The Effects of Ethanol and Boiled Aqueous Extracts of Justicia carnea Leaves on the Male Reproductive Indices: Serum Testosterone and Seminalysis of Male Wistar Albino Rats

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Abstract

Problem: Despite the therapeutic and nutritional benefits of *Justicia carnea*, some of its phytochemicals are antinutritional and harmful if consumed. Reduction in testicular weight, sperm count/motility, testosterone levels, and abnormal testicular histology have been reported following administration of ethanol extracts of *J. carnea* (EJC). Nutraceuticals can utilize these negative effects to benefit mankind as these supposedly harmful effects can be harnessed in the current search for "ideal" male contraceptive. This study aimed at assessing the possible adverse effects of oral administration of boiled aqueous extracts of *J.carnea* leaves (AJC) and the EJC on serum testosterone and seminalysis (sperm count, motility and morphology) of male albino rats as possible candidate male contraceptive. ***Approach:*** Forty-two male Wistar albino rats (12-14 weeks old) in seven groups were used. The control was fed grower feed and water only. Test groups were in addition given 200, 400 and 600mg/kg body weight (BW) of either EJC or AJC for 21*d*(21 days). The first batch (drawn from each group) were sacrificed a day later while the second batch were continued on feed and water only for further 52*d* after discontinuation before sacrificing them. Serum testosterone was determined by ELISA and seminalysis done by mounting semen smear preparations on microscope and viewing. SPSS version 25 was used for analysis of results while ($P< 0.05$) was regarded significant. ***Findings:*** After 21*d* of treatment, no significant change in serum testosterone was noted in comparison with the control for both extracts' treated groups. However, 52*d* after discontinuation, the serum testosterone of rats administered 400mg/kg BW of both extracts were significantly higher than the control and other groups. There was no significant change in total sperm count of all the treatment groups when compared with the control after 21*d* of administration. But 52*d* after discontinuation, the total sperm count for the group administered 400mg/kgBW EJC was significantly lower than the other groups and the control. There was no significant change in sperm motility in all the groups administered both extracts after 21*d* compared to the control. By 52*d* after discontinuation, there was significant reduction in sperm motility in rats administered 400 and 600mg/kg BW AJC. There was no significant change in sperm motility in rats administered 200mg/kg BWEJC, 400mg/kg BW and 600mg/kg BWAJC. There was a significant increase in the abnormal morphology of the group that received 400mg/kg BW AJC compared to the other groups. This was noted to have reversed by 52*d*after discontinuation. The group that received 400mg/kg BW EJC had no significant change in the abnormal morphology at 21*d*after administration, but a significant increase in abnormal morphology was noted 52*d* after discontinuation.

Conclusion: Both extracts of *J.carnea* when administered for 21*d*adversely impact some male reproductive indices in albino rats, such as testosterone level and seminalysis at the doses used. This can be explored in the development of male contraceptive.

Key words: ethanol extract, aqueous extract, *Justiceacarnea*, seminalysis, sperm morphology, sperm motility, sperm count, testosterone, male contraceptive, albino rats.

Introduction:

Reproduction is among the major areas where scientists world-wide engage in research using medicinal plants. This is with the intention of identifying the natural products derived from plants which can have impact on fertility and reproduction (Brandão-Costa et al., 2015). A lot of these products have been tried in a bid finding a lasting solution to fertility and population control. These include both synthetic and traditional drugs. Each has its own merits and demerits.

Species of *J.carnea* are among the biggest genus within the Acanthaceae family (Corrêa&Alcântara, 2012) and one of the most popular species used in alternative and complementary medical practice. They have been found to contain many phytochemicals which are used to treat many diseases while some have adverse effects on health (Anthonia et al., 2019; Corrêa&Alcântara, 2012; Udedi et al., 2020). Akintimehin(Akintimehin et al., 2021)reported hepatotoxicity and renotoxicity effects of *J.carnea* extracts. A lot of substances are known to have both serious side effects and beneficial effects on the human reproductive capacity. These are among the major focus in the industries. The alteration in the spermatogenesis of male albino rats by oral administration of plant extracts have been reported (Ashidi et al., 2019).

