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Ameliorative Potentials of Justicia Carnea and Cnidoscolus Aconitifolius on the Fecundity of Chloramphenicol-Induced Lymphoma Rats

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Abstract

Justicia carnea and Cnidoscolus aconitifolius were tested for their capacity to ameliorate the fecundity of chloramphenicol-induced lymphoma in rats. Seventy male Wistar rats weighing an average of 128g were randomly divided into 14 groups of five rats each. Group 1 was provided with commercial rat diet and water on a daily basis. For 28 days, rats in groups 2-14 received 250mg/kg bodyweight chloramphenicol by oral intubation. Group 2 received no therapy and is thus referred to as the negative control group. The remaining groups (3–14) received aqueous leaf extracts of J. carnea (ALEJC) (Groups 3-6), C. aconitifolius (ALECA) (Groups 7–10), or a combination of both extracts (Groups 11–14) at dosages of 500mg/kg, 1000mg/kg, 1500mg/kg, and 2000mg/kg, respectively, for 28 days. After anaesthesia with ether, blood was drawn from the retro-orbital venous plexus to determine the activities of oxidative stress indicators such as superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and glutathione (GSH). The testes were dissected for histological inspection and the semen were collected for analysis. The results indicated a significant (p0.05) increase in weight across groups, as well as a reduction in sperm volume, viability, and motility in group 2 animals as compared to group 1. SOD and GSH levels increased significantly, but MDA levels increased in a non-significant (p>0.05) manner. The results indicated that the extracts had a beneficial impact on infertility associated with excessive chloramphenicol exposure.

Introduction

Chloramphenicol is a broad-spectrum antibiotic that inhibits protein synthesis and also causes the generation of reactive oxygen species (Tripathi 2003). It inhibits apoptosis, which results in the differentiation of aberrant cells, resulting in the development of a leukaemia-like disease with abnormal lymphoblasts (lymphoma) (Yuan & Shi, 2008). Chloramphenicol induces some of the morphological and functional changes associated with senescence in somatic cancer cells, activating SA- Gal, a senescence biomarker (Duewelhenke et al., 2007; Li et al., 2010). In the short term, even before therapy starts, the presence of lymphoma in the system might reduce sperm count (Louis et al., 2014).

Fertility is a phrase that relates to the actual creation of children, as opposed to the physical capacity to reproduce, which is referred to as fecundity. Fertility can be quantified, but fecundity cannot. Certain factors, such as infection with a venereal disease, lifestyle choices, and inadequate nutrition, may all have an effect on fertility. It is hypothesized that sperm volume, count, motility, and morphology drop with age, with men aged 50–80 years generating sperm at an average rate of 75% compared to men aged 20–50 years, and that significant disparities in the number of mature sperm in the testes' seminiferous tubules are seen (Kidd et al., 2001). A recent research on the quality of sperm in teenage male cancer patients discovered

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that cancer patients had poorer sperm parameters than healthy control participants (Bahadur et al., 2002). However, when the cancer process is directly responsible for the patient's lowered reproductive potential, spermatogenesis may improve after the disease's cure (Schilsky, 1989). The use of herbs and traditional medicine as complementary and alternative therapies dates all the way back to ancient times, particularly in continents such as Asia and Africa. The traditional system of medicine has been designed in such a way that a greater proportion of inhabitants in Africa and Asia rely on it to alleviate symptoms of different ailments. Justicia carnea is one of the plants that has been found as having therapeutic properties such as antihypertensive and haematinic properties (Udedi et al., 2020), igniting interest in its biochemical examination. Cnidoscolus aconitifolius has many of the same biochemical features as J. carnea, including well-documented hypolipidemic qualities (Fagbohun et al., 2021). It is a member of the Euphorbiaceae family and grows to a height of 6m with alternating palmate lobed leaves and milky sap. On the succulent petiole, the leaves are huge, measuring 32cm × 30cm. It evolved as a domesticated leafy green vegetable in the Maya area of Guatemela, Belize, and South–East Mexico during the pre–Cambrian era (Fagbohun et al., 2012), and owing to its ease of cultivation and potential production, the plant spread across the planet, including the tropics. The leaves and shoots are used as laxatives, diuretics, circulatory stimulants, lactogenic stimulants, and to harden fingernails. Tannins, saponins, cardiac glycosides, deoxy sugar, and terpenes have been discovered in plant leaves in previous investigations (Fagbohun et al., 2012).

