Combined Effect of Ethanolic Leaf Extracts of Carica Papaya and Newbouldia Laevis on Hematological Parameters and Sperm Quality of Alloxan-Induced Rats

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Abstract:

Objective*: This study was carried out to investigate the effect of ethanolic leaf extracts of *C. papaya* and *N. laevis* on hematological parameters and sperm quality of alloxan-induced diabetic wistar rats. Methodology: Forty male rats weighing 130-180g were procured, acclimatized for two weeks, after which, were divided into eight groups of five rats each, and were housed in cages. The groups were designated as groups A - H. Group A served as the control group and received distilled water only. Groups B – H were induced with diabetes using alloxan. Group B did not receive any treatment, while the groups C - H received 400mg/kg of *C. papaya* leaf extract, 600mg/kg of *C. papaya* leaf extract, 400mg/kg of *N. laevis* leaf extract, 600mg/kg of *N. laevis* leaf extract, 200mg/kg of *C. papaya* + 200mg/kg of *N. laevis,* and 300mg/kg of *C. papaya* + 300mg/kg of *N. laevis* leaf extract respectively for 21 days orally with oral gastric tube. On the 22nd day, the animals were sacrificed via chloroform inhalation and blood samples were collected through ocular puncture for hematological analyses, and epididymis were collected for sperm quality study. All data were tabulated and statistically analyzed using SPSS version 25.0. Result: The levels of WBC, RBC, HGB, PCV, sperm motility and sperm count were significantly (P<0.05) decreased in group B (48.0 ± 2.25) (28.0 ± 2.25) (24.0 ± 1.87) and (50.86 ± 3.18), and D (44.0 ± 3.39) (30.0 ± 2.92) (26.0 ± 2.0) and (42.44 ± 2.17) (for sperm motility and count) when compared to group A (71.0 ± 2.25) (15.0 ± 1.58) (14.0 ± 1.87) and (68.74 ± 2.30), and significantly (P<0.05) increased in groups F (89.0 ± 1.0) (6.0 ± 1.0) (5.0 ± 0.01) and (82.74 ± 3.19), G (91.0 ± 3.30) (5.0 ± 2.81) (4.0 ± 1.8) and (88.78 ± 2.50), and H (88.0 ± 1.60) (7.0 ± 0.80) (5.0 ± 0.01) and (80.92 ± 0.26) (for sperm motility and count), groups D, F, G and H (for WBC, HGB, PCV) and groups DEFGH (for RBC) when compared to the control group A. However, there was no significant difference on the levels of WBC, RBC, HGB, and PCV, sperm motility and sperm count for groups C and E when compared with the control group A. Conclusion: *C. papaya* and *N. laevis* leaf extracts have ameliorating effects on diabetes and increased serum levels of hematological parameters and sperm quality. The ameliorating effects of the combined doses to the diabetic rats were better at lower dosages than when the individual leaf extracts were administered.

***Keywords*: *Carica papaya, Newbouldia laevis,* hematological parameters, sperm quality**

I.INTRODUCTION

Diabetes can be defined as a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves [1]. According to WHO [1] about 422 million people worldwide have diabetes, with majority of them living in low-and middle-income countries; and with 1.5 million deaths are directly attributed to it each year. Both the number of cases and the prevalence of diabetes have been steadily increasing over the past few decades [1]. Its symptoms include excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger, weight loss, vision changes, and fatigue [2]. Hyperglycaemia or raised blood sugar is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels [2].

Uncontrolled diabetes mellitus (DM) is associated with multiple disorders including metabolic, cellular, and blood disturbances leading to vascular complications [3]. The underlying biochemical and hematological changes in type 2 diabetes mellitus (T2DM) patients may lead to the development of long-term complications and poor quality of life or death [4]. Hematological changes in diabetes may be caused by several factors including increased production of reactive oxygen species (ROS) and the formation of advanced glycation end products (AGEs) as a result of the long-term hyperglycemia. Increased production of ROS resulting in oxidative stress, which is implicated in tissue damage and

