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TESTICULAR TOXICITY INDUCED BY CYCLOPHOSPHAMIDE AMELIORATED BY HERBAL EXTRACT (JALIN): AN EXPERIMENTAL STUDY

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ABSTRACT

This study aimed to evaluate cyclophosphamide induced testicular toxicity ameliorated by herbal extract (Jalin) (Ginseng, Lepediummeyenii, Liriosmaovata and Honey). Fifteen (15) albino male rats were grouped into three (3) and treated with cyclophosphamide and Jalin extract. Distilled water was given to group one, which served as the control. Group two received 100mg/kg bwt of cyclophosphamide per body weight of albino rats and group three received cyclophosphamide 24 hours before the administration of 2ml/kg of Jalin extract per body weight of albino rats. Male reproductive hormones and body weight were measured in blood samples (testosterone, luteinizing hormone and follicle stimulating hormone). Results showed a significant decrease ($p<0.05$) reproductive hormones (testosterone, luteinizing hormone and follicle stimulating hormone). Testosterone decreased from (151.60±7.60) to (94.65±9.60), luteinizing hormone decreased from (29.33±3.92) to (11.79±4.17), follicle stimulating hormone decreased from (19.76±3.21) to (8.90±2.60) when compared to control. Male reproductive hormones (testosterone, luteinizing hormone, and follicle stimulating hormone) are more active when Jalin extract and cyclophosphamide are administered compared to controls. Testosterone increased from (147.20±8.10) to (151.60+7.60), luteinizing hormone increased from (11.79±4.17) to (27.25±2.70), follicle stimulating hormone increased from (8.90±2.60) to (17.30±2.43). In Conclusion, as indicated by the manufacturers, Jalin herbal mannex liquid extract may be a promising herbal cure for male infertility. It should be promoted.

KEYWORDS: Cyclophosphamide, Hormones, Testicular Toxicity, Infertility.

INTRODUCTION

Medicinal plants remain beneficial to man despite the development of modern medications.[17] According to[11] about 80% of the 5.2 billion people of the world exclusively depend on medicinal plants for their healthcare needs. Some chemically-active plant components known as secondary metabolites are what give these plants their therapeutic significance.[21] Plants' secondary metabolites, or phytochemicals, offer remarkable medicinal properties that include anti-oxidative, anti-allergic, antibiotic, hypoglycemic, and anti-carcinogenic properties.[4] This plant has received a lot of interest lately because of its possible implications on sexual function.[1]

Jalin extract is a medicinal extract composed of ginseng, maca and honey. Ginseng is a widespread herbal medicine and it has served as an important component of many drug prescriptions since thousands of years.[5] Ginnosides and gintonin are substances that define ginseng. Numerous physiological effects of ginseng, including those on the circulatory, immunological, and neurological systems, have been documented. It has been utilized to improve sex performance and fulfillment.[5] Lepediummeyenii, as commonly known , is a plant used in folk medicine and as a dietary supplement to boost sexual and reproductive health.[19] Maca contains several secondary metabolites, such as macamides, macaridine, alkaloids, and glucosinolates.[19] Benzyl glucosinolate (glucotropaeolin) and m-methoxybenzylglucosinolate are the two aromatic glucosinolates found in the highest concentrations in Maca.[5] Honey is a naturally occurring sweet, viscous substance made by honeybees from the nectar of blossoms, plant secretions, or excretions left behind by insects that feed on living plants. Honeybees collect these substances, transform them, mix them with other substances, then store and allow them to ripen and mature in the honey comb.[16] In addition, honey is one of nature's wonders.[10] Its antibacterial, anti-inflammatory, and anti-oxidant properties, as well as the enhancement of the immune system, have been endorsed for their beneficial effects.[10]

Furthermore, the development of male reproductive systems, as well as their growth and sexual characteristics, is significantly influenced by the reproductive hormones.[3] [6] These hormones are synthesized in the same cells and their synthesis is encouraged by the hypothalamus gonadotropin releasing hormone (GnRH), their expression and circulating levels change in accordance with their various functions.[5] Many tissues contain the male reproductive hormones, such as testosterone (T), follicle-stimulating hormone (FSH), and luteinizing hormone (LH).[8][14]

