects. Therefore this study was undertaken to investigate the effects of ethanol leaf extract of *Justicia carnea*(ELEJC*)* on reproductive function of male wistar albino rats

II. Material And Methods

Twenty adult male wistar albino rats (12 - 14 weeks) of average weight of 100 – 160g were used for this study. All the animals used were obtained from the Chris Animal house, Mgbakwu, Anambra, Nigeria. The rats were acclimatized for 7 days under 12 h dark/light cycle, fed standard grower feed, and allowed free access to drinking water *ad libitum.*

Study Design: *Invitro* and *invivo* study

Plant Materials: *Justicia carnea* leaves used in this study were collected from Ifite - Awka in Anambra State and authenticated at Department of Botany, Nnamdi Azikiwe University, Awka.

Preparation of Ethanolic Extract*: Justicia carnea* leaves were handpicked and dried under shade for 7 daysand milled to coarse powder using a hand mill. The 1 kg leaf powder was soaked in 70 % ethanol (10L) for 48h, filtered using No 1 What man filter paper and concentrated in water bath at 40°C according to the method of Jensen [15].

Study Location: This study was done in the Department of Applied Biochemistry, at Prof S.C Udedi's Research and Development Laboratory

Study Duration: May 2019 to June 2019.
Subjects &Treatment method: Twenty adult male Wistar albino rats (12-14 weeks) of average weight of 100 – 160g were randomly divided into four groups of five rats each. Group I (Control): rats were fed with normal diet, water and administered 0.5ml of water.
Group II: rats were administered with low dose (200mg/kg BW) of the leaf extract.
Group III: rats were administered with medium dose (400mg/kg BW) of the leaf extract.
Group IV: rats were administered with high dose (600mg/kg BW) of the leaf extract.
The extract was administered to the rats orally with gavage tube two times daily (morning and evening) for 21 days.

Sacrificing and Samples Collection: The animals were sacrificed under mild anasthesia with diethyl ether, twenty four hours after the last treatment. Blood was collected via cardiac puncture into bottles containing anticoagulant and mixed gently. Blood sample was centrifuged at 3000 rpm for 10 min. The plasma was carefully transferred into clean vial bottles and kept frozen at -4°C for testosterone assay. For serum preparation , blood was collected from the heart of the animals into plain centrifuge tubes and was allowed to stand for 1 hour. Serum was prepared by centrifugation at 3,000 g for 15 minutes in a Beckman bench centrifuge. The serum was used for the enzymes and MDA assays. All the readings were noted spectrophotometrically at the specified O.D..

Procedure methodology

Proximate analysis

Moisture, ash and fibre content of the sample were determined by method of Association of Official and Analytical chemists (AOAC) [16]. Crude protein was determined by micro Kjedalh method. Crude fat content was analysed by soxhlet method. The total percentage carbohydrate content was determined by difference of 100 as reported by Yerima and Adamu [17].

Phytochemicals analysis

The saponin content was determined using the method of Obadoni and Ochuko [18]. The flavonoid content was determined by the using the slightly modified colorimetry method described by Barros *et al.* [19]. The cyanogenic glycoside content of the samples was determined by the alkaline titration method of the AOAC [16]. Tannin content was determined according to method of AOAC [16]. Oxalate was determined according to Osagie [20]. The phytate content was determined using the method of Young and Greaves [21]. The total phenol content of the samples was determined using the method of Barros *et al.*[22]. Alkaloid was determined as described by Harborne [22].

Sperm function analysis

Sperm count and motility determination was carried according to the method of Pant and Srivastava [24]. Sperm morphology and live -dead ration were determined according to method of Wells and Awa [25].An enzyme based immunoassay system was employed to determine testosterone according to the method described by Ikpeme *et al.* [30]. The histology of the rats'testes was carried out according as described by Akpantah et al. [31]

Antioxidant enzymes and MDA estimation

SOD activity was assayed according to the method described by Sun and Zigma [26] .Catalase activity was assayed according to the method described by Aebi [27]. Glutathione peroxidase activity was assayed according to the method described by Flohe and Gunzler [28] .The level of LPO was measured in the blood serum using malondialdehyde (MDA) concentration as a surrogate measure according to method of Ohkawa *et al* [29]

Statistical analysis

Data were expressed as mean ± SEM. Differences between the mean values of the control and treatment groups were determined by One-way Analysis of Variance with Dunnettpost hoc test using the Graph Pad Prism 5. Significant difference was considered if $p < 0.05$.

