HISTOMORPHOLOGICAL ANALYSIS OF *CARICA PAPAYA* AND *PHYLLANTHUS NIRURI* TREATMENT POTENTIALS ON TESTICULAR TISSUE OF ALBINO RATS PRE-TREATED WITH NITROFURATOIN

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Background: Herbal medicine is a natural product from plants and has proven overtime to be useful in so many areas since the days of old and still in use today.

Aim: The research item is aimed at evaluating the remedial changes associated with *Carica papaya* and *Phyllanthus niruri* treatment on testicular tissue of rats pre-treated with Nitrofuratoin.

Materials and Methods: The research was divided into two stages, in the first stage, group 1, received water and pelleted rat feed, group 2-5 received, 100 mg/kg body weight of nitofuratoin and served as control and test respectively. In the phase 2, group 1, received rat pellet and water *ad lithium*, group 1-2, received rat pellet and water, group 3-5 received 100 mg/kg body weight of *P. niruri, C. papaya*, equal of *P. nuriri* and *Carica papaya* respectively. After two weeks of administration, In stage 1, four animals in control and 4 in test. In the stage 2, all animals were weighed, euthanized and sacrificed and the vas deferens, and other part of the testicular tissues were harvested, analyzed, processed, stained and examine.

Results: The result shows that nitrofurantoin caused distortion in the seminiferous tubule, affected the sperm cell morphology which was in turn remedied by *C. papaya, P. niruri* and combined.

Conclusion: The herbal medicines have remedial potentials on damage tissue.

Keywords: *Phyllanthus niruri, Carica papaya*, Combine extract, herbal, nitrofuratoin

INTRODUCTION

The use of therapeutic plants for healing is as old as humanity itself. There is ample proof that man and his search for medicines in nature have a long history together, including written records, preserved monuments, and even the original plant medicines (Petrovska, 2012). The knowledge of using medicinal plants came about as a result of man's long-standing battles with disease, which taught him to look for drugs in the barks, seeds, fruit bodies, and other parts of plants (Petrovska, 2012). Modern pharmacotherapy now includes a variety of plant-based medications that have been used for millennia and were known to ancient civilizations. Modern science has acknowledged their active action (Petrovska, 2012). The ability of pharmacists and doctors to respond to challenges that have emerged with the spread of professional services in the facilitation of man's life has increased as a result of their knowledge of the development of ideas related to the use of medicinal plants as well as the evolution of awareness (Petrovska, 2012).

Both ayurveda and siddha, the Indian medical systems, emphasize the need of strengthening the immune system (Balkrishna *et al*., 2020). Numerous people have used various plant extracts in varied solvent systems to explore the immunomodulatory effects on rats and mice as model organisms at diverse dosages and with various experimental conditions. Increased delayed type hypersensitivity, enhanced cytotoxicity of natural killer cells, decreased hemagluttination, and increased or decreased delayed type hypersensitivity reaction are some of the immunomodulatory effects of diverse plant extracts (Balkrishna *et al*., 2020). Plant extracts in various solvent systems can either suppress or stimulate the immune system. An immunomodulator is identified by a rise in foot pad thickness/paw value, bone marrow cells, alpha esterase, and immunoglobulin levels. Eight allopathic medicinal herbs were studied for their phytochemistry (Balkrishna *et al*., 2020).

Some of these drugs have been known to have one side effect or the other on various organs, some of these effects are reversible while some can lead to permanent damage. Herbal medicine has long been used for various treatment purposes; *Carica papaya* and *Phyllanthus niruri* are not left out of the equation. The unregulated practices of some of these herbal practitioners and the unguided use of this herbal medicine call for serious concern (Srivastava and Singh, 2016).

The need to source for a cheaper means of treating some of the illnesses and the need for government to put in place control measures on indiscriminate use of synthetic drugs such as nitrofuratoin and herbal medicine necessitated this study.

Carica papaya and *Phyllanthus niruri* have been researched by notable researchers to have some treatment potentials. The root of C. papaya is used for cough, bronchitis, and other respiratory diseases treatment (Ayoola*et al.,* 2010; Moreira *et al*., 2013). The anti-bacterial activities of *P. niruri* extract was reported by Akinjogunla, (2010). Both plants have been used separately for medicinal and nutritional purposes, hence, we decided to see the potency of this herbal medicine when combined, hence, this study is aimed at evaluating the histomorphological changes associated with c*arica papaya* and *Phyllanthusniruri* treatment on testicular tissue of albino rats previously treated with Nitrofuratoin

MATERIALS AND METHODS

Study Design

The research was conducted in an experimental setting. It was carried out at the University of Benin's Department of Medical Laboratory Science, School of Basic Medical Sciences..

Ethical consideration

The National Research Council's Guide for the Care, Handling, and Use of Laboratory Animals served as the study's protocol (NRC, 2012).

Plant Material

The leave of *P. niruri and C.papaya*was identified and authenticated by Mr. H. Akinbosun in the Department of Botany, University of Benin.