The immunosuppressive drug cyclophosphamide (CP), a cytotoxic alkylating agent, is often used to treat systemic lupus erythematosus and other benign diseases as well as antineoplastic treatment for various malignancies.[7] Despite having a wide range of clinical uses, it may have toxic side effects in a number of organ systems, including the testes, which can lead to male infertility in both humans and animals[7] The two major cyclophosphamide metabolites, phosphorylamide mustard and acrolein, are functional alkylating agents that have the potential to connect single strands of DNA or DNA with proteins. Reviewing the negative effects and side effects of damaging anti-cancer treatments on a regular basis is a serious concern.[7]

Based on the above background, this study aimed to investigate the curative effect of jalin extract on cyclophosphamide-induced reproductive toxicity in male albino rats.

AIMS OF THE STUDY

The aim of this study are as follows.

i. To investigate the effects of Jalin on reproductive parameters.
ii. To assess the possible curative potential of Jalin male albino rats.
iii. To determine the effect of Jalin herbal extract on cyclophosphamide induced testicular toxicity

OBJECTIVES OF THE STUDY

The aim of the study would be achieved with the following specific objectives.

i. Male reproductive hormones (LH, FSH and testosterone).
ii. Histopathological studies of the testes.

MATERIALS AND METHODS

Chemicals

The investigation only employed analytical reagent grade chemicals.

Purchase of the Jalin Herbal Mannex Liquid

The Jalin Herbal Mannex Liquid (MFG 8 2020 ExP 8 2022) batch number BNO: 320. With Nafdac registration number A7-2077L was purchased from Cynflac Pharmacy Limited, Yenegoa, Bayelsa State.

Experimental Animals

We got Fifteen (15) healthy adult male wister rats from the Niger Delta University's animal home on Wilberforce Island in Bayelsa state, weighing between 120g and 260g. For a period of six weeks, they were fed with regular feed (pellet) and purified water For the goal of controlling and supervising experiments on animals, all operations were carried out in accordance with the Institutional Animal Ethical Committee's (IAEC) directives.

Experimental Design

A total of fifteen (15) adult albino rats grouped into three (3) groups each having five rats

Group 1: received distilled water and standard feed for 6 weeks

Group 2 (Positive control): received 100mg/kg per body weight (bw) of Cyclophosphoamide (given once via intraperitoneal injection) and distilled water after 24 hours for 6 weeks.

Group 3 (Test group): received 2ml/kg per body weight of jalin herbal mannex liquid after 24 hours of administration of cyclophosphamide for 6 weeks.

The administration of cyclophosphamide was done 24 hours before the administration of Jalin herbal mannex liquid. 24 hours after the last administration, the rats were dissected and blood sample was collected, the testes were also harvested and processed for biochemical analysis. The blood was centrifuged and the serum taken and stored in the refrigerator. Part of the testes was preserved with formalin for histopathological studies, while the other part was mixed phosphate buffer and homogenised.

Sample Collection

Testes

After 6 weeks of oral administration of jalin herbal mannex liquid the rats were slaughtered using chloroform anaesthetization. The testes were obtained, washed in normal saline, weighed, homogenised using phosphate buffer (PH 7.4) and centrifuged for 10 minutes at 2000rpm and their serum was collected. The serum was kept in sample bottles containing formalin and was subjected to histological analysis. The epididymis was excised during the dissection and transferred into petri-dish and crushed using a blunt forceps and 1ml of normal saline was added to the semen and mixed thoroughly using a syringe 5ml to draw and release the mixture continuously.

Biochemical Assay

Estimation of Reproductive Hormones

The levels of testosterone, luteinizing hormone, and follicle stimulating hormone in the blood were assessed using specific commercial kits (IBL-Hambug GmbH, Germany). Standard operating protocols were employed using specific commercial kits that utilized the Enzyme Linked Immunosorbent Assay (ELISA) technique as described by Steyn et al. to measure these hormones in

serum (2013). The tests were carried out in line with the manual-recommended manufacturer methods. With a microtitre plat reader, the absorbances were measured using the appropriate wavelength for each analyte, and the associated concentration was computed.