The significant side effects of *J.carnea* extracts on male fertility indices may be harnessed in the current search for an effective contraceptive for males just like their female counterparts. Most contraceptives available are used by females. Many females are not able to make use of contraceptive because of their state of health, adverse effects, fear or lack of awareness for proper use of contraceptive and hence may have to rely on their male partner's usage of contraceptive.

One of the pathways in which an effective male contraceptive might function is by altering the hypothalamo-pituitary-gonadal axis. Different hormones released by the hypothalamus, pituitary gland, and testis control spermatogenesis (Sofikitis et al., 2008). Under the direction of the pituitary gonadotrophin - luteinizing hormone [LH], Leydig cells produce and secrete the primary male sexual hormone testosterone. There are many plant compounds that are known to target the enzymes needed to synthesize androgens. When gossypol acetic acid, a polyphenolic molecule derived from the seeds of the cotton plant, was incubated with isolated rat interstitial cells at a concentration of 50g/mL, the histochemical stain for 3-β-HSD dramatically decreased, demonstrating the drug's direct inhibitory impact (Paz &Homonnai, 1984). It has been noted that *Quassiaamara's* crude methanol extract lowers levels of testosterone, LH, and FSH(Raji &Bolarinwa, 1997).*Mentha piperitalabiatae* and *Mentha spicata labiatae* were found to elevate FSH and LH levels while lowering total testosterone levels (Akdogan et al., 2004). When primary mouse Leydig cells were incubated with various concentrations of crude *Toonasinensis*, steroidogenic enzymes such as the P450 side-chain cleaving enzyme, 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase, hydroxylase, 20α-hydroxylase, and 17β-hydroxysteroid dehydrogenase were found to have their activities suppressed (Ling Poon et al., 2005). When *Abruspecatorius* was given to male rats, a dose-dependent decrease in the enzyme activity of the 3α, 3β, and 17β hydroxysteroid dehydrogenases and Leydig cell degeneration were observed (Sinha & Mathur, 1990).

A lot of unplanned pregnancies end in abortion of babies or abandoned babies (WHO, 2019). Udedi and co-workers reported that EJC was a male contraceptive candidate due to its adverse effects on the reproductive parameters of male albino wistar rats (Udedi et al., 2020).

There have been lots of studies on the effects of EJC on experimental animals like rats. The study by (Udedi et al., 2020) considered the effects of EJC on reproductive functions of male rats not the AJC. Most people who consume *J.carnea* leaves do so by boiling in water. This study is therefore justified by closing this gap in the knowledge. This study is also aimed at investigating the possible reversibility of the derangements of the reproductive functions of both EJC and AJC on male Wistaralbino rats 52 days (the duration of spermatogenesis in rats) after discontinuation.

Figure I: Justicia carnea plant

Materials and methods:

Materials:

All chemicals used were of analytical grade, obtained from British Drug House Ltd., England, via her sales representative in Nigeria. Testosterone kit was obtained from Monobind Inc., 100 North Points Drive, Lake Forest CA92630, USA. Other materials used include microscope, filter papers (Whatman number 1), oral canula, lyophilizer, microplate reader (RT-2100C, Hamburg, Germany), centrifuge machine (Model 800, China), rotary evaporator (Model: TT-52, USA), water bath (Model: H-H-W470, China), and shaker (Model: 073185, Denley, England). *J.carnea* leaves were obtained from Ahucol phase 2, Udoka housing estate, Awka and identified by the botanist at the department of Botany of the University. Albino rats were obtained from the animal house of the College of Health Sciences, Nnamdi Azikiwe University (NAU), Nnewi Campus.

Methods:

The study location: The study was majorly carried out at Prof. S.C. Udedi's Research Laboratory, in theApplied BiochemistryDepartment, NAU, Awka.

Preparation of the plant extract:*J.carnea* leaves were handpicked and dried under shade at room temperature. The dried leaves were ground into powder using a hand mill. The powder was soaked in 70% ethanol for 48h with occasional shaking and then filtered using Whatman No. 1 filter paper and concentrated in a water bath at 40ºC (Jensen, 2007), to obtain the EJC. The AJC was obtained by boiling the powder in distilled water for 10 min, filtered through muslin cloth and then filter paper. The resultant fluid was then heated in water bath to obtain a concentrated liquid (AJC).