Given that the prognosis for patients with lymphoma has improved significantly over the past decades (Diehl et al., 2003) and that the majority of lymphoma patients are young, on average 32 years old (Greco et al., 1974), long-term treatment side effects are becoming more critical. Infertility after therapy, in particular, imposes a significant mental strain on young patients. According to a recent survey, 51% of men with cancer stated a desire to retain their reproductive potential in the future, including 77% of men who were childless at the time their disease was discovered (Schover et al., 2002). Chemotherapy regimens using alkylating drugs such as cyclophosphamide and procarbazine were shown to be significantly related with infertility (Behringer et al., 2005). Interestingly, the majority of male Lymphoma patients were found to have deficient sperm quality even prior to therapy (Rueffer et al., 2001). There is, however, a dearth of data on the fertility of male patients treated in big, prospective studies. The current research examines the reproductive status of male Wistar Rats before and after treatment with aqueous leaf extracts of Justicia carnea (ALEJC) and Cnidoscolus aconitifolius (ALECA).

Methods

Collection and Identification of Specimens

C. conitifolius and J. carnea leaves were collected in Rumuogba, Rivers State, Obio/Akpor Local Government Area. Dr. Ekeke Chimezie of the University of Port Harcourt's Department of Plant Science Herbarium recognized them and assigned them the Voucher numbers UPH/V/1448 and UPH/V/1449, respectively.

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Figure 1. Leaves of Justicia carnea [A] Cnidoscolus aconitifolius [B]

This research was conducted in two parts, each of which lasted 28 days. The first 28 days were used to generate lymphoma in rats, and the second phase (another 28 days) was utilized to treat them with extracts of J. carnea and C. aconitifolius. Seventy (70) male Wistar rats weighing an average of 128g were randomly divided into 14 groups of five rats each. Group 1 was provided with commercial rat diet and water on a daily basis. For 28 days, rats in groups 2-14 received 250mg/kg bodyweight chloramphenicol by oral intubation. Group 2 received no therapy and is thus referred to as the negative control group. The remaining groups (3-14) received aqueous leaf extracts of J. carnea (Groups 3-6), C. aconitifolius (Groups 7-10), or a combination of both extracts (Groups 11-14) at dosages of 500mg/kg, 1000mg/kg, 1500mg/kg, and 2000mg/kg, respectively, for 28 days. For the course of the experiment, the animals were fed orally once daily via rat oral canula.

Determination of Weight and Oxidative Stress Parameters

The weights of the rats in each group were determined and recorded before and after treatment using a digital laboratory weighing scale prior to anaesthetizing the animals. After anaesthesia with ether, blood was obtained from the retro-orbital venous plexus to determine the activities of oxidative stress indicators MDA, SOD, and Catalase, as well as the levels of glutathione, as reported by Usoh et al (2005). Semen was taken for analysis, and testes were dissected and histopathologically examined.

Histological Procedure And Semen Examination

The caudal portion of each divided epididymis was removed and put in a beaker containing 10 ml diluting solution (sodium bicarbonate; 5g and formalin neutral; 1 ml in 100 ml of distilled water). Each portion was rapidly macerated with a pair of sharp scissors and allowed to rest for a few minutes to allow the spermatozoa to be liberated into the solution.

Sperm Motility

The total spermatozoa in the caudal epididymal sperm sample were counted and the sperm count per epididymis was determined using the enhanced Neubauer haemocytometer slide. Two drops of heated 2.9 percent sodium citrate were applied to the sperm drop on the slide. The slide was covered with a coverslip and inspected with X40 objectives for sperm motility, morphology, abnormalities, sluggishness, and death (Raji & Morakinyo, 2006).