Statistical Analysis

All data obtained were presented as mean and standard deviation (Mean ±SD). The SPSS Software of version 23.0 was used for the analysis of the data obtained. Comparison of result between control and test was done using one-way analysis of variance (ANOVA and group means were compared using Bonferroni multiple comparison. Level of significance was determined at a probability level of $p< 0.05$.

RESULTS AND DISCUSSION

The results for the effect of Jalin herbal mannex liquid on liver of male wistar albino rats is presented in Tables 3.1 and 3.2.

Table 3.1: The effect of administration of cyclophosphamide and the Jalin extract on the body weight and liver weight of albino rats.

Treatment	Body weight (initial) (g)	Body weight (final) (g)	Testis weight (g)	Relative testes weight (g)
Control (distilled water)	170.70±8.15[a]	199.6± 10.03[a]	1.22±0.15[a]	0.60±0.12[a]
Cyclophosphamide alone	195.10±8.04[b]	215.20±10.50[b]	0.93±0.05[b]	0.43±0.04[b]
Cyclophosphamide + jalin herbal extract	171.30±7.50[a]	191.3±9.87[c]	1 .18±0.30[c]	0.62±0.18[a]

Data are mean ±SD (n=5). Mean in the column with a different superscript letter(s) are significant different, (P<0.05) one-way ANOVA.

When albino rats were given cyclophosphamide (100mg/kg body weight), their weight decreased significantly, (P<0.05) compared to the control group.. There was a significant increase (P<0.05) in the weight of albino rats treated with cyclophosphamide and jalin extract compared to positive control (the group with cyclophosphamide alone).

Table 3.2: The effect of cyclophosphamide and Jalin herbal mannex liquid on the reproductive hormones of male albino rats.

Treatment	Testosterone (ng/ml)	Follicle stimulating hormone (mg/L)	Luteinizing hormone(mg/L)
Control group (distilled water)	151.60±7.60[a]	29.33±3.92[a]	19.76±3.21[a]
Cyclophosphamide group (100mg/kg bw) alone	94.65±9.60[b]	11.79±4.17[b]	8.90±2.60[b]
Cyclophosphamide(100mg/kg per body weight) + Jalin herbal mannex liquid (2ml/kg bw)	147.20±8.10[c]	27.25±2.70[c]	17.30±2.43[c]

Data are mean + SD (n=4) mean in the same column with different superscript letter(s) are significantly different (P<0.05) one way ANOVA.

There was a significant decrease (P<0.05) in the level of testosterone, luteinizing hormone and follicle stimulating hormone respectively in the reproductive hormones of the male albino rats treated with cyclophosphamide alone when compared to the control respectively, while there was Significant increase (P<0.05) in luteinizing hormone, testosterone and follicle stimulating hormone respectively in the reproductive hormones of male albino rats treated with cyclophosphamide and Jalin extract when compared to the cyclophosphamide group alone.

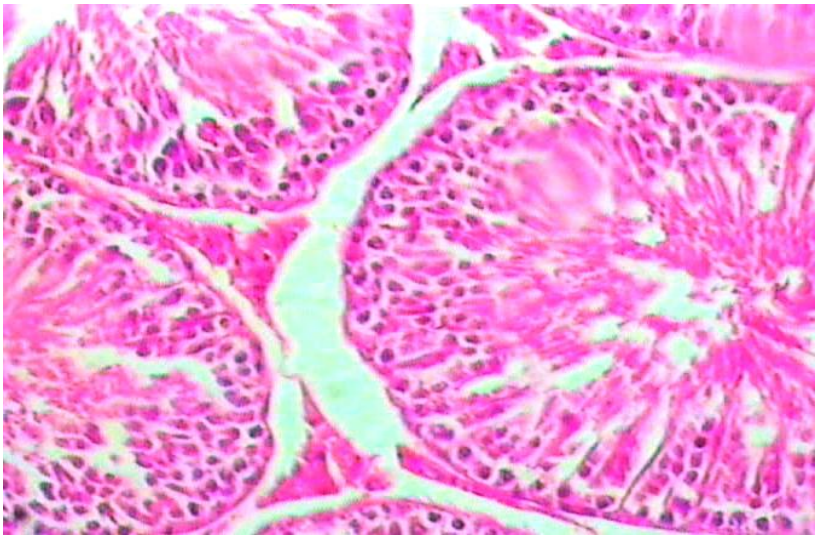

Fig. 1: Photomicrographs of testicular sections of control rats. atrophed seminiferous tubules with widen interstitial space, spermatogonia spermatids and spermatocytes.