Experimental design:Forty-two adult maleWistar albino rats between 12-14 weeks old (180-200g), randomly grouped into seven of six rats each:

Group I (control): grower diet plus 0.5ml of water only.
Group II: low dose (200mg/kg BW) EJC.
Group III: moderate dose (400mg/kg BW) EJC.
Group IV: high dose (600mg/kg BW) EJC.
Group V:low dose (200mg/kg BW) AJC.
Group VI: moderate dose (400mg/kg BW) AJC.
Group VII: high dose (600mg/kg BW) AJC.

The extracts were administered orally with gavage tube twice daily for 21 days.

Animal handling: The rats were fed on a commercial pellet diet (Vital growers obtained from Gland Cereals Ltd., Jos, Plateau State, Nigeria, via her sales representative at Awka, Anambra State. Animal care and handling were done per guidelines recommended by the WHO (WHO, 2004), and Ethical committee of NAU, Awka. The rats were kept in metal cages and fed *ad libitum*. They were acclimatized for two weeks under the same condition of temperature humidity with 12 hours of light and dark cycle.

Blood sample collection: The animals were sacrificed in batches under mild anaesthesia with diethyl ether and blood samples collected via cardiac puncture. The first batch of the samples was collected after 21 days of administration of the extracts. The second batch of rats constituted by half from each of the groups was

sacrificed 52 days after discontinuation. They were equally sacrificed and specimens taken and processed as previously done for the first batch.

Hormonal (testosterone) assay: Determination of serum testosterone level was by enzyme-linked immunosorbent assay (ELISA) using microplate reader (Model: RT-2100C, Hamburg) as described by Ikpeme(Ikpeme E.V et al., 2014).

*Principle:*Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a completion reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This affects the separation of the antibody bound fraction after decantation or aspiration. The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentrations. A dose response curve can be generated by utilizing several different serum references of known antigen concentration of an unknown can be ascertained.

Reagent preparation: Buffer was diluted to 1000ml with distilled water in a storage container and stored at 25ºC. The contents of the amber vial labelled solution "A" was poured into the clear vial labelled solution "B". The yellow cap was placed on the clear vial for easy identification. This was mixed and labelled "working substrate solution". It was stored at 8ºC.

Procedure: All reagents, serum calibrations and controls were brought to room temperature before commencement of the assay. Microplate wells for each serum reference, control, and rat specimen to be assayed were formatted. Ten microlitre (10µl) of the appropriate serum reference, control or specimen was pipetted into the assigned well. Fifty microlitre (50µl) of the ready to use testosterone enzyme reagent was added to all the wells. The microplate was swirled gently for 30sec to mix. Fifty microlitre (50µl) of testosterone biotin reagent was added to all the wells. The microplate was swirled gently for 30sec to mix. The plate was covered and incubated for 60min at room temperature. The content of the microplate was discarded by decantation. It was blotted dry with absorbent paper. Three hundred and fifty microlitre (350µl) of the wash buffer was added to all the wells and decanted. This was blotted dry. This was repeated two more times, making it a total of three washes. Hundred microlitre (100µl) of the working substrate solution was added to all the wells. This was incubated at room temperature for 15min. Fifty microlitre (50µl) of stop solution was added to all the wells and gently mixed for 20 sec. The absorbance was taken at 450nm using a microplate reader. The absorbance for each serum reference was plotted against the corresponding testosterone concentration in ng/ml giving the standard curve. The unknown concentration of the rat serum was extrapolated from this curve.

Seminalysis:

1. Sperm morphology*:* This was determined per the method of (Pant & Srivastava, 2003).

*Procedure:*Clean sterile specimen containers were used to collect the semen samples. A thin smear of the liquefied well mixed semen was made on every slide. The smear was fixed with 95% v/v ethanol for 5 min and allowed to air-dry at room temperature. This was washed with sodium bicarbonate-formalin solution to remove any mucus. The smear was rinsed several times with distilled water. The smear was covered with dilute carbol fuchsin (1 in 20) and was allowed to stain for 3 min. The stain was counterstained by covering the smear with dilute Loeffler's methylene blue for 2 min. The stain was washed off with distilled water. It was drained and allowed toair-dry at room temperature.The slides were examined for normal and abnormal spermatozoa using the 40X objective lens of microscope, while the 100X objective was used to confirm abnormalities. The percentage normal and abnormal sperm were estimated by counting 100 spermatozoa and extrapolating.

