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Combined effect of ethanolic leaf extracts of *Carica papaya* and *Newbouldia laevis* on the histology of testes of Alloxan-induced diabetic rats

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Abstract

Objective: This study was carried out to investigate the combined effect of ethanolic leaf extracts of *C. papaya* and *N. laevis* on the histology of testes of alloxan-induced diabetic rats.

Methodology: Forty male wistar rats weighing 150 -180 g were procured, acclimatized for two weeks, after which were divided into eight groups of five rats each, and housed in cages. The groups were designated as groups A - H. Group A served as the control group, and received distilled water only. Animals in groups B – H were induced with diabetes using alloxan. Diabetic group B received no treatment throughout the experiment, while the diabetic groups C - H received 400 mg/kg of *C. papaya* leaf extract, 600 mg/kg of *C. papaya* leaf extract, 400 mg/kg of *N. laevis* leaf extract, 600 mg/kg of *N. laevis* leaf extract, 200 mg/kg of *C. papaya* + 200 mg/kg of *N. laevis* , 300 mg/kg of *C. papaya* + 300 mg/kg of *N. laevis* leaf extract respectively for 21 days via oral route with the aid of oral gastric tube. On the 22nd day, the animals were sacrificed via chloroform inhalation, and testes were harvested for histological studies.

Result: Histopathological findings showed moderate spermatogenic arrest with interstitial hemorrhage (H) within the basal layer; mild regeneration with moderate spermatogenic arrest (SA), moderate vacoulation (V) of the sertoli cells and interstitial hemorrhage (IH); moderate regeneration with mild spermatogenic arrest (SA), mild vacoulation (V) of the sertoli cells and moderate interstitial hemorrhage (IH); moderate regeneration with active seminiferous tubules, and well enhanced spermatogenesis though there were mild pyknotic (P) appearance of the sertolli cells in some areas and interstitial hemorrhage; mild regeneration with moderate atrophy (A) of the seminiferous tubules, mild arrest of the spermatogenesis (SA) and interstitial hemorrhage (IH); moderate healing with disorganization of the seminiferous tubules and interstitial fibrosis (IF); and moderate healing with distortion (D) of the seminiferous tubules, interstitial fibrosis (IF) and mild arrest of the spermatogenesis (AS) on the histology of testes in groups B - H that received variable doses of *C. papaya* and *N. laevis* leaf extracts respectively.

Conclusion: Combined leaf extracts of *Carica papaya* and *Newbouldia laevis* leaf extracts have antidiabetic and ameliorating effects on the histology of testes of alloxan induced wistar rats.

Keywords: *Carica papaya*; *Newbouldia laevis*; Testes; Alloxan

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1. Introduction

Diabetes mellitus refers to a group of metabolic disorders characterized by chronic hyperglycemia with abnormalities in the metabolism of carbohydrate, lipid, and protein resulting from defects in insulin secretion, action, or both [1], which occurs either when the pancreas does not produce enough insulin (a hormone that regulates blood sugar) or when the body cannot effectively use the insulin it produces [2]. It is characterized by excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger, weight loss, vision changes, and fatigue; and these symptoms may occur suddenly [2]. About 422 million people worldwide have diabetes, with majority of them living in low-and middle-income countries; and 1.5 million deaths are directly attributed to the disease each year [3]. Hyperglycemia or raised blood sugar which is a common effect of uncontrolled diabetes over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels [2]. Many organs can be affected, particularly the brain causing stroke, eyes (diabetic retinopathy) causing blindness, heart causing heart attack, Kidneys (diabetic nephropathy) causing chronic kidney disease, and Nerves (diabetic neuropathy) causing decreased sensation in feet [4]. Also, reproductive complications such as disruption of male fertility, impotence, retrograde ejaculation and hypogonadism may occur due to damage to the beta cells of the pancreas which secret pancreatic insulin [5]. Prolonged DM may be associated with sexual dysfunctions like erectile and testicular dysfunctions, reduced libido, retrograde ejaculation [6], disrupted endocrine control of spermatogenesis [7], impaired sperm DNA integrity [8], reduced sperm count and motility [9]; and low serum testosterone [10] DM induces many functional and structural syndromes and complications in multiple organs, such as testis, brain, heart and retina [11, 12, 13]. Studies have revealed that reduced testicular function was widely observed in animals with hyperglycemia [14], and that in human with diabetes, spermatogenesis, sperm count, sperm motility, seminal fluid volume and testosterone levels are lower compared to healthy individuals [15, 16, 17]. Also, DM can affect the development of sperm and the production of androgens, which finally causes male infertility [18]. Erectile dysfunction, damage to the autonomic nervous system and urologic issues can occur in men with diabetes due to diabetic nerve damage [19].

Medicinal plants according to Ahn, [20] and Smith-Hall *et al.,* [21] are plants that are used to attempt to maintain health by administering them for a specific condition, or both, whether in modern medicine or in traditional medicine. The evaluation of medicinal plants used traditionally in treating diabetes is of growing interest [22, 23]. World Health Organization recommended and encouraged this practice especially in countries where access to conventional treatment of diabetes is inadequate [24], and also has however emphasized the fact that safety should be the over-riding criteria in the selection of herbal medicine for use in healthcare [25]. Such medicinal plants include *Carica papaya* (*C. papaya*) and *Newbouldia laevis* (*N. laevis*).