Results and Discussion

The findings in Figure 2 reveal that the weights of animals treated C. aconitifolius and J. carnea extracts increased significantly (p0.05) when compared to the positive and negative controls. The pH of sperm remained unchanged. The treated groups' sperm volume, viability, and activity were substantially different from the positive and negative control groups (Table 1). The positive control values for these parameters were 0.180.07, 78.334.41, and 71.674.41, respectively, in comparison to the negative control values of 0.120.02, 75.002.89, and

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66.673.33 for the negative control group. When compared to the negative, extracts of J. carnea and C. aconitifolius shown a significant increase (p0.05) in the metrics (Volume, Viability, and Activeness). Group 3 (500mg/kg J. carnea) had the greatest increase in semen volume (0.200.06), viability (85.002.80), and activity (78.332.3) (p0.05).

The impact of J. carnea and C. aconitifolius leaf extracts on oxidative stress indicators in Wistar mice is shown in Figure 3. The Malondialdehyde (MDA) levels were same in the positive and negative control groups. When compared to the negative control, superoxide dismutase, catalase, and glutathione levels were substantially lowered (p0.05) in the treatment groups (Figure 4)

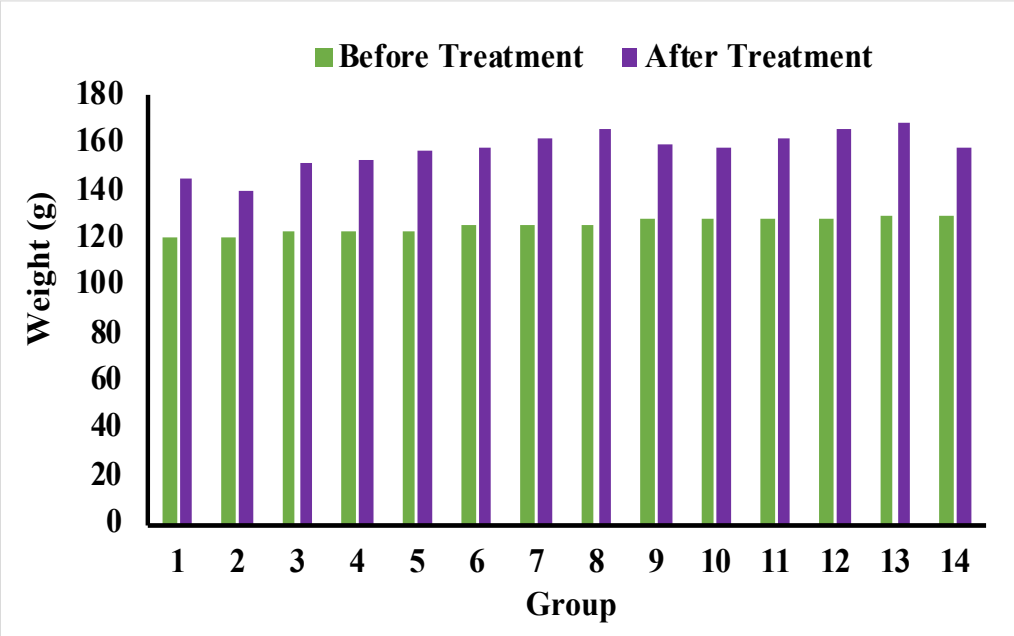

Figure 2. Changes in weight of chloramphenicol-induced lymphoma rats treated with different concentrations of ALEJC and ALECA extracts