hematological changes such as (red blood cell) RBC dysfunction, platelets (PLT) hyperactivity, and endothelial dysfunction [5,6]. These hematological changes may lead to complications such as anemia, and a state of hypercoagulability, and contribute to cardiovascular disease (CVD) in diabetic patients [7]. Karaman *et al*, [8] disclosed that type 2 diabetes (T2DM) is a part of the metabolic syndrome that comprises dyslipidemia, obesity, hypertension, and changes in hematological parameters. While Antwi-Baffour *et al*, [9] opined that hematological changes encountered in T2DM patients include changes in the function, structure, and metabolism of red blood cells (RBCs), white blood cells (WBCs), platelet (PLT) and the coagulation systems. Waggiallah and Alzohairy, [10] stated that these changes may manifest as immunological and coagulation problems, and anemia characterized by a decrease in the RBC count, hemoglobin (Hgb) and hematocrit (Hct) level as compared to non-diabetic individuals. Anemia which is a common hematological change in patients with T2DM is often unrecognized, and the estimates of its prevalence vary widely [11.12, 13].

Diabetes mellitus being a life-long condition requires consistent management and tight glyceamic control to reduce the risk of development of complications sequel to DM [14]. Studies have shown that reproductive complications such as disruption of male fertility, impotence, retrograde ejaculation and hypogonadism are due damage to the beta cells of the pancreas which secret pancreatic insulin that regulate the male hypothalamic-pituitary-gonadal axis which is essential for fertility [15.16,17]. Also prolonged diabetes mellitus is associated with sexual dysfunctions ranging from erectile and testicular dysfunctions, reduced libido, retrograde ejaculation [18], disrupted endocrine control of spermatogenesis [19], impaired sperm DNA integrity [20], reduced sperm count and motility [21,22] and low serum testosterone [23]. Ramalho-Santos *et al.*, [24] also stated that one of the complications of diabetes mellitus is infertility which may be due to other underlying factors such as neuropathies and impaired blood flow that is usually seen in diabetic patients. For people living with diabetes, access to affordable treatment, including insulin, is critical to their survival and there is a globally agreed target to halt the rise in diabetes and obesity by 2025 [25].

Medicinal plants are plants that are used to attempt to maintain health, to be administered for a specific condition, or both, whether in modern medicine or in traditional medicine [26, 27]. The evaluation of medicinal plants used traditionally in treating diabetes is of growing interest [28, 29]. Thus, World Health Organization [30] recommended and encouraged this practice especially in countries where access to conventional treatment of diabetes is inadequate. Plant resources are veritable source of pharmaceuticals and therapeutics, but they have not been adequately documented [31]. Development of drugs for the treatment of diabetes mellitus is one of the major health problems in the world that requires experimental studies using diabetic and anti-diabetic agents [32, 33]. Such medicinal plants include *Carica papaya* and *Newbouldia laevis.*

C. papaya plant is a large, single–stemmed herbaceous perennial tree having 20–30 feet height, with its leaves 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 [34]. *Papaya* is a juicy and tasty fruit that belongs to the family *Caricaceae*. It is scientifically known as *Carica papaya Linn* [35]. It has its origin in the tropics of Americas, perhaps from southern Mexico and neighboring Central America [36]. Chinnappan *et al.*, [37] revealed that the phytochemical composition of its leaves include the presence of alkaloids, Carbohydrates, saponins, glycosides, Proteins and aminoacids, Phytosterol, Phenolic compounds, flavonoids, Terpinoids Terpinoids, and Tannins. Udoh *et al.*, [38] revealed that *papaya* leaves are made into tea as a treatment for malaria. Bergonio and Perez, [39] and Kavimandan and Saraf, [40] revealed that its leaves are used for colic, fever, beriberi, abortion, asthma in India, and cancer in Australia; and that the leaves are used traditionally in treatments of jaundice, malaria, dengue immunomodulatory and antiviral activity. They leaves also have medicinal properties like anti-inflammatory hypoglycaemic, anti-fertility, abortifacient, and hepatoprotective, wound healing, antihypertensive and antitumor activities [41]. *C. papaya* young leaves are used in cooking and are eaten like spinach in East Indies and are also used to treat stomach ache and serve as a tonic for heart disease; have antioxidant, anticancer, antiseptic and analgesic properties, immunomodulatory, antitumour, antiplasmodial, antibacterial and anti dengue; and also used in the treatment of wounds, burns and malignant tumours [42, 43]. Dried leaves of *papaya* are known as blood purifiers and taken as tonic. Nugroho *et al.*, [43] disclosed that *papaya* leaf tea is used to treat obesity [44]. It helps in losing weight, acts against chronic indigestion, high blood pressure and arteriosclerosis weakening of heart; while the fresh leaves are used to cure gastrointestinal problems and hepatic disease; acts as anti-inflammatory and natural abortion agent. In India, traditional Siddha medicinal systems prescribe *C. papaya L* leaf extract to patients with dengue fever symptoms According to Imaga and Adepoju [45], daily consumption of *papaya* leaves helps to prevent malaria in Indonesia, increases platelets, WBC and neutrophils counts (especially in dengue patients), while its dried leaves have been indicated in sickle cell anemia management. Extract of *C. papaya* reversed the damage associated with alloxan-induced diabetes revealing its hypoglycemic, liver and renal function integrity [46, 47]. *C. papaya* leaves exert insulin-like effect on peripheral tissues by either promoting glucose uptake metabolism [48], or stimulate regeneration process and revitalization of the remaining beta cells through the absorption of glucose into the muscle and adipose tissue [49] and also have significant hypoglycaemic, hepatoprotective and nephroprotective effects [50]. Studies have also revealed that *C. papaya* leaf extract has the ability to increase platelet and RBC counts thus it can be used as a