Preparation of sample

The *Phyllanthusniruri* leaf was washed with water, cut into small pieces, and dried in the shade for three days before being ground into powder and kept in an airtight container..

Extraction Procedure

The powder was extracted with an ethanol: water (3:1) mixture for three hours on a reflex water bath. The cycle was run through three times. The extract was dried by air and concentrated on a rotary flash evaporator to create the semisolid extract.

Phytochemistry analysis

To identify the phytochemical components in the blended powder of the seeds, a qualitative phytochemical analysis was carried out using a technique described by Owoyele et al., (2011).

Research Tools

Hypodermal needles, EDTA bottles, sample bottles, gavage (orogastric tube), microtiter plates, and vernier caliper

Acute Toxicity Study

The acute toxicity study was carried out using Lorke's methodology.

The formula LD50 = (Highest Nonlethal Dose) x was used to calculate the median lethal dose (LD50) (Lowest lethal dose). The LD50, the effective dosage used in this experiment, was informed by the potential dosages that an animal might be exposed to intentionally or concurrently with the LD50 (Lorke, 1983).

The LD50 and the possible dose animals and humans could be exposed to at a time or accidentally formed the basis for the experimental doses administered.

Nitrofuratoin dosage

This was calculated using the following formula:

Animal body weight divided by 1000 grams times the dosage in milligrams gives the dosage in mg (Erhirhie, et al., 2014)

Experimental Animals

Twenty eight (28) healthy albino ratswere housed in polyvinyl cages under typical laboratory conditions, including temperature 250^0C, 10^0C and light and dark cycles (12h light and 12h dark). In addition to having access to clean water at all times, they were fed pelleted rat food. To avoid conception, male and female rats were housed in separate cages both before and during the study period. Prior to the experiment, the animals were given a two-week period of acclimatization.

Grouping and Management

A total of 28 healthy albino rats were divided into five groups (5). The research was in two phases..

Phase 1:

The animals in group 1, has 8 rats while the group 2 to 5 has five rats each.

Group 1, which served as the control group, received water and pelleted rat food; group 2, 100mg/kg body weight of nitrofuratoin; group 3, 100mg/kg body weight of nitrofuratoin; group 4, 100mg/kg body weight of nitrofuratoin; and group 5, 100mg/kg body weight of nitrofuratoin. After two weeks of administration, 4 animals from each group—4 rats from the test and 4 rats from the control—were randomly chosen, sacrificed, the vas deferens and testicular tissues were harvested. Seminar fluid analysis was then performed on the testicular tissues, which were then processed, stained, and examined for structural changes.

Phase 2: GROUP 1, continued to receive lithium along with rat pellets and water, served as the positive control; GROUP 2, had previously received nitrofuratoin and had only received rat pellets and water; GROUP 3, received 100 mg/kg of *P. nuriri*; GROUP 4, which received 100 mg/kg of C. papaya; and GROUP 5, received an equal concentration of *P. nuriri and C. papaya*. Following the intervention for another 14 day, all of the animals were weighed, put to sleep, and sacrificed. The vas deferens and other testicular tissues were harvested, seminar fluid analysis was performed, and testicular tissues were processed, stained, and examined**.**

Sperm Analysis

Isolation of Sperm Cells

The vas deferens was located and ligated for a minimum of 36mm length, both extremities were cut, and the vas deferens was placed in a sterile petri dish. The petri dish was filled with 6 microliter (ul) of standard saline that had already been heated to 37 + 2°C. It was teased to allow the sperm cell to diffuse out of the Vas deferens. Onto a grease-free, spotless slide, which was then covered with a clear cover slip, a drop of sperm was transferred from the petri dish.

Study of motility of Spermatozoa

Sperm cell motility was established using the relationship between fertility and the progression of sperm cell motility following ejaculation (Ibeh *et al.,* 2019). Three variables—progressive motility, non-progressive motility, and immotile spermatozoa—were used to measure the motility of spermatozoa, and the results were expressed as percentiles (Ibeh *et al*., 2019).

Study of morphology of Spermatozoa

Using Improved Eosin and Leishman stain, the morphology of sperm cells was examined on the stained slide (Ibeh *et al*., 2019).

Haematoxylin and Eosin

Omorodion *et al.* (2020) standard .'s procedure for hematoxylin and eosin staining was previously discussed and was adapted.

Analysis of statistics

GraphPad Prism 8's one-way ANOVA and Turkeys multiple comparison tests were used to contrast the *Carica papaya, Phyllanthus niruri*, and the combined extract treatment groups with the control group (GraphPad Software, Inc., CA, USA). Any differences that had a p-value of 0.05 or less were considered statistically significant.