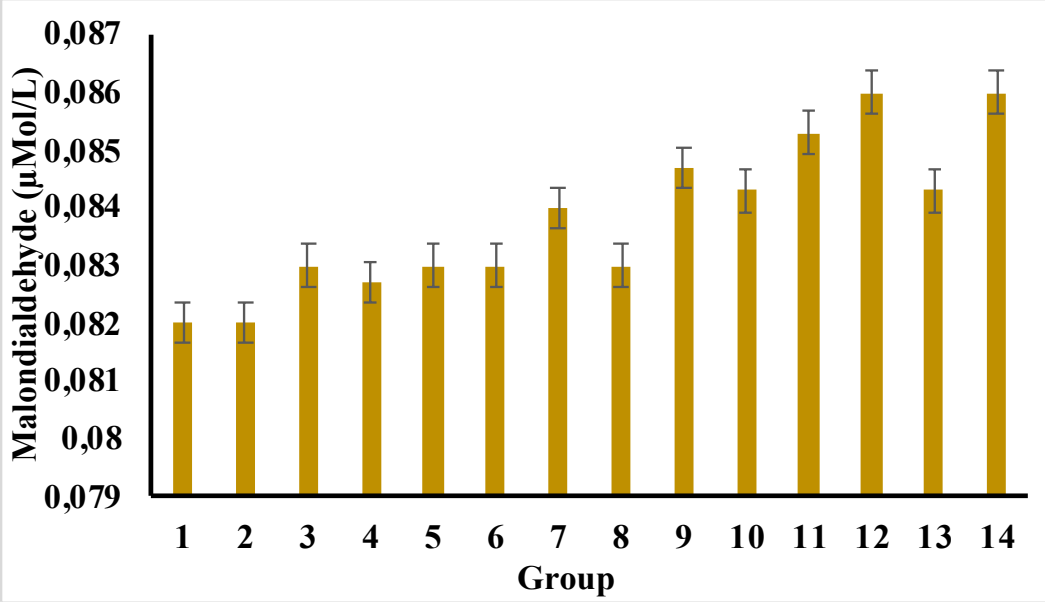

Figure 3. Effect of ALEJC and ALECA extracts on malondialdehyde - a lipid peroxidation marker of chloramphenicol-induced lymphoma rats

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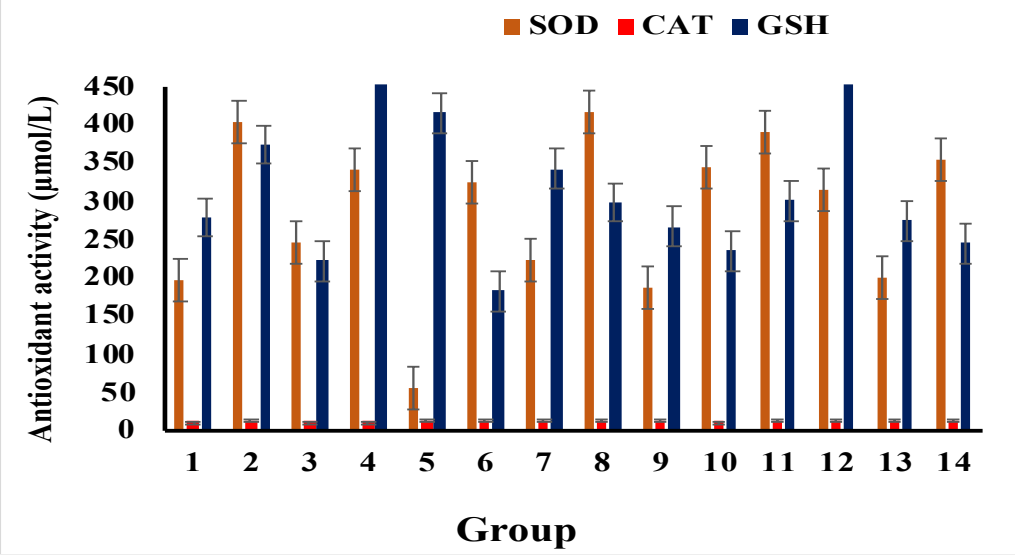

Figure 4. Effect of ALEJC and ALECA extracts on oxidative stress markers of chloramphenicol-induced lymphoma rats

Table 2. Effect of ALEJC and ALECA extracts on Semen of chloramphenicol-induced lymphoma Rats