medicine to boost haemopoiesis and thrombopoiesis when these have been suppressed by disease [51] because of its immunological effect [52]; and they possess and confer erythropoietic properties on pretreated rat groups as evident in the increased levels of Hb, PCV, RBC and lymphocytes [53]. Likewise, it has also be shown that leaf extract of *N. laevis* has low toxicity profile regarding hematological, blood biochemical and histological parameters [54]; and improves liver and kidney functions as well as improves antioxidant status are beneficial in the management of chronic diseases such as diabetes [55, 46, 56].

N. laevis belongs to the family *Bignoniaceae.* It is a non-leguminous, medium sized angiosperm, commonly called boundary tree (planted as hedgerows and as a life fence), chieftaincy tree (used in chieftaincy and traditional religious ceremonies) [57], or tree of life (possibly because of its longevity). Different local names of *N. laevis* are Ogirisi (Igbo), Akoko (Yoruba), Aduruku (Hausa) [58]. The phytochemical analyses of *N. laevis'* leaf showed the presence of flavonoids, terpenoids, tannin, alkaloids, phytic acid, trypsin inhibitor, phenols, antioxidants, carotenoids, oxalate and cyanide [59]. Its leaf extract is employed in the treatment of coughs, diarrhoea and dysentery, whilst it is also given to children for treating epilepsy and convulsions [60]. Some other 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, sore-feet, 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 [61, 62. 63. 64, 65] Also *N. laevis* leaf is used to manage hyperglycemia, improve haematological and biochemical derangements, control muscle wasting, induce adipogenesis (66) and has antidiabetic effect [55]. Pharmacological studies on extracts of different parts of *N. laevis* have revealed the antioxidant and free radical scavenging, antimicrobial and anti-malarial [67] sedative and anticonvulsant [68], analgesic, antinociceptive and an-tiinflamatory [69], hepatoprotective [70, 76. 56]), anticancer [72], uterine contraction [73], wound healing and antiulcer [74], antisickling [75], hypoglycemic [66], ac-tivities among others. Recently, the antihyperglycemic activity of the leaf extract and active fractions of the plant was reported [76] and apigenin was reported to be one of the active metabolites responsible for the antihyperglycemic activity [55]. It has low toxicity profile regarding hematological, blood biochemical and histological parameters [54]; and improves liver and kidney functions as well as improves antioxidant status are beneficial in the management of chronic diseases such as diabetes [55].

Research has shown that ethanolic leaf extracts of *C. papaya* and *N. laevis* have ameliorative effects on the body weight and serum levels of urea and creatinine [77], histology of liver [46] kidney [56] and cerebellum [78] of alloxan-induced diabetic male Wistar rats.

Therefore, this study was carried out to investigate the combine effect of ethanolic leaf extracts of *C. papaya* and *N. laevis* on hematological parameters and sperm quality of alloxan-induced diabetic male rats since no work has been carried out on this.

II. MATERIALS AND METHODS

2.1 Animal procurement, care and treatment

Forty (40) male wistar rats weighing between 130g to 180g 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 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 4^0C 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. 250mg of these extracts /kg body weight were dissolved in 10mls 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 150mg of alloxan per kg body weight of rat (150mg/kg body weight) [79]. 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 200mg/dl [80].

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 + 400mg/kg of *C. papay* leaf extract.
Group D Diabetic + 600mg/kg of *C. papay* leaf extract.

Group E Diabetic + 400mg/kg of *N. laevis* leaf extract.