RESULTS

Sperm Analysis

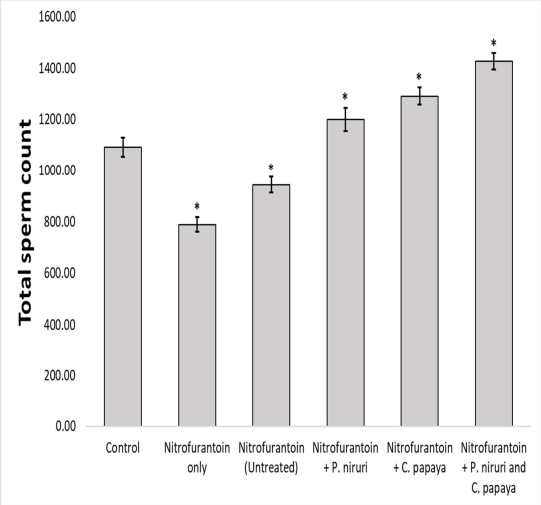


Figure1: Chart showing total sperm count in per ml
*significantly different from the control group

In contrast to the control group, Groups B and C experienced statistically significant drops in total sperm count (P 0.05), while Groups D, E, and F experienced statistically significant gains (P 0.05).

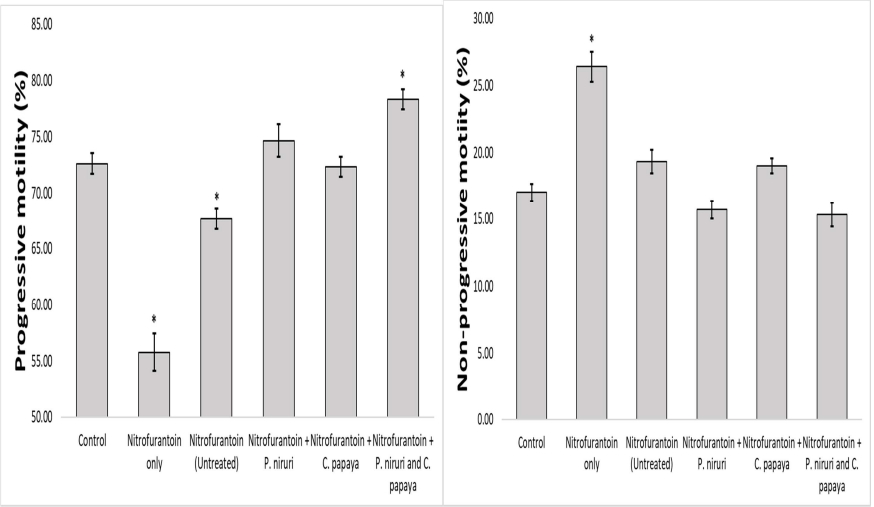


Figure 2: Chart showing progressive motility and non-progressive motility
*substantially different from the control group

When compared to the control group, there were statistically significant increases (P0.05) of non-progressive motility in Groups 2 and 3, and statistically significant decreases (P0.05) of progressive motility in Groups 2 and 3.

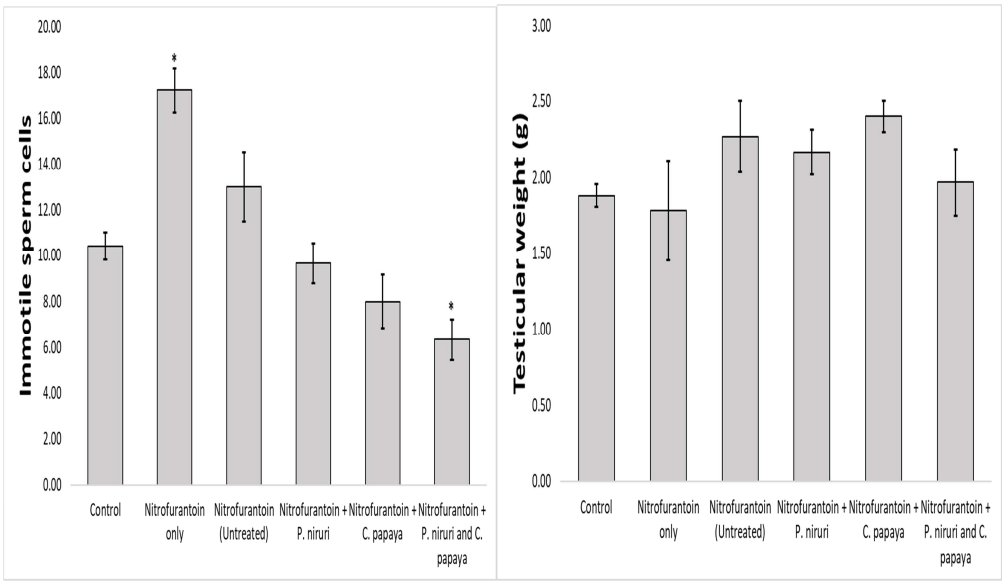


Figure 3: Chart showing immotile sperm cells and testicular weight

A significant difference from the control group

In comparison to the control group, immotile sperm cells in Group 2 showed a statistically significant increase (P0.05), while immotile sperm cells in Group F showed a statistically significant decrease (P0.05). However, there were no statistically significant differences in testicular weight between the groups (P>0.05).