VOL mL	PH	VIABILITY %	NORMAL %	ABNORMAL %	ACTIVE %	SLUGGISH %	DEAD %
0.18±0.017^{a}	8.00±0.00^{a}	78.33±4.41^{a}	78.33±3.33^{a}	21.67±3.33^{a}	71.67±4.41^{a}	11.67±1.67^{a}	16.67±3.33^{a}
0.12±0.02^{b}	8.00±0.00^{a}	75.00±2.89^{a}	70.00±2.88^{b}	30.00±2.89^{b}	66.67±3.33^{a}	11.67±1.67^{a}	21.67±4.41^{a}
0.20±0.06^{a}	8.00±0.00^{a}	85.00±2.89^{b}	81.67±4.41^{a}	18.33±4.41^{a}	78.33±3.33^{b}	8.33±3.33^{b}	13.33±3.33^{b}
0.12±0.02^{b}	8.00±0.00^{a}	68.33±1.67^{c}	68.33±1.67^{b}	31.67±1.67^{b}	65.00±2.89^{c}	11.67±1.67^{a}	23.33±3.33^{c}
0.07±0.01^{c}	8.00±0.00^{a}	65.00±2.89^{c}	56.67±1.67^{c}	43.33±1.67^{c}	48.33±1.67^{d}	10.00±0.00^{a}	41.67±1.67^{d}
0.08±0.02^{c}	8.00±0.00^{a}	73.33±3.33^{a}	75.00±2.89^{a}	25.00±2.89^{d}	75.00±2.89^{a}	11.67±1.67^{a}	13.33±3.33^{b}
0.18±0.06^{a}	8.00±0.00^{a}	75.00±2.89^{a}	71.67±4.41^{b}	28.33±4.41^{d}	68.33±6.01^{a}	10.00±0.00^{a}	21.67±6.01^{a}
0.17±0.02^{a}	8.00±0.00^{a}	85.00±2.89^{b}	80.00±2.89^{a}	20.00±2.89^{a}	71.67±4.41^{a}	10.00±0.00^{a}	18.33±4.41^{a}
0.27±0.03^{d}	8.00±0.00^{a}	78.33±6.01^{d}	75.00±2.89^{a}	25.00±2.89^{d}	71.67±4.41^{a}	11.67±1.67^{a}	16.67±3.33^{a}
0.17±0.03^{a}	8.00±0.00^{a}	85.00±2.89^{b}	86.00±3.06^{d}	14.00±3.06^{e}	78.33±4.41^{b}	8.33±1.67^{b}	13.33±3.33^{b}
0.17±0.02^{a}	8.00±0.00^{a}	73.33±4.41^{a}	68.33±4.41^{b}	31.67±4.41^{b}	65.00±2.89^{c}	11.67±1.67^{a}	23.33±3.33^{c}
0.08±0.02^{c}	8.00±0.00^{a}	60.00±2.89^{e}	60.00±2.89^{c}	40.00±2.89^{c}	53.33±4.41^{d}	11.67±1.67^{a}	35.00±5.00^{d}
0.15±0.03^{ab}	8.00±0.00^{a}	78.33±1.67^{a}	80.00±2.89^{a}	20.00±2.89^{a}	75.00±2.89^{a}	11.67±1.67^{a}	13.33±3.33^{b}
0.08±0.02^{c}	8.00±0.00^{a}	71.67±1.67^{a}	70.00±2.89^{b}	30.00±2.89^{b}	63.33±1.67^{c}	11.67±1.67^{a}	25.00±2.89^{c}

Data are expressed as mean± standard error mean (SEM) of n=5. Values in the same column having the same alphabet superscript are termed not to be statistically significant with each other at 0.05 significant level.

Histology

Photomicrographs of the testes of rats in the control and treatment groups are presented in Plates I – 4