Group F Diabetic + 600mg/kg of *N. laevis* leaf extract.

Group G Diabetic + 200mg/kg of *C. papaya* and 200mg/kg of *N. laevis* leaf extracts.

Group H Diabetic + 300mg/kg of *C. papaya* and 300mg/kg of *N. laevis* leaf extracts.

The acute toxicity tests of ethanolic leaf extracts of *C. papaya* and *N. laevis* were calculated to be above 6,000mg/kg body weight and 5000 mg/kg body weight using the methods employed by Lorke [81] and Nofal *et al.,* [82] respectively.

2.5 Sample collection

The extracts were administered for twenty one (21) days. On the 22nd day, blood samples were collected through cardiac puncture for hematological assay. The serum sample obtained was put in an ethylene-diaminetetra-acetic acid (EDTA) bottles. White blood cell (WBC) and red blood cell (RBC) counts were estimated using the improved Neubauer counting chamber [83. 84], Haemoglobin concentration was determined using cynomethaemoglobin method [84] and Packed Cell Volume (PCV) determination was carried out using the hematocrit method as described by Schalm *et al.* [85], and Dacie and Lewis [83].

Evaluation of epididymal sperm concentration was carried out as described by Saalu *et al.* [86], spermatozoa in the right epididymis were counted by a modified method of Yokoi and Mayi [87]. Sperm motility was evaluated by Sonmez *et al*., [88] method. The fluid obtained from the left cauda epididymis with a pipette was diluted to 0.5 mL with Tris buffer solution. A slide was placed on light microscope with heater table, an aliqout of this solution was on the slide, and percentage motility was evaluated visually at a magnification of x400. Motility estimates was performed from three different fields in each sample. The mean of the three estimations was used as the final motility score. Samples for motility evaluation were stored at 35°C. Sperm morphology was evaluated with the aid of light microscope at x400 magnification. Caudal sperm was taken from the original dilution for motility and diluted 1:20 with 10% neutral buffered formalin (Sigma-Aldrich, Oakville, ON, Canada). Five hundred sperm from the sample were scored for morphological abnormalities. Briefly, in wet preparations using phase contrast optics, spermatozoa were categorized. In this study a spermatozoon were considered for abnormal morphology, if it has a rudimentary tail, round head or detached head and was expressed as a percentage of morphologically normal sperm.

2.6 Statistical Analysis

All data were tabulated and statistically analyzed using SPSS version 25.0. Results were expressed as Mean ± standard error of mean (SEM). Comparative analysis amongst groups was done using one-way analysis of variance (ANOVA). A post-hoc analysis using Bonferoni multiple comparative tests was performed to identify significant groups. $P<0.05$ was taken as statistically significant.

III. RESULTS

The results of the effects of ethanolic leaf extracts of *C. papaya* and *N. laevis* on blood haematological parameters and sperm quality of alloxan induced rats are as shown below:

TABLE 3.1: A table showing the effect of ethanolic leaf extracts of *C. papaya* and *N. laevis* on white blood cells (WBC) of alloxan induced diabetic wistar rats.

Groups	Dosage of ethanolic leaf extracts	WBC (10^9\L)
A	Control (Distilled water)	8.08 ± 0.43
B	Diabetic (Distilled water)	5.51 ± 0.45*
C	400mg/kg *C. papaya*	9.81 ± 0.30
D	600mg/kg *C. papaya*	11.01 ± 0.28*
E	400mg/kg *N. laevis*	9.90 ± 0.28
F	600mg/kg *N. laevis*	11.58 ± 0.50*
G	200mg/kg *C. papaya* + 200mg/kg *N. laevis*	12.56 ± 0.41*
H	300mg/kg *C. papaya* + 300mg/kg *N. laevis*	12.43 ± 0.58*

Table 3.1 showed a significant decrease ($P<0.05$) on WBC in group B that received no treatment when compared to the control group A; and a significant increase ($P<0.05$) on WBC in groups D, F, G and H that received 600mg/kg of *C. papaya*, 600mg/kg of *N. laevis*, 200mg/kg of *C. papaya* + 200mg/kg of *N. laevis*, and 300mg/kg of *C. papaya* + 300mg/kg of *N. laevis* respectively when compared with the control group A. However, there was no significant difference ($P>0.05$) on WBC in groups C and E that received 400mg/kg of *C. papaya*, and 400mg/kg *N. laevis* respectively when compared with the control group A.