2. Total sperm count: A graduated cylinder was filled to 1ml mark for every specimen with well-mixed liquefied semen. Sodium bicarbonate-formalin diluting fluid was added and mixed. A Pasteur pipette was used to fill a Neubauer-ruled chamber with well-mixed diluted semen. The spermatozoa were allowed to settle for 4 min. The 10X objective lens with condensed iris closed enough to give good contrast was used to count the number of spermatozoa in an area of 2sqmm.

Calculation: Number of spermatozoa in 1ml = Number counted x 100,000 (Spermatozoa/ml)

3. Sperm motility: Determined by adding 1drop (10-15µ) of well-mixed liquefied semen on a slide and cover with 20X20mm cover glass. The specimen was focused using the 10X objective lens. The condenser adjusted sufficiently to give good contrast. Several fields were examined to assess motility using the 40X objective. A total of 100 spermatozoa were counted. Those that were actively motile and non-motile were noted out of the hundred and was used to determine percentage motility.

Statistical analysis:

Statistical Package for the Social Sciences (SPSS) version 25 was used for analysis of the results and ($P< 0.05$) was regarded as significant.

Results:

The serum testosterone of rats administered EJC and AJC for 21 days and 52 days after discontinuation are shownin table 1. After 21 days of treatment, there was no significant change in serum testosterone amongthe treatment groupsin comparison with the control for both EJC and AJC. But 52 days after discontinuation, the serum testosterone of rats administered 400mg/kg BW of both extracts were significantly higher compared with the control. There was no significant change in the serum testosterone of rats in other groups when compared with the control.

Table 1: Serum testosterone 21 days after EJC and AJC administration and at 52 days after discontinuation

Groups		Mean (ng/ml)	±Std	P-Value	F-Value
21*d*	I (control)	1.06	±0.70		
	II	1.40	±1.56	0.876 [a]	
	III	2.10	±1.50	0.629 [a]	
	IV	4.66	±6.37	0.107 [a]	0.927
	V	0.96	±0.15	0.963 [a]	
	VI	1.13	±0.20	0.975 [a]	
	VII	0.33	±0.11	0.731 [a]	
52*d*	I (control)	0.60	±0.20		
	II	0.66	±0.46	0.888 [a]	
	III	2.00	±0.80	0.009 *	
	IV	0.87	±0.30	0.576 [a]	6.853
	V	0.35	±0.05	0.600 [a]	
	VI	2.73	±0.90	0.000 *	
	VII	1.20	±0.69	0.219[a]	

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p<0.05$.

(*: significant; a: not significant)

Table 2 shows the total sperm count of rats administered EJC and AJC for 21 days and 52 days after discontinuation. There was no significant change in total sperm count inthe treatment groups compared with the control after 21 days of administration. Fifty-two days after discontinuation, the total sperm count for the group administered 400mg/kg BW EJC was significantly lowercompared to other groups and the control. The total sperm count of other groups did not show any significant change in comparison with the control.

Table 2: Total Sperm Count 21 days after EJC and AJC administration and 52 days after discontinuation

Groups		Mean (X10^6/ml)	±Std	P-Value	F-Value
21*d*	I (control)	66.50	±6.38		
	II	62.16	±6.44	0.586 [a]	
	III	77.66	±6.63	0.173 [a]	2.72
	IV	73.50	±12.75	0.384 [a]	
	V	83.00	±3.46	0.052 [a]	
	VI	57.06	±10.85	0.245 [a]	
	VII	66.00	±14.7	0.950 [a]	
52*d*	I (control)	66.5	±6.38		
	II	74.16	±2.02	0.624 [a]	
	III	36.67	±1.52	0.001 *	8.106
	IV	59.16	±25.35	0.223 [a]	
	V	80.26	±1.70	0.241 [a]	
	VI	86.67	±2.88	0.067 [a]	
	VII	80.13	±1.67	0.247 [a]	

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p<0.05$.
(*: significant; a: not significant).