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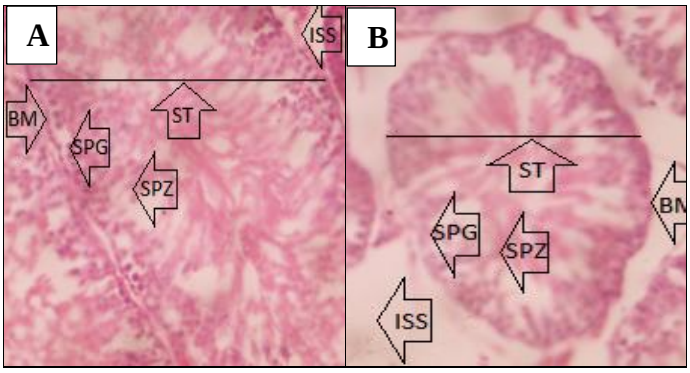
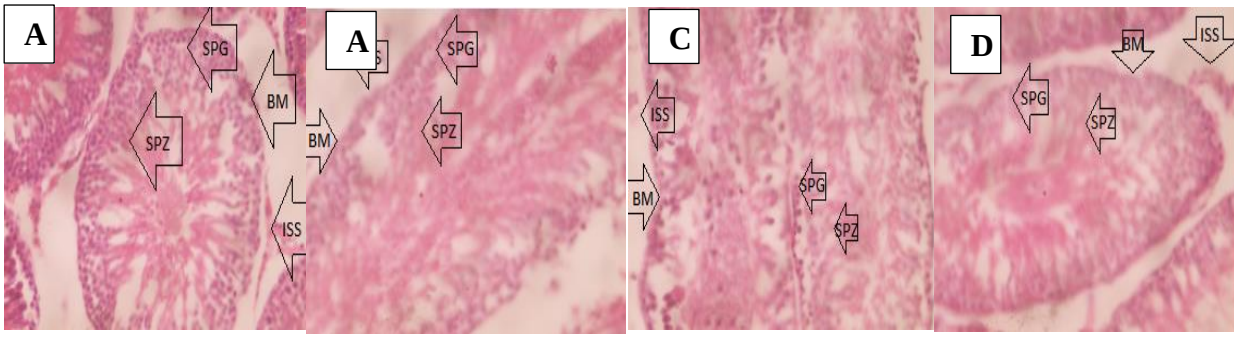


X 400

Figure 5. A - Testis photomicrograph of Group 1 rats demonstrating normal testis, seminiferous tubules (ST) containing spermatozoa (SPZ), and spermatogenic cells (SPG). A basement membrane surrounds seminiferous tubules (BM). Leydig cells are found in the interstitial space. B - Histologically deformed testis, enlarged interstitial gaps, and reduced seminiferous tubules in Group 2 rats. Spermatogenic cells and spermatozoa have normal histological characteristics.



X 400

Figure 7. A - The testis of Group 3 rats is photomicrographed, revealing histologically deformed testis, wider interstitial gaps, and reduced seminiferous tubules. Spermatogenic cells and spermatozoa have normal histological characteristics. B - Histologically deformed testis, enlarged interstitial gaps, and reduced seminiferous tubules in Group 4 rats. Spermatogenic cells and spermatozoa have normal histological characteristics. C - Histologically normal testis of Group 5 rats with seminiferous tubules (ST) containing spermatozoa (SPZ) and spermatogenic cells (SPG). A basement membrane surrounds seminiferous tubules (BM). Leydig cells are found in the interstitial space. D - Histologically normal testis of Group 6 rats with seminiferous tubules (ST) containing spermatozoa (SPZ) and spermatogenic cells (SPG). A basement membrane surrounds seminiferous tubules (BM). Leydig cells are found in the interstitial space

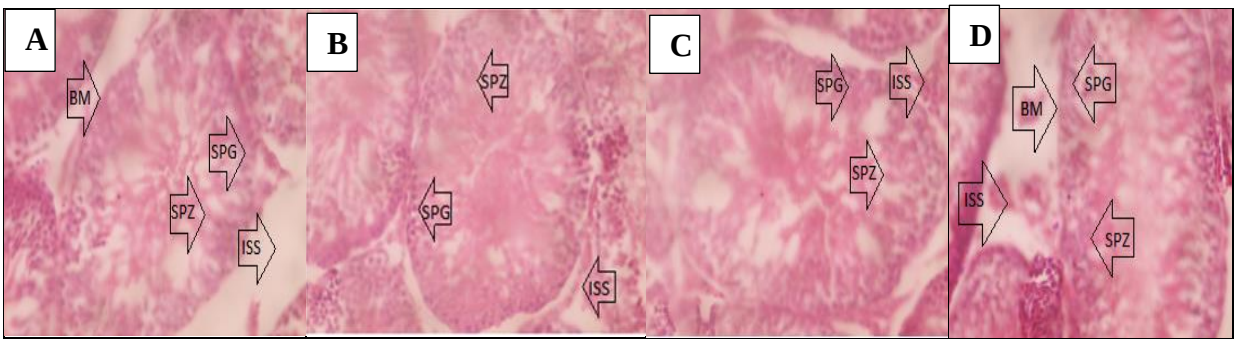


Figure 8. A - Photomicrograph of the testis of Group 7 rats showing histologically distorted testis, widened interstitial spaces, shrunken seminiferous tubules. Spermatogenic cells and spermatozoa are histologically normal. B - Testis of Group 8 rats showing histologically normal testis, seminiferous