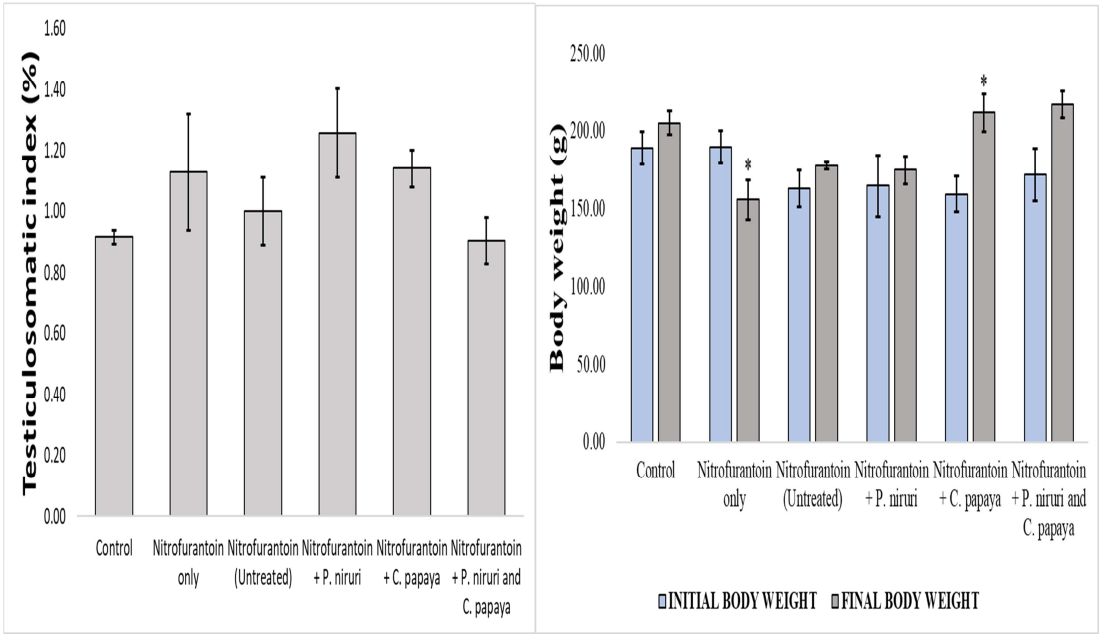


Figure 4: Chart showing testiculosomatic index and body weight

When comparing the initial and final body weights, there were no statistically significant differences (P>0.05) in the testiculosomatic index between the groups, but there was a statistically significant decrease (P0.05) in body weight in Group 2 and an increase (P0.05) in body weight in Group 5

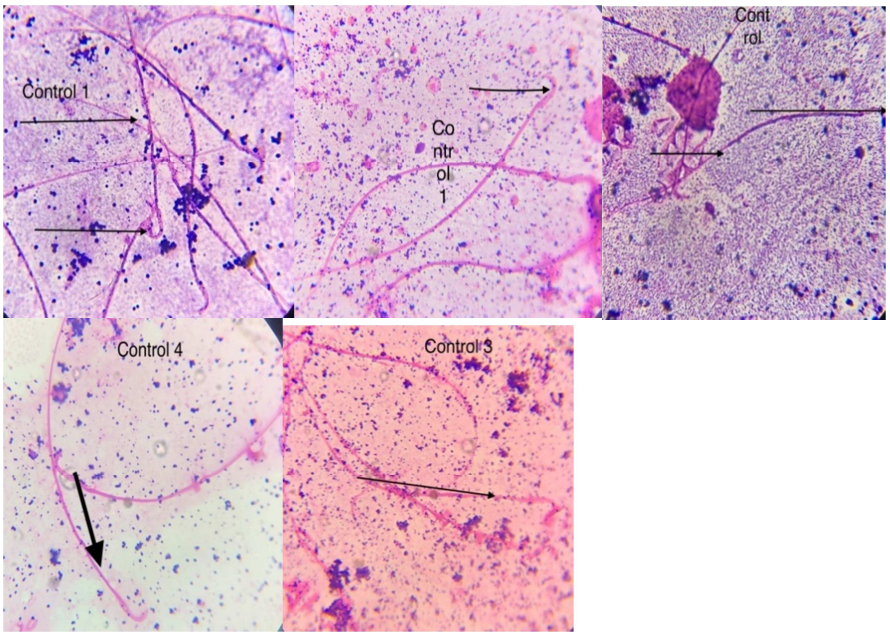


Plate1::Sperm cell morphology of rats treated with rat pellet and water only with form sperm cells. Note the normal shape of head and tail. Leishman and eosin stain. xX1000mag.