C. papaya is one of the 22 accepted species in the genus *Carica* of the family *Caricaceae that* is scientifically known as *Carica papaya Linn* [26]. It originates in the tropics of the Americas, perhaps from Central America and southern Mexico [27], and spread during the 16th century by Spanish explorers, from Central America to the Caribbean and South-East Asia. Its common names include *Papaya,* Pawpaw, Kates and Papaw [28]. *C. papaya* is now widespread in the tropics (between 32°N and S) in South America, Africa and Asia, and in the warm subtropics (Oceania). *Papaya* plant is a large, single–stemmed herbaceous perennial tree having 20–30 feet height. Its leaves are very large (up to 2½ feet wide), palmately lobed or deeply incised with entire margins and petioles of 1-3 feet in length. Its stems are hollow, light green to tan brown in color with diameter of 8 inches and bear prominent of scars [28]. According to Ansah *et al.,* [29] the LD_{50} (lethal dose) of the aqueous leaf extract of *C. papaya* is above 5000 mg/kg. Papaya leaf extract has strong medicinal properties such as antibacterial, antiviral, antitumor, hypoglycaemic and anti-inflammatory activity [30]. Research has proven that *C. papaya* leaf extract has antidiabetic [31], hypoglycemic [32, 33], antioxidant, immunomodulatory, hypolipidemic [33], hepatoprotective effects [34, 35], antidiabetic [36] and is also helpful in preventing diabetic complications by dyslipidemia improvement [37].

N. laevis is a medium sized angiosperm in the *Bignoniaceae* family, found in the tropical Africa, and grows to a height of about 10 meters with a cauliferous habit, and is ever green, though its leaves turn somewhat dark purple during the cold seasons [38]. Different African countries have different names for the plant, for example Togo call it lifui, Ghana call it sesemasa, Hausa call it Aduruku, Igbo call it ogilisi or ògírìsì, Senegal call it gimgid, Gambia call it kallihi, Yoruba call it Akoko, Guinea call it canhom, Urhobo call it Ogiriki, Sierra Leone call it Sherbro, Mali call it kinkin, Edo state call it íkhímì, Tiv call it Kontor, while the Ibibio call it itömö [38]. Its phytochemical screening revealed the presence of flavonoids, terpenoids, tannin, alkaloids, phytic acid, trypsin inhibitor, phenols, antioxidants, carotenoids, oxalate and cyanide [39]. It is used by African traditional healers to treat various ailments like diabetes, rheumatism and toothache, in Nigeria, herbalists' use decoctions of the bark to treat epilepsy and convulsions in children, the leaves are soaked in ethanol for the treatment of diabetes and sickle cell disease [40]. Studies have shown that some of its medical uses include folk treatment of fevers (including yellow fever), malaria, stomach ache, cough, sexually transmitted infections, skin infections, tooth ache, breast cancer, constipation, pain (pelvic pain in females, chest pain, ear ache), gonococcal orchitis,

elephantiasis, sorefeet, ulcer, epilepsy, convulsion, migraine, sickle cell anaemia, as a febrifuge, as a vermifuge, in female reproductive healthcare (fibroids, infertility, hemorrhage), as aphrodisiacs, eye problems, snake bites, wound healing, diabetes, arthritis, rheumatism and other inflammatory conditions [41, 42, 43, 44 and 45]. Its leaf extract is locally used amongst the Binins, Ibos and Yourubas speaking areas in Nigeria to treat diabetes mellitus [46]. According to Osigwe *et al.,* [47], *N. laevis* leaf possesses the ability of managing hyperglycemia, improve haematological and biochemical derrangements in alloxan induced-diabetic rats, control muscle wasting and induce adipogenesis and also has antidiabetic effect. Also, its different parts have antioxidant and free radical scavenging [48], antimicrobial and antimalarial [49], sedative and anticonvulsant [50], analgesic, antinociceptive and antiinflamatory [51], hepatoprotective [35], anticancer [40], uterine contraction [52], wound healing and antiulcer [53], antisickling [54], hypoglycemic [55] activities among others. Leaf extract of *N. laevis* can be used to manage hepatotoxicity and testicular toxicity [56], exhibits protective effect on testicular damage [57], improves erectile function [58], prevents oxidative stress in testes and improves fertility outcomes [59]. It can also boost FSH and LH levels [60] which act on Sertoli and Leydig cells respectively stimulating the process of spermatogenesis.

Lastly, research studies have shown that combined leaf extracts of *C. papaya* and *N. laevis* have ameliorating effects on the histology of liver [61], kidney [62] and cerebellum [63], lipid profile [64], body weight [65], serum levels of urea and creatinine [65]**,** exhibits hepatoprotective effect [35], increases serum levels of hematological parameters and sperm quality [66] of alloxan-induced diabetic rats.