III. Result

Quantitative Phytochemical Constituents of Ethanol Leaf Extract of *Justicia carnea.*

Table 1 shows that phenols and flavonoids were the dominant phytochemicals in the ethanol leaf extract of *Justicia carnea* having values of 13.8 ± 0.02mgGAE/g and 12.12 ± 0.29 mg/g respectively. Cyanogenic glycosides were not detected; oxalate was present in moderate concentration while saponin, tannins, alkaloids and phytate were present in low concentrations.

Table no 1: Shows quantitative phytochemical constituents of ethanol leaf extract of *Justicia carnea.*

Phytochemicals	Flavonoids (	Phytate	Saponins	Tannins	Oxalate	Phenols(mgGAE/g)	Alkaloids
Mean ± SEM	12.12 ± 0.29	0.10 ±	0.55 ± 0.09	0.9 ± 0.01	2.97 ± 0.30	13 .8 ± 0.02	0.28 ± 0.03

Sem = standard error of means (Each value is expressed as mean ± standard error of mean (n = 2))

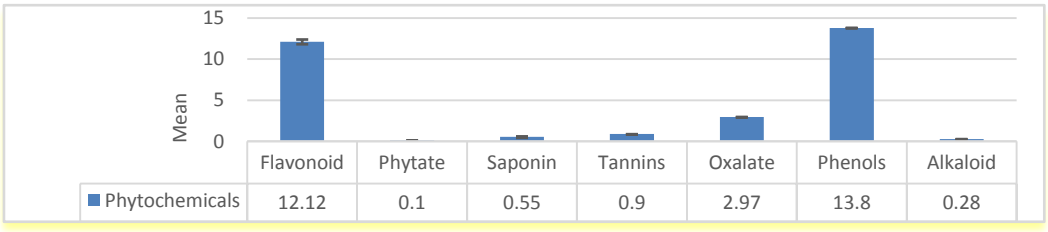


Table 2 shows that ethanol leaf extract of *Justicia carnea* had high carbohydrate content. Protein and moisture contents were moderate while fat, ash and crude fibre contents were low.

Table no2 : Records proximate composition of *justicia carnea* leaves**.**

Proximate Composition	Moisture (%)	Fats (%)	Ash (%)	Crude fibre (%)	Protein (%)	Carbohydrate (%)
Mean ±SEM	10.19±0.01	2.22 ± 0.14	5.48 ± 0.06	4.03 ± 0.02	14.66±0.46	63.45 ± 0.46

Sem = standard error of means (Each value is expressed as mean ± standard error of mean (n = 2))

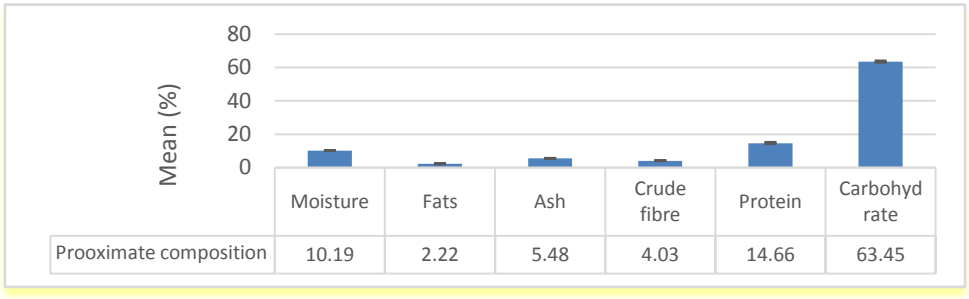


Table 3 shows that there was significant decrease in the weights of animals treated with 200 mg/kg BW of the extract; whereas there was insignificant increase in the weights of animals treated with 400 mg/kg, and insignificant decrease in weights of animals treated with 600 mg/kg BW of the extract when compared to the control, after 21 days administration of ELEJC.