The sperm motility ofrats administered EJC and AJC for 21 days and at 52 days after discontinuation are presented in tables 3 and 4. There was no significant change in sperm motility of all the rat groups administered EJC and AJC at 21 days after administration when compared the control. At 52 days after discontinuation, there was significant reduction in the sperm motility (table 4) of rats administered 400 and 600mg/kg BW EJC. There was no significant change in number of actively motile sperm cells of rats administered 200mg/kg BW of EJC, 400mg/kg BW and 600mg/kg BW of AJC when compared with the control.

Table 3: Sperm motility 21 days after EJC and AJC administration

Groups		Mean (%)	±Std	P-Value	F-Value
Actively motile	I (control)	90.00	±5.00		
	II	86.67	±5.77	0.508 [a]	
	III	95.00	±0.00	0.326 [a]	
	IV	90.67	±9.29	0.894 [a]	2.134
	V	97.00	±0.00	0.176 [a]	
	VI	83.33	±5.77	0.326 [a]	
	VII	85.00	±8.66	0.508 [a]	
Non-	I (control)	10.00	±5.00		

motile	II	13.33	±5.77	0.508 [a]	
	III	5.00	±0.00	0.326 [a]	2.134
	IV	9.33	±9.29	0.894 [a]	
	V	3.00	±0.00	0.176 [a]	
	VI	16.67	±5.77	0.326 [a]	
	VII	15.00	±8.66	0.508 [a]	

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p<0.05$.
(*: significant; a: not significant).

Table 4: Sperm motility 52 days after EJC and AJC discontinuation

Groups		Mean (%)	±Std	P-Value	F-Value
Actively motile	I (control)	88.33	±2.88		
	II	85.00	±5.00	0.277 [a]	
	III	70.67	±1.15	0.000 *	
	IV	76.67	±5.77	0.001 *	13.333
	V	88.33	±2.88	1.000[a]	
	VI	92.33	±2.51	0.196[a]	
	VII	86.67	±2.88	0.580[a]	
Non-motile	I (control)	11.67	±2.88		
	II	15.00	±5.00	0.277 [a]	
	III	29.33	±1.15	0.000 *	13.333
	IV	23.33	±5.77	0.001 *	
	V	11.67	±2.88	1.000[a]	
	VI	7.67	±2.51	0.196[a]	
	VII	13.33	±2.88	0.580[a]	

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p<0.05$.
(*: significant; a: not significant).

Tables 5 and 6 show the spermmorphology of rats 21 days after administration of both extracts (table 5) and 52 days after discontinuation (table 6) There was a significant increase in the percentage of abnormal morphology in the group that received 400mg/kg BWAJC when compared to the other groups (table 5). This was noted to have reversed by 52 days after discontinuation (table 6). For the group that received 400mg/kg BW of EJC, there was no significant change in the percentage of abnormal sperm cells at 21 days after administration (table 5), however, a significant increase in the percentage of abnormal cells was noted 52 days after discontinuation (table 6).

Table 5: Sperm morphology 52 days after EJC and AJC discontinuation

Groups		Mean (%)	±Std	P-Value	F-Value
Normal sperm cells	I (control)	85.00	±5.00		
	II	81.67	±5.77	0.417 [a]	
	III	86.67	±2.88	0.682 [a]	
	IV	83.33	±7.63	0.682 [a]	2.783
	V	86.67	±2.88	0.682 [a]	
	VI	73.33	±2.88	0.011 *	
	VII	80.00	±5.00	0.230 [a]	
Abnormal sperm cells	I (control)	15.00	±5.00		
	II	18.33	±5.77	0.417 [a]	
	III	13.33	±2.88	0.682 [a]	2.783
	IV	16.67	±7.63	0.682 [a]	
	V	13.33	±2.88	0.682 [a]	
	VI	26.67	±2.88	0.011 *	
	VII	20.00	±5.00	0.230 [a]	

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p<0.05$.
(*: significant; a: not significant).