Therefore, this study was carried out to investigate on the combined effect of leaf extracts of *C. papaya* and *N. laevis* on the histology of testes of alloxan-induced diabetic wistar rats since no work has been carried out on it.

2. Material and methods

2.1. Animal procurement, care and treatment

Forty (40) male wistar rats weighing between 150 g to 180 g were procured and housed at the Animal house of Anatomy Department, Abia State University; Uturu with wire gauze cages in a well-ventilated area, were maintained under standard laboratory conditions of temperature (22+2 ^{0}C), relative humidity (55-65 %) and 12 hours light/dark cycle. They were fed with standard commercial pellet diet and water *ad libitum* and were also acclimatized for two weeks before the experiment. Their health statuses were closely monitored before and during the experiment. All procedures were carried out in strict accordance with the Institutional guidelines on the care and use of experimental animals.

2.2. Collection, identification and preparation of plant material

Fresh leaves of *C. papaya* and *N. lavis* leaves were plucked from a farm at Elughu Nkporo in Ohafia L.G.A., Abia State, and were authenticated at Herbarium unit, Botany Department, Abia State University, Uturu, Abia State. The leaves were air dried and crushed using laboratory blender. Extractions were done using ethanol. The crude ethanol extracts were kept in an air-tight container and stored in a refrigerator at 4 ^{0}C until time of use. At the time of use, the ethanol extracts were filtered into a stainless basin with a white cloth and placed in a water bath so as to dry up the ethanol. 250 mg of these extracts /kg body weights were dissolved in 10 mls of distilled water and were administered to the animals.

2.3. Induction of diabetes

The rats were divided into non-diabetic control group and experimental groups. The baseline blood glucose level of the experimental group to be inducted was determined before the induction of diabetes. The rats were allowed to fast over night prior to injection of alloxan and diabetes was induced by intra-peritoneal administration of 150 mg of alloxan per kg body weight of rat (150 mg/kg body weight) [67]. After the induction, the rats were allowed to have free access to the same feed and water. After 72 hours, blood samples obtained through tail tip puncture of the rats were used to confirm diabetes in the rats by testing for hyperglycemia using Glucometer. Diabetes was confirmed at fasting blood glucose levels greater than 200 mg/dl [68].

2.4. Experimental protocol

The animals were grouped into eight (8) groups of five (5) rats each. Different doses of the leaf extracts were administered via oral route with the aid of oral gastric tube as shown below:

- **Group A** The control group + distilled water.
- **Group B** Diabetic group + No treatment
- **Group C** Diabetic + 400 mg/kg of *C. papaya* leaf extract.

- **Group D** Diabetic + 600 mg/kg of *C. papaya* leaf extract.
- **Group E** Diabetic + 400 mg/kg of *N. laevis* leaf extract.
- **Group F** Diabetic + 600 mg/kg of *N. laevis* leaf extract.
- **Group G** Diabetic + 200 mg/kg of *C. papaya* and 200 mg/kg of *N. laevis* leaf extracts.
- **Group H** Diabetic + 300 mg/kg of *C. papaya* and 300 mg/kg of *N. laevis* leaf extracts.

Acute toxicity tests of ethanolic leaf extracts of *C. papaya* and *N. laevis* were carried out and results obtained were calculated to be above 6,000 mg/kg body weight and 5000 mg/kg body weight respectively using the methods employed by Lorke [69] and Nofal *et al.,* [70].

2.5. Sample collection and analysis

The extracts were administered for twenty-one (21) days. On the 22nd day, the animals were sacrificed by anaestethizing under chloroform vapour and dissected. Testes harvested from the wistar rats, and were fixed in 10% formal saline for four hours. This was followed by histological and histochemical methods of tissue processing.

3. Results

3.1. Histopathological findings

Micrograph 1 is the result of the microscopic examination of the testicular cells of the animals in group A (GPAR1R2) (x400) (H/E) showing normal testicular architecture with seminiferous tubules that are lined with interstitial cells of the leydig (ICL), sertoli cell (SC) and well enhanced spermatogenesis (WES).