Table no 3 : Shows body weights of the control and experimental groups

Week no	Group 1	Group 2	Group 3	Group 4
0	155.38 ±16.40	138.02 ± 27.07	102.62 ± 15.73	143.55 ± 6.99
1	168.18 ± 25.44	92.03 ± 19.17*	128.67 ± 18.73	146.30 ±10.58
2	173.36 ± 20.56	98.90 ± 18.10*	98.90 ± 18.10*	161.56 ± 9.85**
3	182.10 ± 18.07	98.46 ± 17.32*	143.46 ± 18.76	135.10 ± 9.85

*Datapresentedasmean±SEM. *P < 0.05 (compared to control); **P≤0.05 (compared to 200mg/kg)*

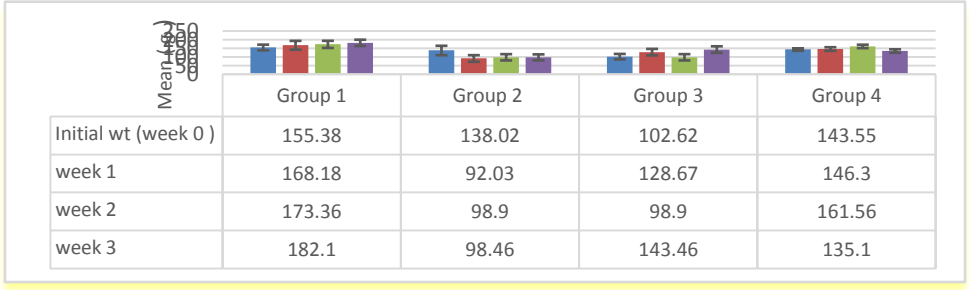


Table 4 below shows that there was a significant decrease in the testes weights of rats treated with 200 mg/kg BW of the extract when compared to the control, whereas there were no significant differences between the testes weights of rats treated with 400 mg/kg, and 600 mg/kg BW of the extract when compared to the testes weight of the control rats. There was no significant difference between the epididymis weight values and testes body weight ratios of all the test groups, although the rats treated with 200 mg/kg BW presented the least epididymis weight value and testes body weight ratio.

Table 4: Effect of ethanol leaf extract of *justicia carnea* on testes weight, epididymis, and testes –body weight ratio

	Group 1	Group 2	Group 3	Group 4
Testes weight (g)	2.13±0.11	0.96±0.26*	2.24±0.29	2.66 ±0.09
Epididymis weight (g)	0.66 ±0.14	0.26 ±0.05	0.51±0.25	0.25±0.02
Testes - body weight ratio	0.0106 ±0.00055	0.0095±0.000955	0.0156±1.5E-05	0.0197±0.000775

*Datapresentedasmean±SEM. *P < 0.05 (compared to control)*

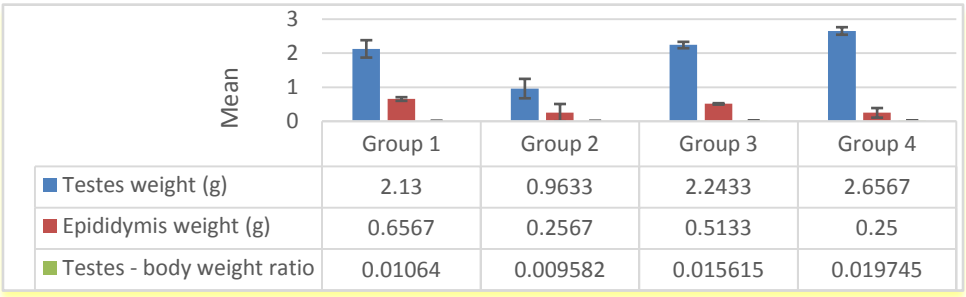


Table 5 shows that there was no significant difference in the sperm count and live –dead ratio values of the experimental groups and that of the control, although the group 2 (200 mg/kg BW) had the least values. There was a significant increase (P < 0.05) in head defects, and a significant decrease in tail defects in the experimental groups as compared to the control group. There was no significant difference (P < 0.05) between sperm motility values in all the experimental groups and when compared to the control group.There was significant increase in percentage normal sperm in group 2 and 3 rats when compared to the control, where as there was no statistical difference between percentage normal sperm values of group 4 and group 1 (control) rats.