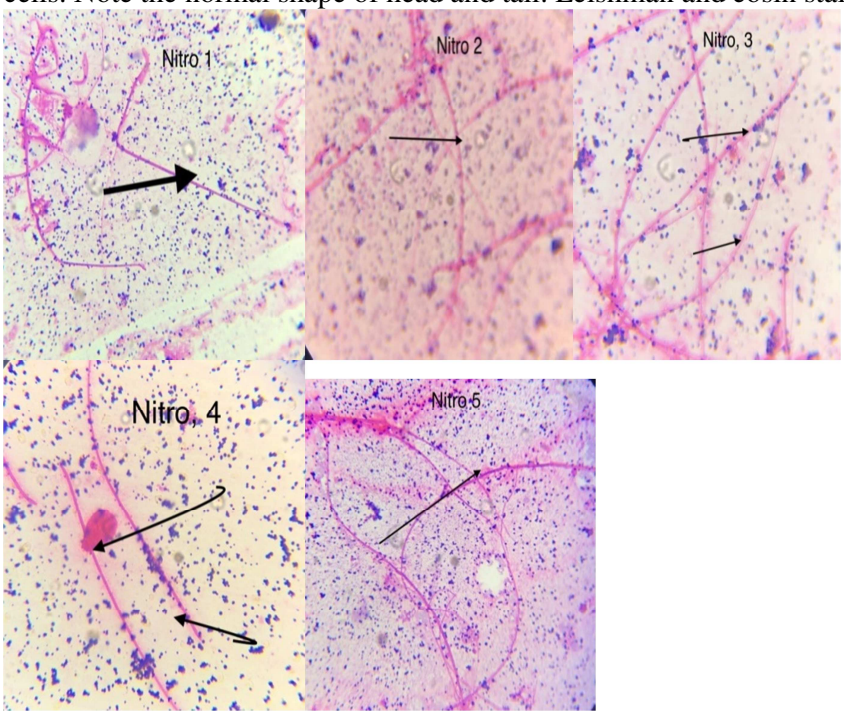


Plate 2: Sperm cell morphology of rat treated with nitrofuratoin. Note the unformed sperm cell with tailless sperm cell (dark arrows). Leishman and eosin stain. x 1000mag

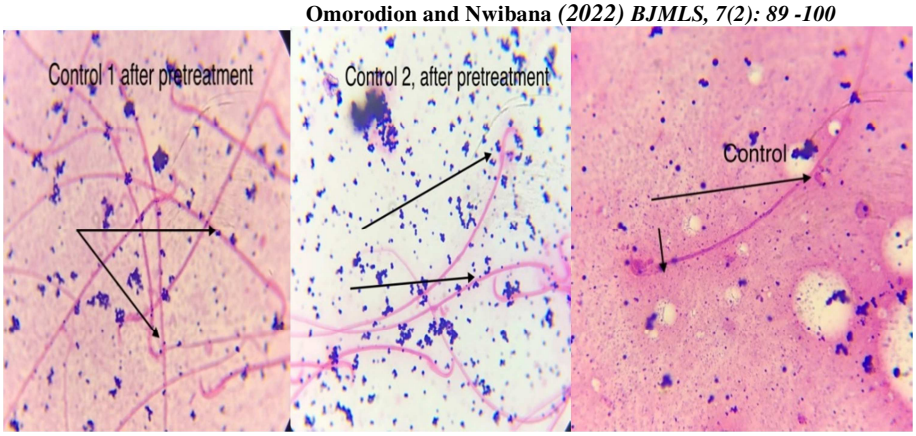


Plate 3: Sperm cell morphology with form sperm cells in untreated rats previously treated with nitrofuratoin . Note the normal shape of head and tail. Leishman and eosin stain. X 1000mag

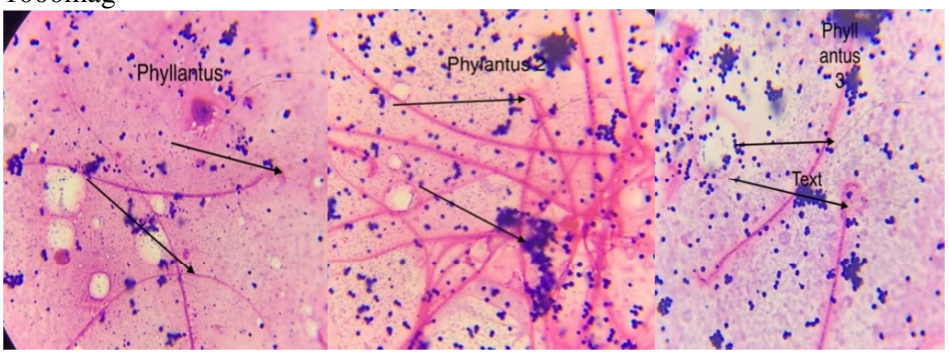


Plate 4: Sperm cell morphology of rat treated with *Phyllanthus niruri* after pretreatment with nitrofuratoin . Note the normal shape of head and tail. Leishman and eosin stain. X 1000mag