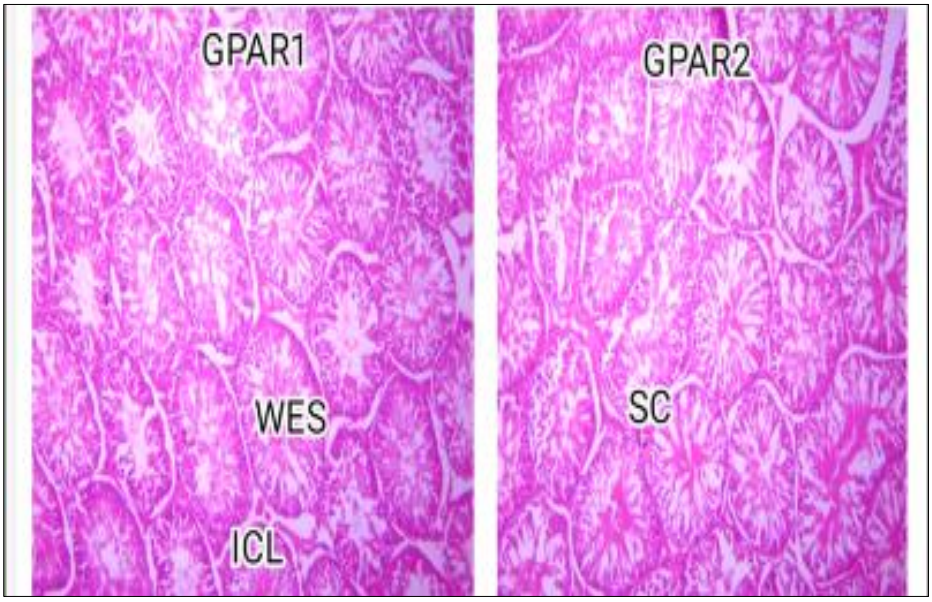

Figure 1 Micrograph 1 showing normal testicular architecture with seminiferous tubules that are lined with interstitial cells of the leydig (ICL), sertoli cell (SC) and well enhanced spermatogenesis (WES)

Micrograph 2 is the result of the histology of the testicular cells of the animals in group B (GPBR1R2) induced with alloxan only (x100) (H/E) without treatment showing moderate spermatogenic arrest with interstitial hemorrhage (H) within the basal layer.

Micrograph 3 is the result of the histology of testicular cells of the animals in group C (GPCR1R2) induced with alloxan and treated with 400 mg/kg of *C. papaya* leaf extract (x100) (H/E) showing mild regeneration with moderate spermatogenic arrest (SA), moderate vacuolation (V) of the sertoli cells and interstitial hemorrhage (IH).

Micrograph 4 is the result of the histology of testicular cells of the animals in group D (GPDR1R2) induced with alloxan and treated with 600 mg/kg of *C. papaya* leaf extract (x100) (H/E) showing moderate regeneration with mild spermatogenic arrest (SA), mild vacuolation (V) of the sertoli cells and moderate interstitial hemorrhage (IH).

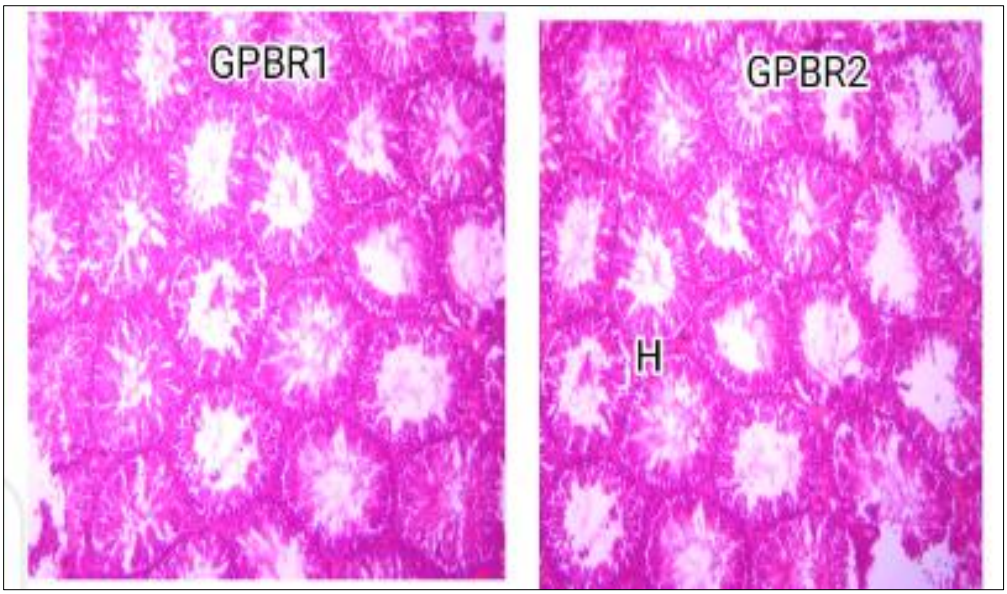

Figure 2 Micrograph 2 showing moderate spermatogenic arrest with interstitial hemorrhage (H) within the basal layer