Table no 5 : Shows effects of ethanol leaf extract of *justicia carnea* on sperm function

	Sperm count (X 10^6 cells /ml)	Live -dead ratio ± Sem	Sperm motility	Head defect	Tail defects ± Sem	Normal sperm ± Sem
Group 1	72.75± 8.3	3.2±0.05	55 ± 2.87	2.64±0.37	9.23±0.09	88.13±0.46
Group 2	65.20±2.5	2.83±0.07	54 ± 1.88	2.83±0.59*	6.5±0.31*	91.17±0.22*
Group 3	71.68±1.2	3.19±0.14	57 ± 0.81	3.24±0.19*	4.95±0.25*	91.815±0.065*
Group 4	71.87±0.03	3.17±0.05	56 ± 0.002	2.87±0.36*	7.75±0.35*	89.38±0.01

***P< 0.05 (compared to control)**

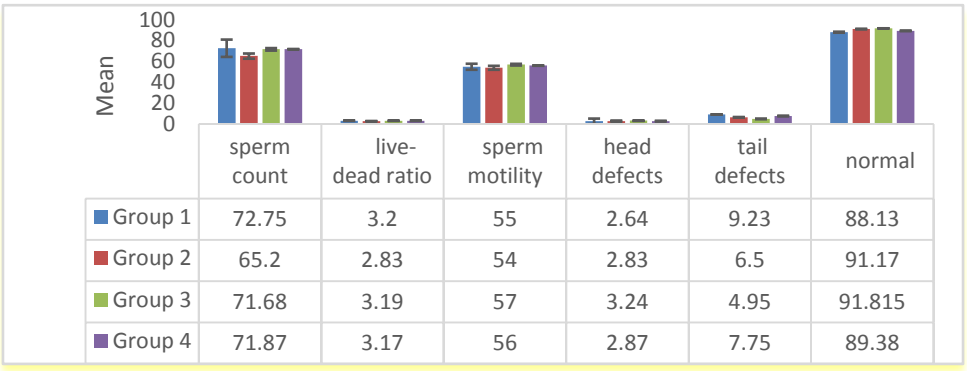


Table no 6 shows that there was a decrease in the testosterone levels of the rats in the experimental groups when compared to those in the control, although it was not statistically significant ($P< 0.05$). The rats treated with the 200 mg/kg BW of the extract presented with the least level of testosterone

Table no 6 : Shows effects of ethanol leaf extract of *justicia carnea* on testosterone levels

	Group 1	Group 2	Group 3	Group 4
Testosterone conc (ng/ml)	1.174±0.20	0.674±0.10	0.806±0.15	0.788±0.11

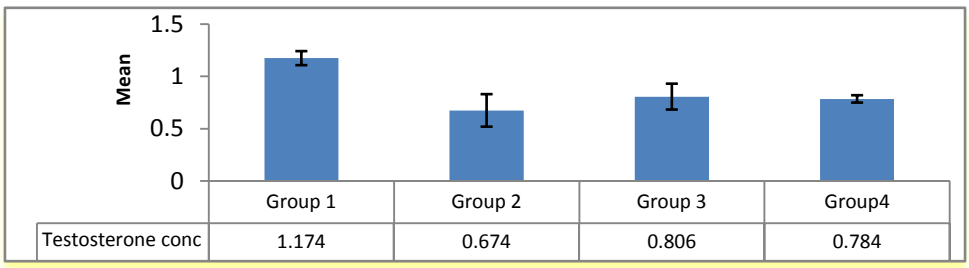
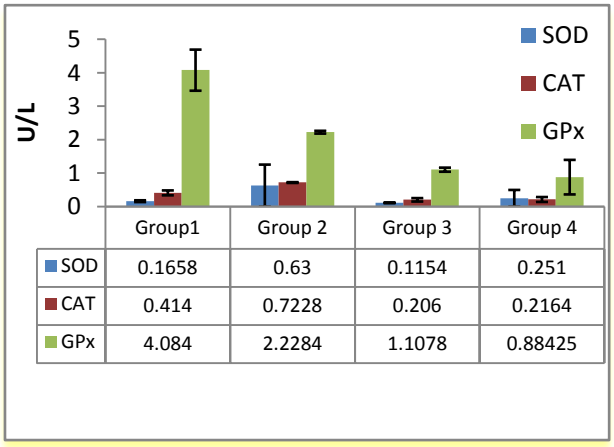