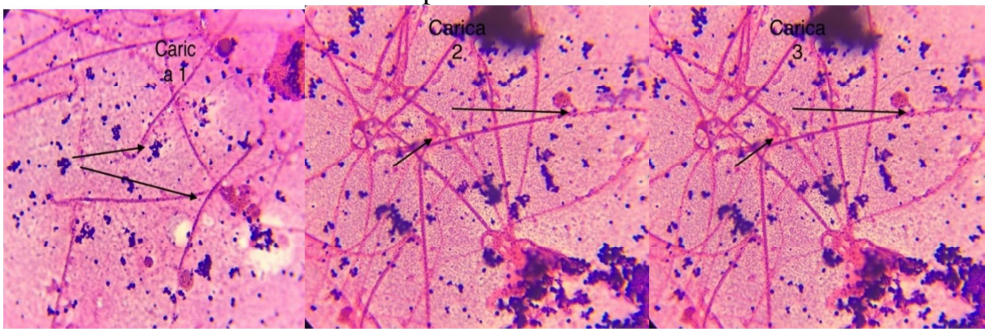


Plate 4: Sperm cell morphology of rats treated with *Carica p.* with form sperm cells. Note the normal shape of head and tail.Leishman and eosin stain. X 1000mag

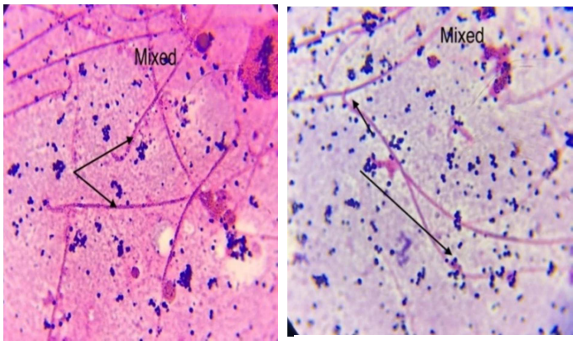
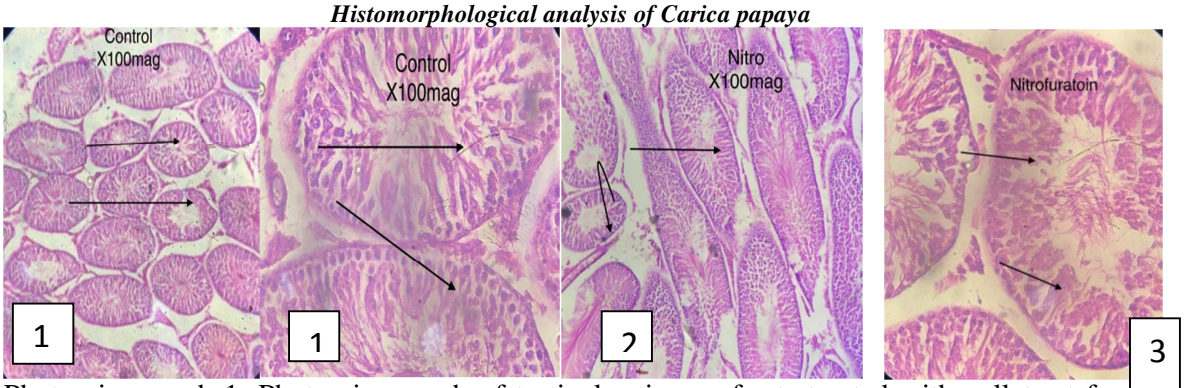
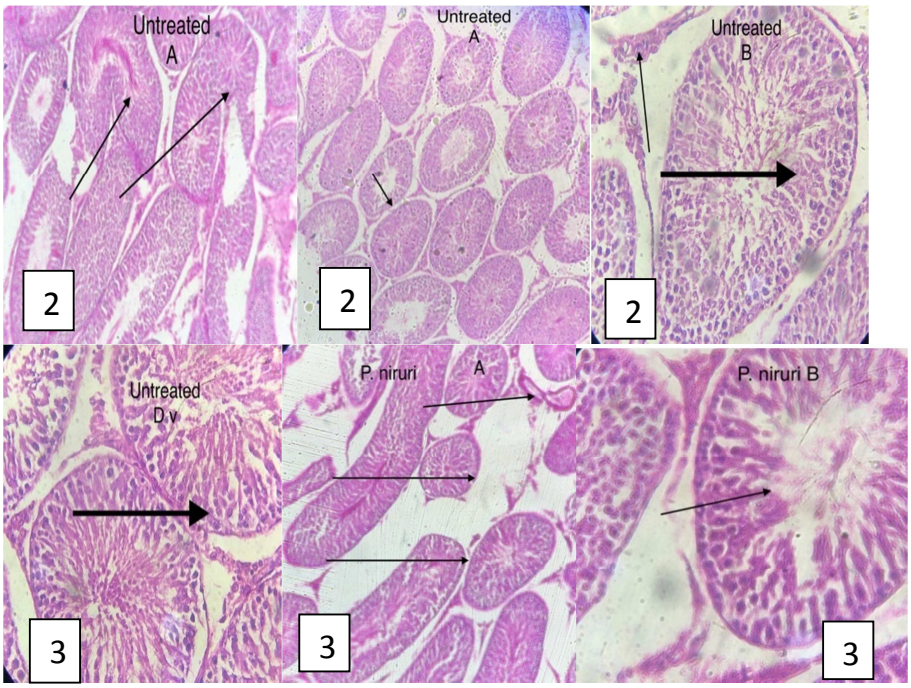