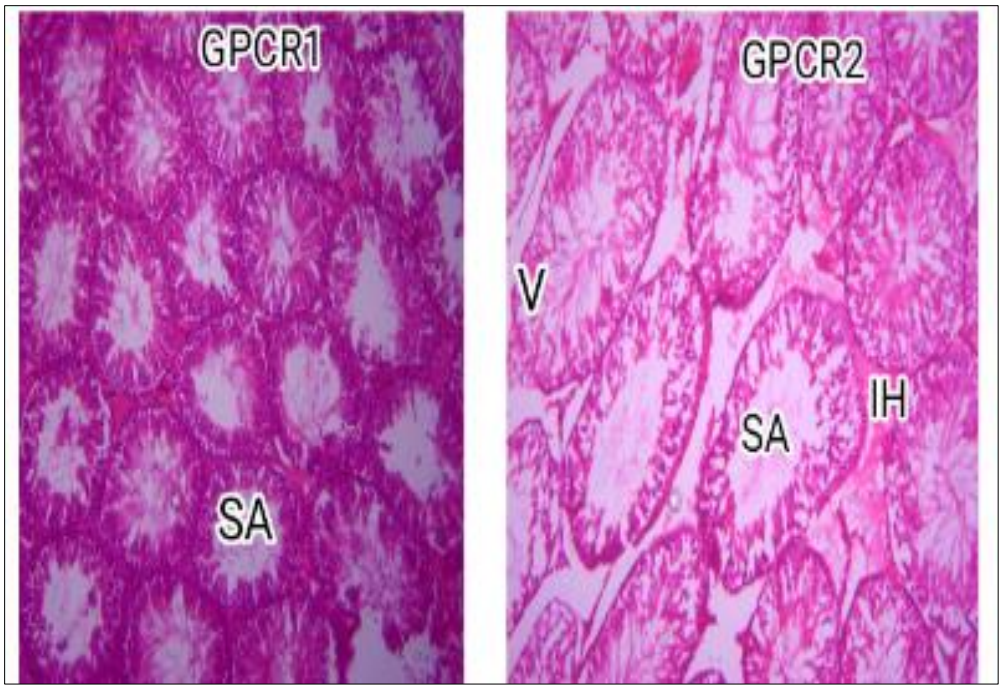

Figure 3 Micrograph 3 showing mild regeneration with moderate spermatogenic arrest (SA), moderate vacuolation (V) of the sertoli cells and interstitial hemorrhage (IH)

Micrograph 5 is the result of the histology of testicular cells of the animals in group E (GPER1R2) induced with alloxan and treated with 400 mg/kg of *N. laevis* leaf extract (x100) (H/E) showing moderate regeneration with active seminiferous tubules, and well enhanced spermatogenesis. However, there are mild pyknotic (P) appearance of the sertolli cells in some areas and interstitial hemorrhage (H).

Micrograph 6 is the result of the histology of testicular cells of the animals in group F (GPFR1R2) induced with alloxan and treated with 600 mg/kg of *N. laevis* leaf extract (x100) (H/E) showing mild regeneration with moderate atrophy of the seminiferous tubules, mild arrest of the spermatogenesis (SA) and interstitial hemorrhage (IH).

Micrograph 7 is the result of the histology of testicular cells of the animals in group G (GPGR1R2) induced with alloxan and treated with 200 mg/kg of *C. papaya* + 200 mg/kg *N. laevis* leaf extract (x100) (H/E) showing moderate healing with disorganization of the seminiferous tubules and interstitial fibrosis (IF).

Micrograph 8 is the result of the histology of testicular cells of the animals in group H (GPHR1R2) induced with alloxan and treated with 300 mg/kg of *C. papaya* + 300 mg/kg *N. laevis* leaf extract (x100) (H/E) showing moderate healing with disorganization of the seminiferous tubules, and interstitial fibrosis (IF).

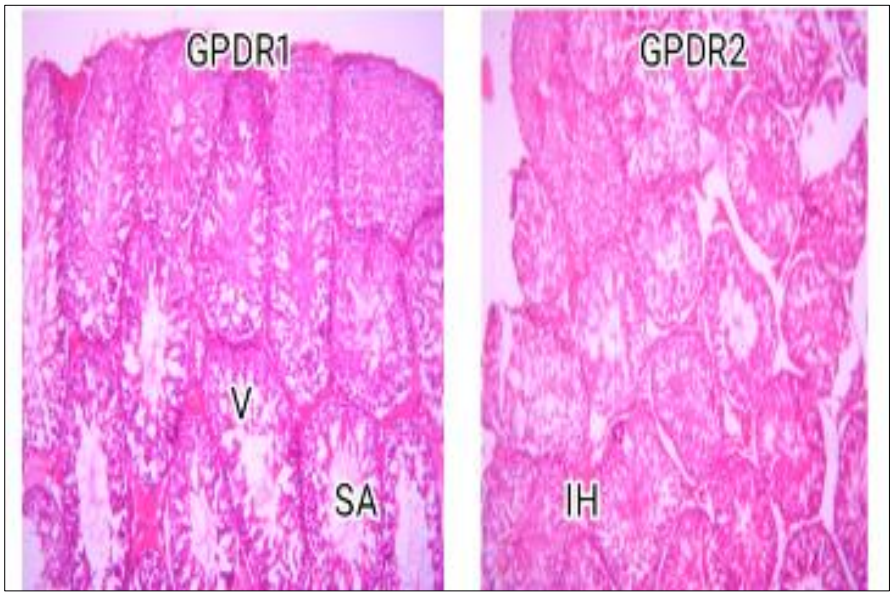

Figure 4 Micrograph 4 showing moderate regeneration with mild spermatogenic arrest (SA), mild vacuolation (V) of the sertoli cells and moderate interstitial hemorrhage (IH)