Table 6: Sperm morphology 52 days after EJC and AJC discontinuation

Groups		Mean (%)	±Std	P-Value	F-Value
Normal sperm cells	I (control)	86.67	±5.77		
	II	86.33	±5.50	0.947 [a]	
	III	63.33	±10.40	0.000 *	
	IV	81.67	±5.77	0.324 [a]	6.696
	V	88.33	±2.88	0.739 [a]	
	VI	85.00	±5.00	0.739 [a]	
	VII	89.00	±3.60	0.641 [a]	
Abnormal sperm cells	I (control)	13.33	±5.77		
	II	13.67	±5.50	0.947 [a]	
	III	36.67	±10.40	0.000 *	6.696
	IV	18.33	±5.77	0.324 [a]	
	V	11.67	±2.88	0.739 [a]	
	VI	15.00	±5.00	0.739 [a]	
	VII	11.00	±3.60	0.641 [a]	

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p<0.05$.
(*: significant; a: not significant).

Discussion and conclusion:

We investigated the effects ofEJC and AJC on the male Wistar albino rats' reproductive indices at 200 mg/kg BW, 400 mg /kg BW and 600 mg/kg BW. Assessment of reproductive functions in animals have been used as one of the major ways of determining the fertility status of animals (Pant & Srivastava, 2003; WHO, 2019).

There was no significant change in total sperm count of all the treatment groups when compared with the control (66.50 x 10^6/ml ± 6.38) after 21 days of administration. However, 52 days after discontinuation, the total sperm count for group administered 400 mg/kg BW EJC was significantly lower when compared with other groups and the control. The reason for this delay in the manifestation is not clear but may be that the implicated agents are slow-acting and the impact persists long after discontinuation. Again, the suppression of sperm count was not noted at higher concentration (600mg/kg BW) of EJC implying there is a critical level beyond which the suppression does not occur suggesting a U-shaped dose-response relationship. The results are in keeping with the findings by (Udedi et al., 2020), who found no significant difference between the sperm count in the experimental group when compared to the control group after 21 days. In contrast, the earlier work by Udedi and co-workers did not involve a longer period of monitoring after discontinuation of the extracts and hence could not document the slow-onset of sperm count suppression noted in this study. Total sperm count is one of the major indices used in accessing the fertility status in males (Kuchakulla et al., 2021). Decrease in total sperm count means decrease in semen quality. This could be harnessed by the pharmaceutical industries to design male contraceptives. The use of contraceptive has been the most effective method of birth control (WHO, 2019). Therefore, suppression of sperm count can potentially impair male fertility and achieve contraception.It will also serve to educate those who use the herbal products derived from *J.carnea* on the need to be cautious due to the potential for causing male infertility.This work observed that AJC did not result in any change on the total sperm count of rats at both 21 days and even 52 days following discontinuation of the varying doses of AJC. This could mean that AJC may not contain the active fraction in large enough quantity as to have effect on the total number of sperm cells at the above-stated doses or it doesn't contain the factor responsible for the deranged sperm count found in the EJC.

The sperm motility showed no change in the percentage motility of sperm cells of rats in all the groups when compared with the control at 21 days of administration. This agrees with findings on other *Justicia species*(Corrêa&Alcântara, 2012). However, 52 days after discontinuation, there was decrease in the number of actively motile sperm cells of rats administered 400 and 600mg/kg BW EJC but not in other groups. This implies that the 400 and 600 mg/kg BW EJC may cause decrease in the movement of sperm cells and thereby impact fertility by impairing movement through the female genital tract. The EJC contains Naphthalene, Dibutyl phthalate and 1,4-dichlorobenzene all of which are known to impair sperm count and motility (Eastmond DA & Balakrishnan S, 2010; Latini et al., 2006; Nayak et al., 2023; Yost et al., 2021). Similar findings have been reported earlier (Udedi et al., 2020), using EJC. The AJC did not have similar effect possibly because the active ingredient may be absent or not substantial enough to be impactful. For an egg to be fertilized, sperm cells must be mobile enough to be able to swim efficiently through the female reproductive system. The more actively motile sperm cells are, the higher the chances of reaching the eggs being fertilized. This have been shown in other reports (Akintimehin et al., 2021; Kuchakulla et al., 2021). When sperm cells don't move, they are either dead or that they are blocked from moving their flagella, thereby reducing the chances of conception taking place in the animals involved.