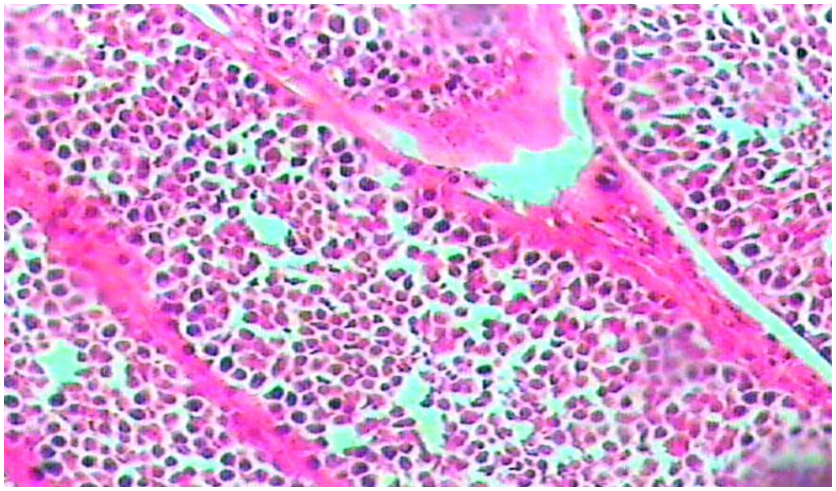

Fig. 2: Photomicrograph of testicular section cyclosphosphamide(100mg/kg) treated rats demonstrates noticeably reduced semeniferous tubules with significant germ cell hyperplasia and thickening of the basement membrane.

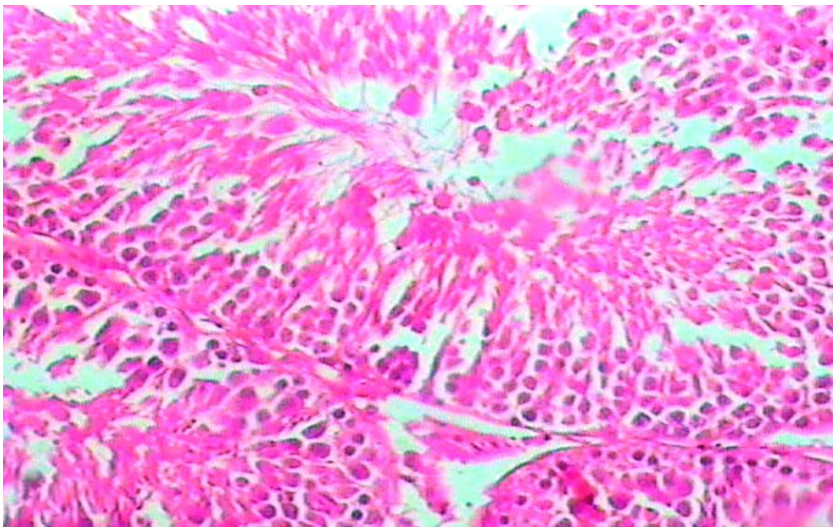

Fig. 3: Photomicrographs of testicular sections of rats treated with Jalin herbal mannexliquid(2ml/kg) + cyclosphosphamide(100mg/kg). Normally occurring seminiferous epithelium and interstitial tissue with active spermatogenesis in the testes.