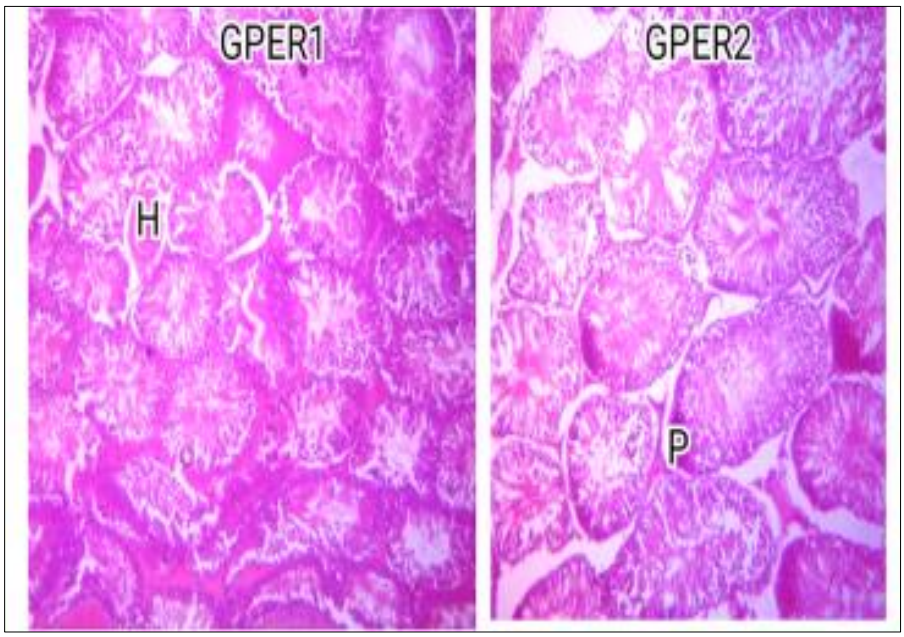

Figure 5 Micrograph 5 showing moderate regeneration with active seminiferous tubules, and well enhanced spermatogenesis. However, there are mild pyknotic (P) appearance of the sertolli cells in some areas and interstitial hemorrhage (H)

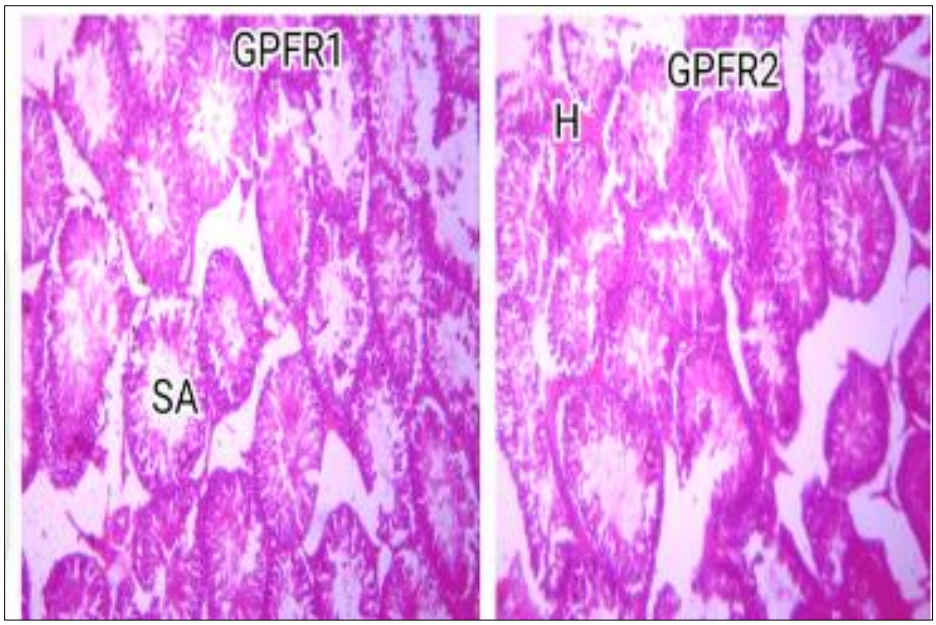

Figure 6 Micrograph 6 showing mild regeneration with moderate atrophy of the seminiferous tubules, mild arrest of the spermatogenesis (SA) and interstitial hemorrhage (IH)