There was a significant increase in the percentage of cells with abnormal morphology among the rats that received 400mg/kg BW AJC after 21 days when compared to the control and other groups. This agrees with earlier documentation using *J.carnea*(Udedi et al., 2020) and *Daucus carota*(Kausar, 2016). This was reversed after 52 days of discontinuation. This has the potential as a target for reversible contraceptive.

Again, the group that received 400mg/kg BW EJC did not show any significant change in the percentage of sperm cells with abnormal morphology after 21 days. However,52 days after discontinuation, the percentage of sperm cells with abnormal morphology was significantly increased in the group that received 400mg/kg BW EJC when compared to the control and other groups. The reason for these variations in the period of manifestation of these abnormalities in the groups that received EJC and AJC are not clear but may not be unconnected with different active ingredients at play in the two extracts. The active ingredients in the extracts may have caused oxidative damage leading to abnormality in the sperm cell morphology. This was shown by an article in press following a work done in our laboratory. Defective spermatogenesis affects sperm DNA integrity, thereby affecting embryology (Kuchakulla et al., 2021).Defects in sperm cells such as abnormal heads, and tails may affect the ability of the sperm cells to reach an egg and penetrate it. This will make the egg not to be fertilized by the abnormal sperm cells by slowing down the movement of the sperm cells towards the egg. Higher number of abnormal sperm cells are associated with low sperm count. This quality once again may endear the extracts to researchers in the pharmaceutical industriesin male contraceptive design.

The results of the serum testosterone levels of male Wistar Albino rats determined indicated that there was no change in the values for rats administered both extracts of *J.carnea* after 21 days. This is suggesting that 21 days may not be enough time for the manifestation of the impact of these extracts on the serum testosterone. It also shows that the impact on morphology stated earlier in this work by 21 days after administration of 400mg/kg BWAJC was not necessarily through suppressing testosterone. Higher testosterone levels were noted in rats administered with 400mg/kg BW of both extracts 52 days after discontinuation when compared to the control and other groups. Again, the higher level of testosterone noted in these two groups may be a rebound effect/negative feedback to boost impaired reproductive function caused by the administration of 400mg/kg BW of both extracts. Such higher level of testosterone was not observed in the groups administered with 600mg/kg BW of either extracts. This is suggesting there may be a critical dosage beyond which this negative feedback does not occur, giving an "inverted" U-shaped dose-response curve. Alteration in serum testosterone of albino rats administered with EJChave been reported (Udedi et al., 2020). The genes responsible for the production of testosterone may have been altered, leading to alteration in the production of testosterone and sperm integrity (Kuchakulla et al., 2021). Testosterone is a sex hormone found in larger quantities in male animals and for male fertility, testosterone is a crucial hormone. The American Society for Reproductive Medicine (ASRM) states that testosterone is important for sperm cell development and health maintenance (Hsieh et al., 2013). Nevertheless, taking exogenous testosterone supplements may result in lower endogenous testicular testosterone production and reduced male fertility by lowering levels FSH, which is crucial for promoting sperm formation. Testosterone production is a tightly controlled process involving the hypothalamic-pituitary-gonadal axis. Up to 30% of male infertility patients have hypogonadism, which can impede spermatogenesis (Hsieh et al., 2013).

Conclusion:

The overall findings show that both EJC and AJC when administered for 21 days adversely impact some reproductive indices in male Wistar albino rats, such as serum testosterone, total sperm count, morphology and motility. There appears to be an inverted "U-shaped" dose-response curve between the concentration of the extracts and the impact on the reproductive indices. It thus appears there is a critical dose beyond which the derangement of these male reproductive indices does not occur. EJCs appears to have a greater impact than AJC on the reproductive indices assessed. Noteworthy is that the alteration in morphology occasioned by AJC was reversed by 52 days after withdrawal of the extract making it a potential target for pharmaceutical industries to exploit in reversible male contraceptive development. These extracts may not have any obvious impact on the reproductive parameters at 21 days or after 52 days of withdrawal when given at the concentration of 200mg/kg BW. This observation is important especially for locals who consume these extracts for other medicinal benefits can do so if a critical dosage is not attained to avoid male infertility. Further studies should be carried out especially using higher doses, administration/and

follow up for longer duration to critically harmonize the grey areas and answer some questions raised by this study.

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