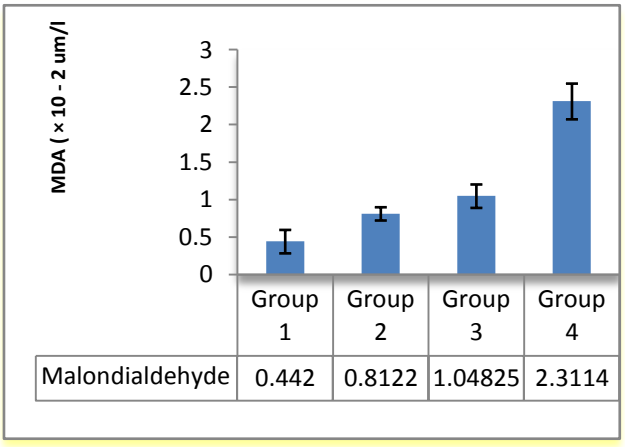
Table 7 shows that there was no significant difference between the SOD activities of the experimental groups when compared to that of the control; although rats in control group, and group 2 (200 mg/kg) showed higher SOD activities than the rest of the groups. There was a decrease in catalase activities in rats treated with 400 mg/kg, and 600 mg/kg BW of the extract when compared to the control, although this decrease was not statistically significant. The catalase activity of rats treated with 200 mg/kg (BW) of the extract was slightly higher than that of the control, it was however not statistically significant. Therewas a dose dependent decreasein glutathione peroxidase activities, with the activities of the enzyme decreasing as the dose increases. Although this decrease was not statistically significant when compared to the control. The malondialdehyde increased in a dose - dependent manner.

Table no 7 : Shows effects of ethanol leaf extract of *justicia carnea* on antioxidant enzymes activities and malondialdehyde levels.

	SOD	CAT	GPx	MDA
Group 1	0.17 ±0.08	0.41±0.07	4.08±1.71	0.44±0.18
Group 2	0.63±0.40	0.72±1.27	2.23 ±2.71	0.81±0.75
Group 3	0.12 ±0.05	0.21 ±0.06	1.11 ±0.29	1.05 ±0.22
Group 4	0.25±0.22	0.22 ±0.12	0.89 ±0.30	2.31±0.72



	Group1	Group 2	Group 3	Group 4
SOD	0.1658	0.63	0.1154	0.251
CAT	0.414	0.7228	0.206	0.2164
GPx	4.084	2.2284	1.1078	0.88425



	Group 1	Group 2	Group 3	Group 4
Malondialdehyde	0.442	0.8122	1.04825	2.3114

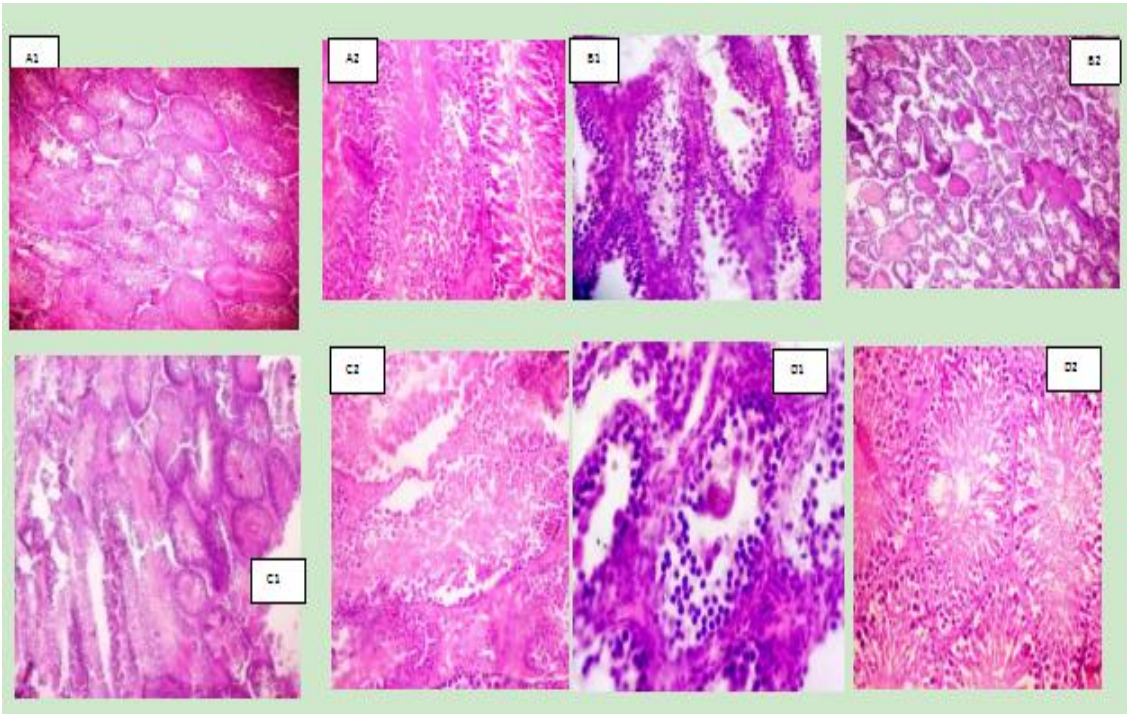


Figure 3 : shows the histology of the testes in experimental and control rats

A1 & A2 show the normal histoarchitecture and spermatogenesis in the testes of control rats. B1 & B2 show spermatogenic arrest, empty lumen, depletion of leydig cells, and absence of elongated spermatids in the testes of rats that received 200 mg/kg BW (group 2) of ELEJC. C1 & C2 show sloughing and degeneration of