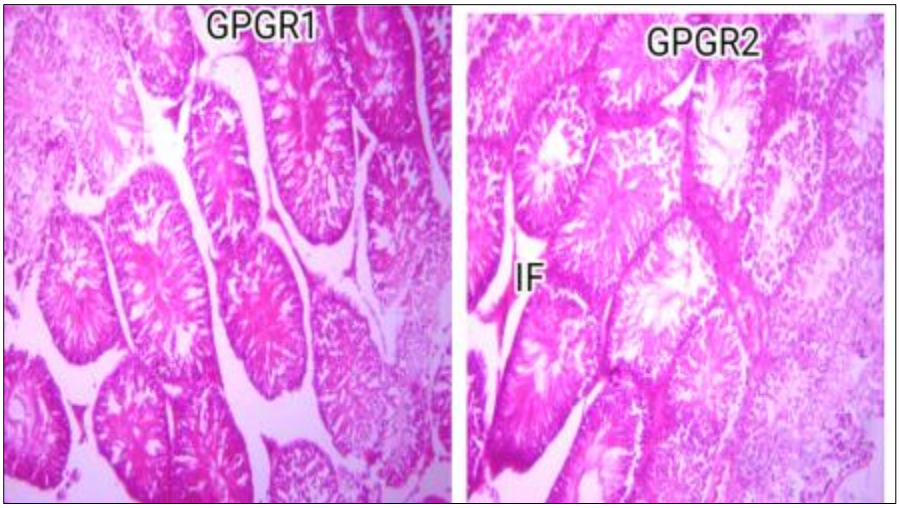

Figure 7 Micrograph 7 showing moderate healing with disorganization of the seminiferous tubules and interstitial fibrosis (IF)

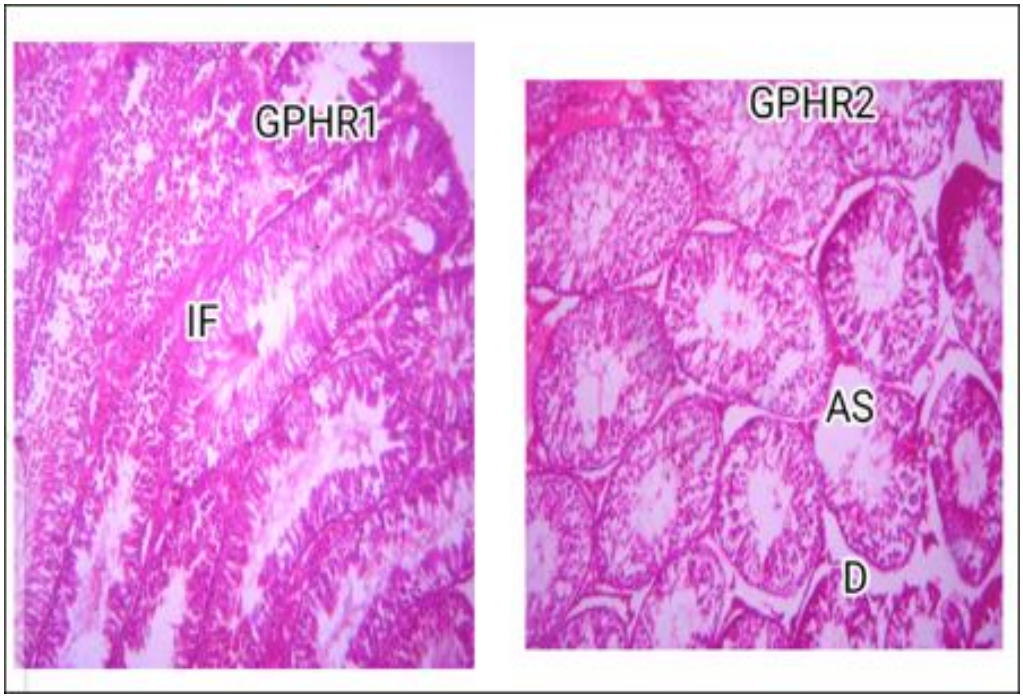

Figure 8 Micrograph 8 showing moderate healing with disorganization of the seminiferous tubules, and interstitial fibrosis (IF)

4. Discussion

The histopathological finding of the present study of group A (GPAR1R2) (x400) (H/E) (Micrograph 1) indicated normal (testes) showed normal testicular architecture with seminiferous tubules that are lined with interstitial cells of the leydig (ICL), sertoli cell (SC) and well enhanced spermatogenesis (WES). In a normal testicular architecture, there exist a normally functioning hypothalamic pituitary gonadal axis in which the hypothalamus releases GnRH pulses that stimulate the pituitary to secrete both luteinizing hormone (LH) and follicular stimulating hormone (FSH). LH and FSH act on the Sertoli cells and the Leydig cells, respectively, to stimulate the process of spermatogenesis [71].

The histopathological finding of group B (Micrograph 2) indicated moderate spermatogenic arrest with interstitial hemorrhage (H) within the basal layer. This could be attributed to the alloxan monohydrate which damages the pancreatic cells leading to diabetes and its effects on the testicular cells. According to Lenzen *et.al*, [72] alloxan monohydrate induces diabetes in the rats by destroying the insulin producing beta-cells of the pancreas causing cell necrosis. However, the results of these researchers suggest that the testicular morphological alterations that were observed during the early stages of treatment with alloxan or streptozotocin may be more related to the toxic action of these drugs than to the effects of diabetes mellitus [73]. The diabetic condition in this group which is left untreated could have led to low serum testosterone with low LH and FSH, hypercholesterolemia, hypertriglyceridemia, hypoalbuminemia and increased oxidative stress as seen in diabetic men [74]. It has been shown that there exists a link between diabetes and low testosterone, men with diabetes are more likely to have low testosterone; and men with low testosterone are more likely to later develop diabetes [75]. Testosterone helps the body's tissues take up more blood sugar in response to insulin, men with low testosterone more often have insulin resistance and need to produce more insulin to keep blood sugar normal [75]. Research has revealed that the onset of Type I diabetes disrupts the HPG axis, resulting in impaired spermatogenesis and subsequent sub-fertility, thus disruptions in any part of the HPG axis impair fertility [71].