DISCUSSION

The importance of medicinal plants cannot be overlooked, since most herbal plants are used for sustaining health and alleviating or treating health issues[2] Some of the herbal plants have been certified as a drug and are claimed to be safe and effective.[4] This plant has received a lot of interest lately because of its possible effects on sexual function. According to reports, people with infertility issues who consumed American ginseng saw an improvement in their sperm's characteristics.[9] It demonstrates that the primary active components in American ginseng are responsible for the pharmacological actions of the herb (ginsenoside).[5] Cyclophosphamide, a widely used anticancer medication, has a variety of antitumor activity in the treatment of human cancer.[15] stated that the kind and cumulative dose in addition to the period of exposure determine how quickly these side effects are reversed. The duration and amount of anticancer drug usage can result in various adverse effects.In this study, treatment with cyclophosphoamide (100mg/kg per body weight) + Jalin extract (2ml/kg bw) increased the body weight and testes weight from 171.30±7.15 to 195. 10±8.04;1.18±0.30 to 1.22 ±0.15 respectively when compared to the cyclophosphamide (alone) group (Table 3.1).Also, cyclophosphamide decreases the testosterone, FSH and LH levels of reproductive system of male albino rats from 151.60 ± 7.60 to 94.65 ±9.60•, 29.33±3.93 to 11.79±4.17 and 19.76 ±3.21 to 8.90 ±2.60 respectively when compared to the control group. While the treatment with Jalin extract + cyclophosphamide increased the levels of testosterone, FSH and LH of reproductive system of male albino rats from 94.65 ±9.60 to 147.20±8.10; 11.79 ±4.17 to 27. 25 ±2.70; and 8.90 ±2.60 to 17.30 +2.43 respectively when compared to the rats treated with cyclophosphamide alone (Table 3.2).Treatment with Jalin herbal extract did not cause significant changes in the parameters compared to the treatment with cyclophosphamide alone which caused significant changes in the parameters. While on the other hand, the treatment with cyclophosphamide and Jalin herbal extract maintained the levels of all the parameters closer to control group.

This findings revealed that testosterone levels in the cyclophosphamide-treated rats dropped statistically significantly. This result is consistent with earlier research by[7], which showed that cyclophosphamide treatment significantly increased DNA damage, increased malondialdehyde levels, and decreased serum testosterone concentrations. However, ginseng co-administration reduced these effects and boosted recovery in the cyclophosphamide+ginseng group. Our histology results, which are consistent with the reduction in plasma testosterone, suggest that the drug's toxic effects on leydig cells may be the cause. Treatment with Jalin herbal extract reduced the cytotoxic effect of cyclophosphamide on male hormones of reproductive system of male albino rats.

Photographs of control rat testicular slices taken under a microscope match Figure 3 and the histopathology report of Figure 1 (control rats). Rats treated with Jalin herbal mannex liquid and cyclosphosphamide had their testicular sections photomicrographed in Figure 3. Seminiferous epithelium and interstitial tissue, both of which are typical characteristics of testes, are present. Spermatogenesis is also active. This shows that Jalin Herbal Mannex liquid treatment on the rats had a positive effect similar to the control. This is in line with the findings of[12] who reported an improved sperm parameter, specifically sperm concentration and vitality, treated with green tea and appears to be safe in rats.

This study demonstrated that oxidative stress, which negatively affects testicular structure and function, is greatly increased by cyclophosphamide (CP) therapy, and that Jalin extract has curative and protective effects on CP-induced toxicity. Ginseng, maca, and honey have an antioxidant impact against cellular oxidative damage in tissues, according to research to date. Most likely, the source of its antioxidant activities is its capacity to scavenge free radicals and reduce DNA oxidative damage. These properties of the jalin extract may have several positive impacts in preventing organ damage. As a result, using Jalin herbal extract as a prophylactic measure reduces cyclophosphamide adverse effects. These findings may aid cancer patients who have received cyclophosphamide in recovering from reproductive gonad failure.

CONCLUSION

This study demonstrates that the use of jalin herbal extract improves the situation where the administration of cyclophosphamide therapy significantly increased oxidative stress, which severely damaged the function and structure of male reproductive hormones. This is most likely because of its antioxidant qualities, which result from its capacity to scavenge free radicals and reduce DNA oxidative damage. This supports the assertion that the jalin herbal extract may have therapeutic promise, improving oxidative damage while also enhancing male fertility.

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