germ cells, seminiferous tubules, edema, vacuolisation of leydig cells and hypospermatogenesis in a few seminiferous tubules in the testes of rats that received 400 mg/kg BW (group 3) . D1 & D2 shows hypospermatogenesis, presence of sloughed dead cells in the lumen of rat that received 600 mg/kg BW (group 4)

IV. Discussion

Plantshave playedamajor role inmaintaininghumanhealthandimprovingthe qualityofhuman life forthousandsofyearsandthis is attributed to their nutraceutical potentials[32]. The nutritive potentials of leaves of *J. carnea* were studied. Proximate analysis of the *J. carnea* leaves showed that it contains protein, fiber, ash, fats/oil as well ascarbohydrate as shown in table 2. Plantproteins are sources of nutrientespecially for people in developing countries such as Nigeria. Fibredetoxifies the digestive system and precludesthe absorption of excess cholesterol. The ashcontent suggests the availability of mineral elements in the leaves. The plant is a good source of carbohydrate when consumed because it meets the recommended dietary allowance (RDA) values [33]. The plant is also a good source of protein because it provides more than 12% of caloric value from protein [34]. Dietary fat improves the organoleptic quality of food by absorbing and retaining flavours[35]. A diet that provides 1 - 2% of its caloric of energy as fat is good enough for humans as excess fat consumption is implicated in certain cardiovascular disorders [34].The high levels of flavonoids and phenols observed in this study shows richness of ethanol extract of *J. carnea* leaves in important pharmacologically active bio-constituents that could promote quality human health. The phytoconstituents in the extract suggests their fertility regulatory potentials, as a number of plant metabolites – alkaloids, saponins, flavonoids and phenolic acids – have been shown to exert fertility regulatory effect[36, 37, 38, 39]. Ethanol leaf extract of *Justicia carnea* was also studied to investigate its effects on body weight and reproductive organs of male wistar albino rats.At the onset of the experiment the mean body weight was 155.38 ±16.40 g, while just prior to their sacrifice, it was 182.10 ± 18.07 g in the control rats. The increase in the body weight in these animals was not statistically significant. The mean body weights of animals in group 2 (200mg/kg) , group 3 (400mg/kg) , and group 4 (600mg/kg) were 138.02 ± 27.07 g , 102.62 ± 15.73 g , 143.55 ± 6.99 g respectively, at the beginning of the experiment; and after 21 days were 98.46 ± 17.32 g , 143.46 ± 18.76 g , 135.10 ± 9.85 g respectively. The results showed that there was significant decrease($P \leq 0.05$)in body weights of animals treated with 200 mg/kg of the extracts as compared to the control. However there was no significant difference ($P< 0.05$) between the mean body weights of the group 1 (control) rats and the mean body weights of group 3 (400mg/kg) and group 4 (600 mg/kg) rats after 21 days of treatment. The decrease in body weight, at 200 mg/kg BW , suggests the anti-androgenic effects of ELEJC, as testosterone has anabolic effect; hence decrease in testosterone level, as observed in rats that received 200 mg/kg BW of ELEJC , could have negative effects on body weight. The improvement in body weights at medium and higher doses of the extract could be due to improvement in testosterone at these doses. Normal testis weight varies only modestly within a given test species [40]. This relatively low inter animal variability suggests that absolute testis weight should be a precise indicator of gonadal injury. However, damage to the testes may be detected as weight change only at doses higher than those required to produce significant effects in other measures of gonadal status [41]. This contradiction may arise from several factors, including a delay before cell deaths are reflected in a weight decrease (due to preceding edema and inflammation, cellular infiltration or leydig cell hyperplasia). The effect of ethanol leaf extract of *Justicia carnea* on reproductive organ weights of wistar albino rats showed that there was significant decrease in testes weight of rats treated with 200 mg/kg BW as compared to the control rats and other experimental groups (600mg/kg & 400mg/kg). The slight increase in testes weights of rats treated with 400mg/kg and 600 mg/kg BW was not significant as compared to the control. This slight increase in testes weight observed in group 3 and 4 could be due to blockage of the efferent ducts by cells sloughed from germinal epithelium or the efferent ducts themselves which led to an increase in testes weight due to fluid accumulation (which is in tandem with the edema observed in the testes of group 3 (400mg/kg) and group 4 (600 mg/kg) rats [42], an effect that can offset depletion of germinal epithelium on testis weight.Thus while testis weight measurements may not reflect certain adverse testicular effects and do not indicate the nature of an effect, a significant increase or decrease is indicative of an adverse effect[43]. The difference between the testes – body weight ratio of the rats in control groups and rats in all the experimental groups was not statistically significant, although that of the group 2 (200mg/kg BW) showed the least value. The rats treated with 200 mg/kg and 600 mg/kg BW showed the highest decrease in epididymis weight as compared to the control rats, but the decrease in weight was not significant. The rats treated with 400mg/kg BW showed slightly higher epididymis weight value than that of the control rats, the difference is not statistically significant.Effect of ethanol leaf extract of *Justicia carnea* on sperm dynamics of male wistar albino rats after 21 days treatment showed the highest reduction in sperm count in the rats treated with 200mg/kg BW as compared to control groups and other experimental groups. The sperm counts of rats treated with 400mg/kg and 600 mg/kg BW albeit still lower showed sperm count values closer to that of the control groups. However the