Micrographs 3 and 4 treated with 400 mg/kg and 600 mg/kg of *C. papaya* leaf which showed mild regeneration with moderate spermatogenic arrest (SA), moderate vacuolation (V) of the sertoli cells and interstitial hemorrhage (IH) and moderate regeneration with mild spermatogenic arrest (SA), mild vacuolation (V) of the sertoli cells and moderate interstitial hemorrhage (IH) respectively when compared with the control group could be due to anti-diabetic and healing/ameliorating effects as the leaf extract has proven to have ameliorative effect on the histology of liver of alloxan-induced Wistar rats [57]. The administration of the leaf extract of *C. papaya* to this group could have ameliorated the effect of the induced-alloxan thereby resulting to the mild regeneration with moderate spermatogenic arrest (SA), moderate vacuolation (V) of the sertoli cells and interstitial hemorrhage (IH) and moderate regeneration with mild spermatogenic arrest (SA), mild vacuolation (V) of the sertoli cells and moderate interstitial hemorrhage (IH)

respectively. This could also be due to the ameliorating effect of the leaf extract to the HPG axis which enhances the release of GnRH pulses from the hypothalamus, thus stimulating the pituitary to secrete both luteinizing hormone (LH) and follicular stimulating hormone (FSH). The released LH and FSH act on the Sertoli cells and the Leydig cells, respectively stimulating the process of spermatogenesis. The result of this research showed that *C. papaya* leaf at low dose in few days may not deter fertility as this may affirm to its use for decades [76].

The result of Micrographs 5 and 6 treated with 400 mg/kg and 600 mg/kg of *N. laevis* leaf extracts showed moderate regeneration with active seminiferous tubules, and well enhanced spermatogenesis. However, there are mild pyknotic (P) appearance of the sertolli cells in some areas and interstitial hemorrhage (H); and mild regeneration with moderate atrophy of the seminiferous tubules, mild arrest of the spermatogenesis (SA) and interstitial hemorrhage (IH) when compared with group A could be due to anti-diabetic and healing/ameliorating effects as the leaf extract. Research has shown that the ethanolic leaf extract of *N. laevis* can be used to manage hepatotoxicity and testicular toxicity [56], exhibits protective effect on testicular damage [57], prevents oxidative stress in testes and improves fertility outcomes [59] and improves erectile function [58]. *N. laevis* can also boost the FSH and LH levels [53] which act on the Sertoli cells and the Leydig cells, respectively stimulating the process of spermatogenesis. The leaf extract of *N. laevis* could have ameliorated effect of the induced-alloxan to the HPG axis by enhances the release of GnRH pulses from the hypothalamus, thereby stimulating the pituitary to secrete both luteinizing hormone (LH) and follicular stimulating hormone (FSH) and serum insulin. Research has shown that serum insulin has long been known to affect the central nervous system, and these effects could mediate whole body energy homeostasis, including the reproductive axis through further signaling to the pituitary and ultimately, the gonads [5].

Micrographs 7 and 8 treated with 200 mg/kg of *C. papaya* + 200 mg/kg *N. laevis* and 300 mg/kg of *C. papaya* + 300 mg/kg *N. laevis* leaf extracts showed moderate healing with disorganization of the seminiferous tubules and interstitial fibrosis (IF); and moderate healing with disorganization of the seminiferous tubules, and interstitial fibrosis (IF) respectively when compared the control group A could be due to the anti-diabetic and healing/ameliorating effects as the leaf extracts. The combined leaf extracts of *C. papaya* and *N. laevis* could have moderately ameliorated effect of the induced-alloxan to normalcy. According to Schoeller *el al.,* [71], in a normally functioning hypothalamic pituitary gonadal axis, the hypothalamus releases GnRH pulses that stimulate the pituitary to secrete both luteinizing hormone (LH) and follicular stimulating hormone (FSH). The leaf extracts could have also enhanced serum insulin. Research has shown that serum insulin has long been known to affect the central nervous system, and these effects could mediate whole body energy homeostasis, including the reproductive axis through further signaling to the pituitary and ultimately, the gonads [71]. It has equally been revealed that the peripheral insulin injection caused an increase in insulin levels in cerebral spinal fluids, suggesting that insulin could potentially be a signal to the brain regarding energy stores and promoting whole-body energy homeostasis [77]. However, the combined leaf extracts produced better healing/ameliorating effect at lower dosages than the individual leaf extracts.

5. Conclusion

Combined leaf extracts of *Carica papaya* and *Newbouldia laevis* leaf extracts have antidiabetic and ameliorating effect on the histology of testes of alloxan induced wistar rats.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

Approved by Institutional ethical approval.

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