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tubules (ST) contain spermatozoa (SPZ) and spermatogenic cells (SPG). Seminiferous tubules are surrounded by a basement membrane (BM). The interstitial space contains Leydig cells. C - Testis of Group 9 histologically normal testis, seminiferous tubules (ST) contain spermatozoa (SPZ) and spermatogenic cells (SPG). Seminiferous tubules are surrounded by a basement membrane (BM). The interstitial space contains Leydig cells. D - Testis of Group 10 histologically normal testis, seminiferous tubules (ST) contain spermatozoa (SPZ) and spermatogenic cells (SPG). Seminiferous tubules are surrounded by a basement membrane (BM). The interstitial space contains Leydig cells

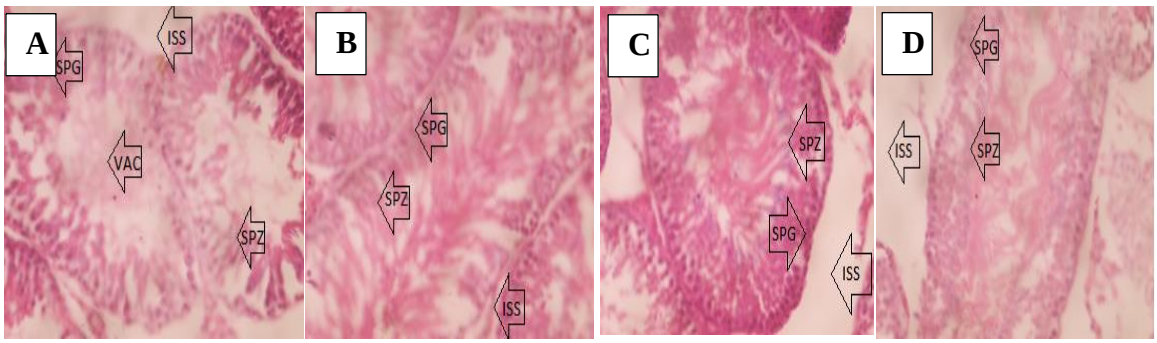

Figure 9. A - Photomicrograph of the testis of Group 11 histologically distorted testis, spermatozoa destroyed in most of the seminiferous tubules creating vacuolization (VAC), and normal spermatogenic cells. B - Testis of Group 12 histologically normal testis, seminiferous tubules (ST) contain spermatozoa (SPZ) and spermatogenic cells (SPG). Seminiferous tubules are surrounded by a basement membrane (BM). The interstitial space contains Leydig cells. C - Testis of Group 13 histologically distorted testis, widened interstitial space, shrunken seminiferous tubule, histologically normal spermatogenic cells, and spermatozoa. D - Testis of Group 14 histologically distorted testis, widened interstitial space, histologically normal spermatogenic cells, and spermatozoa.

Blood disorders and infertility have had a crippling impact on families and countries, prompting the quest for alternate sources of medicinal substances. Due to the phytochemical makeup of ethnomedicinal herbs, they have significant potential in haematological treatment. Pharmaceutical agents used to treat blood infections have very specific effects, which severely restricts their capacity to counteract the establishment of resistance, particularly in monotherapy. On the other hand, herbal products provide a complimentary or synergistic effect as a result of the many secondary metabolites contained in plant materials (Hajian, 2013). While the general population perceives herbs as having no adverse effects, herbal medicines have not been extensively investigated or standardized to permit clinical implementation. Certain therapeutic plants have been documented to have morphological, histopathological, hematological, and biochemical effects (Eboh & Ekundina, 2012; Chris-Ozoko et al., 2013; Ebeye et al., 2014; Ekundina et al., 2014).