difference between sperm count values of both the control and experimental groups was not statistically significant. This result suggests that ELEJC can reduce sperm count at 200 mg/kg BW. The rats treated with the 200 mg/kg BW showed the least live – dead ratio value. The sperm live – dead ratio values of rats treated with 400mg/kg and 600 mg/kg BW though lower than that of control group can approximate to it. However the difference between live – dead ratio values of all the groups was not statistically significant. The effect of ethanol leaf extract of *Justicia carnea* on sperm morphology showed that the head defects in experimental groups were higher than head defects observed in control group. The head defect was highest in rats treated with 400mg/kg BW. There was significant increase in head defects in all experimental animals compared to the control. The tail defect was highest in control group. There was significant decrease in tail defects in all experimental groups as compared to control. However the percentage total defects in each of the groups is not up to 12 %, showing the extract has no much deleterious effect on sperm cells [44]. There was no significant difference ($P< 0.05$) between the percentage sperm motility values of the control and experimental groups.Spermatogenesis and male infertility are dependent upon presence of testosterone in the testes. In the absence of testosterone or androgen receptor, spermatogenesis does not proceed beyond meiosis stage. The major cellular target and translator of testosterone signals to developing germ cells is the sertoli cell. In the sertoli cell, testosterone signals can be translated directly to changes in gene expression or testosterone can activate kinases that may regulate processes required to maintain spermatogenesis[45]. The effect of ethanol leaf extract of Justicia carnea on testosterone concentration showed that the extract induced a decrease in testosterone levels in all the experimental groups (200 mg/kg, 400 mg/kg, 600 mg/kg BW) as compared to control, but the decrease in testosterone was not significant. The testosterone concentration was least in group 2 (200 mg/kg). The seemingly improved testosterone levels and some sperm dynamics at 400 and 600 mg/kg BW , albeit still diminished in performance than the control group, show that there could be an interplay between testosterone pathway and COX 2 - initiated signalling cascade , more so, when ELEJC contains large amounts of AA synthesis inhibitors and appreciable amounts of testosterone inhibitors. It has been reported of a regulatory loop in which testosterone stimulates COX 2 expression, and hence PGD_2 (which maintains testosterone levels at basal conditions) and PGF_2 alpha (which in turn down - regulates testosterone levels through inhibition of 17 β HSD and StAR protein). Therefore with increase in doses of ELEJC (whose arachidonic acid synthesis inhibitors constituents outstripes the testosterone (17 β HSD) inhibitors constituents) there will be lesser fluxes through COX 2 - initiated pathway, leading to diminished levels of PGF_2 alpha and hence relaxation of the brakes mounted on testosterone biosynthetic pathway; this could account for the improved testosterone levels at higher doses of ELEJC than 200 mg/kg BW. Superoxide dismutase, catalase and glutathione peroxidase are the first line defense antioxidants. They play indispensable role in antioxidant protective capacity of biological systems against free radical attack. SOD is the first detoxification enzyme and most powerful antioxidant in the cell. It catalyzes the dismutation of two molecules of superoxide anion to hydrogen peroxide and molecular oxygen, consequently rendering the potentially harmful superoxide anion less hazardous. Catalase catalyzes the degradation or reduction of hydrogen peroxide to water and molecular oxygen, consequently completing the detoxification process initiated by SOD. It is located primarily in peroxisomes but absent in mitochondria of mammalian cells. This implies that the breakdown of hydrogen peroxide to water and oxygen is carried out by another enzyme known as glutathione peroxidase in mammalian mitochondria. Glutathione peroxidase is an important intracellular enzyme that breaks down hydrogen peroxidase to water; and lipid peroxides to their corresponding alcohols mainly in the mitochondria and sometimes cytosol[46]. The effect of ethanol leaf extract on antioxidant enzymes in male wistar albino rats showed that superoxide dismutase activity in group 2 (200 mg/kg BW and group 4 (600mg/kg BW) was higher than that of group 1 (control) and group 3 (400mg/kg) .Whereas SOD activity is highest in group 2 (200mg/kg BW). it is lowest in group 3. The result showed that catalase activity in group 1 (control) and group 2 (200 mg/kg BW) was higher than that of group 3 (400 mg/kg BW) and group 4 (400 mg/kg BW) whereas catalase activity was highest in group 2 (200 mg/kg BW) it is lowest in group 3 (400 mg/kg BW). The results showed that the glutathione peroxidase activity was highest in group 1 (control) and group 2 (200 mg/kg BW) than in group 3 (400 mg/kg) and group 6 (600 mg/kg BW). While the glutathione peroxidase activity is highest in group 1 (control), it is lowest in group 4 (600 mg/kg BW). The Malondialdehyde (MDA) concentration in group 3 (400 mg/kg BW) and group 4 (600 mg/kg BW) was higher than MDA concentration in group 1 (control) and group 2 (200 mg/kg BW). While group 4 (600 mg/kg) has the highest MDA levels group 1 (control) has the lowest MDA levels. This result showed that the ELEJC could cause increase in oxidative stress at higher doses, but could help mop up reactive oxygen species at low dose of 200 mg/kg BW and more likely at a dose lower than 200 mg/kg BW. While group 2 (200 mg/kg) rats responded positively to the oxidative stress in order to rein it in , the antioxidant first line of defense in group 3 (400 mg/kg) and group 4 (600 mg/kg) rats seemed overwhelmed by ELEJC, which is evident in the increased MDA levels observed in these groups. This increase in MDA levels at 400 mg/kg and 600 mg/kg BW doses could be due to the presence of iron (Fe) in the extract, as ELEJC has been shown to have high iron content [46]. Iron, which though maintains proper cell function, could also be a pro-