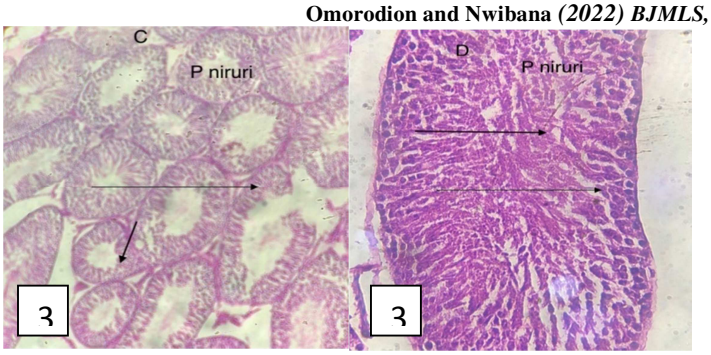
Plate 5: Sperm cell morphology of rats treated with equal mixture of *P. niruri*. And *Carica papaya.* Note the normal shape of head and tail. Leishman and eosin stain. X1000mag



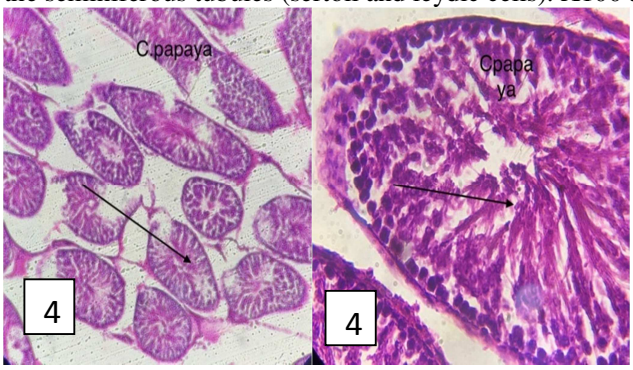
Photomicrograph 1: Photomicrograph of testicular tissue of rats treated with pellet rat feed and water only. Note the clarity of the features seminiferous tubules (sertoli and leydig cells). X100 and 400mag. H and E, Photomicrograph of testicular tissue of rats treated with nitrofuratoin, pelleted rat feed and water only. Note the erosion of the features and cells in the seminiferous tubules (sertoli and leydig cells). X100 and 400mag. H and E



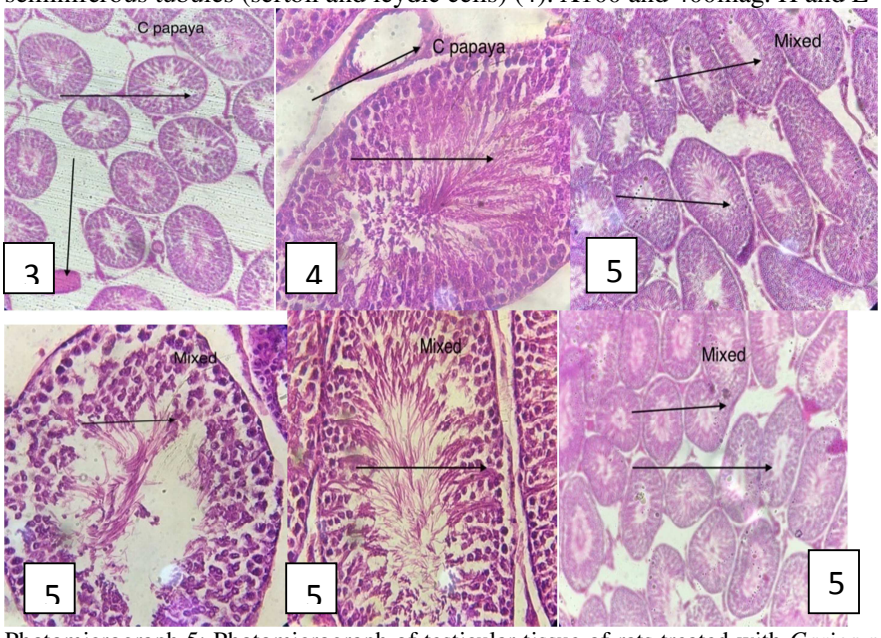
Photomicrograph 2: Photomicrograph of testicular tissue of rats treated with pellet rat feed and water only after pretreatment with nitrofuratoin. Note the slight erosion of the features and cells in the seminiferous tubules (sertoli and leydig cells). Photomicrograph of testicular tissue of rats treated with pellet rat feed and water only after pretreatment with nitrofuratoin. Note the slight degeneration in seminiferous tubules (sertoli and leydig) (2), Photomicrograph of testicular tissue of rats treated with *Phyllanthus niruri,* pellet rat feed and water only after pretreatment with nitrofuratoin. Note the remarkable features and cells in the seminiferous tubules (normal sertoli and leydic cells) (3). X100 and 400mag. H and E