The current investigations investigated the biochemical consequences of chloramphenicol-induced lymphoma and the ameliorative effects of J. carnea and C. aconitifolius on male Wistar rat fertility. The weights of animals provided C. aconitifolius and J. carnea extracts were significantly increased (p0.05) as compared to the control. This rise in weight demonstrates the plant's nutritional importance as a source of proteins, minerals such as calcium, phosphorus, magnesium, and iron, as well as vitamins A, B, and C. (Orjiakor et al., 2019). Semen analysis revealed that aqueous extracts of J. carnea and C. aconitifolius had a beneficial impact on the rats' semen profiles. Following chloramphenicol-induced lymphocytosis, sperm motility and count decreased, as did the proportion of live/dead spermatozoa.

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However, the number of aberrant spermatozoa rose significantly (Azeez et al., 2010). This is a sign that the animals' fertility has been lowered. However, these effects were ameliorated to some extent after treatment with the extract (particularly 500mg/kg of J. carnea and 1500mg/kg of C. aconitifolius). The extract had a significant influence on the percentage motility and live/dead ratio of spermatozoa, although the extract of C. aconitifolius had a greater ameliorative effect on the percentage motility than the other extracts or the combined extract.

The pH of the semen of the positive, negative, and treatment groups remained constant at 8.00. When compared to the positive and negative control groups, the volume, viability, and activeness of the sperm were considerably different. When compared to the negative, extracts of J. carnea and C. aconitifolius shown a considerable increase in all metrics (volume, viability, and activity). Lymphoma and its treatment have been demonstrated to affect some sperm properties, consequently lowering male fertility. Additionally, the results suggested that the majority of patients ultimately recover their sperm, although the extent and timing of that recovery may vary depending on the patient's diagnosis and therapy (Louis et al., 2014).

The plant extracts had no beneficial impact on the treated groups, as their values continued to climb unabated throughout the treatment period. While all aerobic cells produce ROS, severe oxidative stress may result in a variety of clinical diseases. The primary factor affecting the redox environment is the generation and clearance of reactive oxygen species (ROS), and antioxidants play a critical role in ROS elimination, hence preserving the normal physiological state (Valko et al., 2007).

These findings indicate that Wistar rats with lymphoma are under oxidative stress, as shown by a considerable rise in ROS plasma levels and a reduction in antioxidant capacity. Numerous oxidative pathways have been found in cancer patients that contribute to oxidative stress (Mantovani et al., 2002). Excessive reactive species formation and subsequent depletion of antioxidants may result in oxidative stress, a potentially harmful situation for cells and organic tissues (Valko et al. 2006; 2007). By contrast, oxidative stress mediates the actions of a large number of cytostatic medicines by generating sublethal DNA damage and so initiating apoptosis (Peroja et al. 2012). Vajdovich et al. (2005) discovered that lymphoma patients had a depleted antioxidant capacity, and that free radical concentrations were associated with a more proliferative phenotype. Additionally, tumors with a poor capacity for oxidative burst and a high ratio of reduced to oxidized glutathione reacted better to chemotherapy, and the afflicted blood and lymph nodes were subjected to severe oxidative stress.

J. carnea and C. aconitifolius both include alkaloids, flavonoids, glycosides, carbohydrates, saponins, tannins, terpenoids, phenols, steroids, and resins, according to preliminary phytochemical investigation (Onyegeme-Okerenta et al., 2019). It asserts that flavonoids are abundant. Flavonoids are the most abundant class of plant phenols and contribute significantly to the taste and color of fruits and vegetables (Tanwar & Rajni, 2012, Orjiakor et al., 2019). This may be because to the powerful flavor and bright crimson color of Justicia carnea despite its green leaf when boiled as swallowed. Flavonoids and phenols are a diverse group of compounds that contribute significantly to the protection of biological systems. They act as potent water-soluble antioxidants and free radical scavengers, preventing oxidative cell damage, lowering the risk of heart disease, and exerting strong anticancer activity (Okwu, 2004). Thus, the aqueous leaf extracts of J. carnea and C. aconitifolius contain certain physiologically active chemicals that may be used as a source of medications or as a preventative treatment against oxidative stress in animals

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Disclosure of conflict of interest

The Authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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