oxidant when administered at high doses [48, 49] .The ethanol leaf extract of *Justicia carnea* induced significant histological changes in the testes of male wistar albino rats. The group 2 (200 mg/kg BW) rats showed spermatogenic arrest decreased height of germinal epithelium, empty lumen, depletion of leydig cells, and absence of elongated spermatids . This could be caused as a result of lower levels of testosterone observed. The group 3 (400mg/kg BW) showed show sloughing and degeneration of germ cells, seminiferous tubules, edema, vacuolisation of leydig cells and hypospermatogenesis in a few seminiferous tubules. The group 4 (600 mg/kg BW) shows hypospermatogenesis, presence of sloughed dead cells in the lumen of rat. The increased destruction of basement membrane corroborates with the increased MDA levels observed in these groups as the destruction could be linked to increased lipid peroxidation due to oxidative stress. The significant alteration in histology of testes of experimental rats portends that the sperm function could diminish further at extended treatment period.

V. Conclusion

Justicia carnea is one plant that has nutritional importance and has been proven to cure myriad of ailments including rheumatism, arthritis, anemia, cancer, and inflammation, respiratory and gastrointestinal disorder. In this study, ethanol leaf extracts of *Justicia carnea* has been shown be rich in pharmacologically bioactive compounds (flavonoids, and phenols) and nutrients. ELEJC has an antioxidant activities at low doses, but induces the opposite effect at higher doses which could be due to its high iron content.. ELEJC causes a reduction in performance of most sperm dynamics and alteration of normal histoarchitecture of the testes of experimental rats, more so at 200 mg/kg BW. This suggests its aspermatogenic potentials.

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