Photomicrograph 3: Photomicrograph of testicular tissue of rats treated with *Phyllanthus niruri,* pellet rat feed and water only after pretreatment with nitrofuratoin. Note the remarkable features and cells in the seminiferous tubules (sertoli and leydic cells). X100 and 400mag. H and E



Photomicrograph 4: Photomicrograph of testicular tissue of rats treated with *Carica papaya,* pellet rat feed and water only after pretreatment with nitrofuratoin. Note the remarkable features and cells in the seminiferous tubules (sertoli and leydic cells) (4). X100 and 400mag. H and E



Photomicrograph 5: Photomicrograph of testicular tissue of rats treated with *Carica papaya,* pellet rat feed and water only after pretreatment with nitrofuratoin. Note the remarkable cellular architectures with intact seminiferous tubules (sertoli and leydic cells), Photomicrograph of testicular tissue of rats treated with a mixture of *Phyllanthus niruri.+ Carica papaya.,* pellet rat feed and water only after pretreatment with nitrofuratoin. Note the remarkable cellular architectures with intact seminiferous tubules (sertoli and leydiccells), Photomicrograph of testicular tissue of rats treated with a mixture of *P. niruri.+ C. papaya.,* pellet rat feed and water only after pretreatment with nitrofuratoin. Note the remarkable cellular architectures with intact seminiferous tubules (sertoli and leydic cells). X100 and 400mag. H and E

DISCUSSION

In our study, the seminiferous tubule of the testicular tissues, which can be seen to showed signs of cellular degeneration. Additionally, there was a significant decrease in the total sperm count ($P < 0.05$), a progressive increase in immotile sperm cells ($P < 0.05$). Researchers have noted a slight improvement in the total sperm count and motility after the administration of nitrofuratoin was stopped, suggesting that the drug's constituents may be to blame for the group's decreased progressive motility, sperm count, and testicular weight.

The decrease in testicular cells, reduction in motility count, total seminal count and morphology corroborated with the findings of the National Toxicology Program, (1989) wherein, they found out that, there were degenerative cells in the seminiferous tubules of nitrofurantoin-treated rats, which was consistent (National Toxicology Program, 1989). In two-year studies by the same organization, testicular atrophy, aspermatogenesis, degeneration of the germinal epithelium and atypical cells, and epididymis depletion at increased incidences in high-dose male mice were all observed. It is therefore known that nitrofuratoin can affect sperm production as well as have detrimental effects on the architectures of the testicular tissues (National Toxicology Program, 1989)

Cohen, (1978), also in their research findings, noted, that testes are poisoned by nitrofurantoin (Cohen, 1978). Rats given nitrofurantoin by gastric intubation at doses of 10 or 85 mg/kg body weight per day for a month displayed a decrease in spermatogenesis, primarily at the primary spermatocyte stage; spermatogonia also experienced some deleterious effect (Cohen, 1978).

There were decrease in sperm count and progressive motility but increased in non-progressive motility (sluggish) and immotile sperm cells but not significant in animals previously treated with nitrofuratoin (untreated with extract) but increased in comparison with nitrofuratoin treated rats.

There were significant increase ($P < 0.05$) in the sperm count and progressive motility in comparison with untreated and control, slight increase in non-progressive sperm cells in comparison with the control. There were decrease in non-progressive sperm cell in comparison with control and untreated rats previously treated with nitrofuratoin, decrease in immotile but not significant in comparison with control and untreated.

There were increased (($P < 0.05$) in total sperm count, progressive motility were observed in rats treated with *C. papaya, P. niruri* and the combine extract (*C. papaya* and *P. niruri*). The sperm cells were better improved with the combine extract in comparison with singular administration. There were unformed sperm cell morphology (tailless sperm cell and curvy sperm cell). The sperm cells are well formed in the rats treated with *C. papaya, P. niruri* and combine extract with normal head and tail.

The results of this study's histopathology were in line with those of Dosumu et al. (2014), Szeto et al. (2002), and Oremusu and Akang (2015), who discovered that ethanol toxicity in animals causes extensive cellular necrosis, seminiferous tubule degeneration, and defoliation (Dosumu, et al., 2014; Szeto, et al., 2002; Oremusu and, Akang 2015)

CONCLUSION

Combination of these two herbal medicines has shown to be very effective in relieving the effect of some drugs and having reparative potential on damage testicular tissue. These traditional herbs if given in right dosage can go a long way in solving some of our little health issues. *P. niruri and C. papaya* can also serve as a source of food or supplement due to its richness in phytonutrients. From our research findings, it can be said that *C. papaya* and *P, niruri* is more effective and efficient in treatment when combined than when used separately.

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