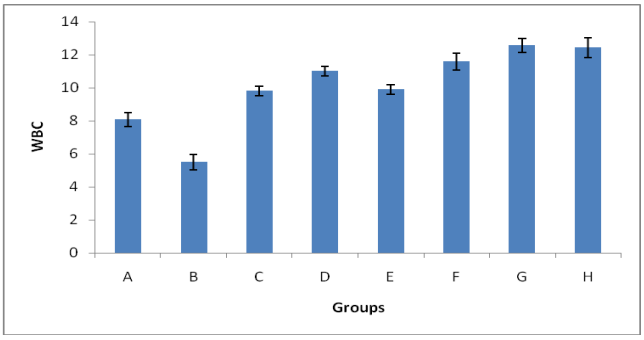

Figure 3.1: The effect of ethanolic leaf extracts of *C. papaya* and *N. laevis* on white blood cells (WBC) of alloxan induced diabetic wistar rats.

TABLE 3.2: A table showing the effect of ethanolic leaf extracts of *C. papaya* and *N. laevis* on red blood cells (RBC) of alloxan induced diabetic wistar rats.

Groups	Dosage of ethanolic leaf extracts	RBC (10^{12}\L)
A	Control (Distilled water)	6.91 ± 0.31
B	Diabetic (Distilled water)	5.17 ± 0.27*
C	400mg/kg C. papaya	7.10 ± 0.21
D	600mg/kg C. papaya	8.20 ± 0.26*
E	400mg/kg N. laevis	8.91 ± 0.14*
F	600mg/kg N. laevis	8.33 ± 0.28*
G	200mg/kg C. papaya + 200mg/kg N. laevis	9.36 ± 0.28*
H	300mg/kg C. papaya + 300mg/kg N. laevis	9.35 ± 0.25*

Table 3.2 showed a significant decrease (P<0.05) on RBC in group B that received no treatment when compared to the control group A; and a significant increase (P<0.05) on RBC in groups D, E, F, G and H that received 600mg/kg of *C. papaya*, 400mg/kg *N. laevis*, 600mg/kg of *N. laevis*, 200mg/kg of *C. papaya* + 200mg/kg of *N. laevis*, and 300mg/kg of *C. papaya* + 300mg/kg of *N. laevis* respectively when compared with the control group A. However, there was no significant difference (P>0.05) on RBC in group C received 400mg/kg of *C. papaya* when compared with the control group A.

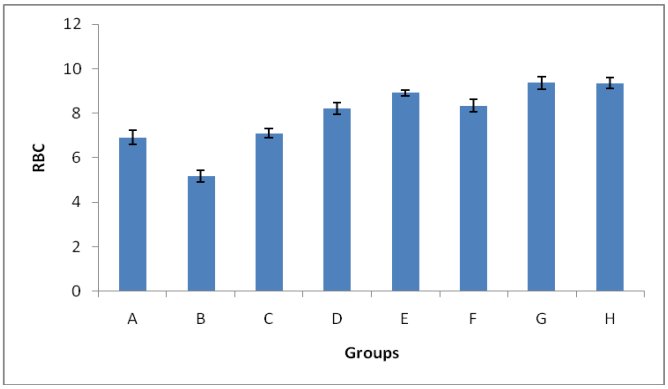

Figure 3.2: The effect of ethanolic leaf extracts of *C. papaya* and *N. laevis* on red blood cells (RBC) of alloxan induced diabetic wistar rats.

TABLE 3.3: A table showing the effect of ethanolic leaf extracts of C. papaya and N. laevis on hemoglobulin (HGB) of alloxan induced diabetic wistar rats.

Groups	Dosage of ethanolic leaf extracts	HGB (%)
A	Control (Distilled water)	14.20 ± 0.37
B	Diabetic (Distilled water)	10.80 ± 0.31*
C	400mg/kg C. papaya	14.40 ± 0.51
D	600mg/kg C. papaya	16.80 ± 0.37*
E	400mg/kg N. laevis	14.80 ± 0.37
F	600mg/kg N. laevis	16.0 ± 0.51*
G	200mg/kg C. papaya + 200mg/kg N. laevis	17.20. ± 0.37*
H	300mg/kg C. papaya + 300mg/kg N. laevis	17.10 ± 0.37*

Table 3.3 showed a significant decrease (P<0.05) on HGB in group B that received no treatment when compared to the control group A; and a significant increase (P<0.05) on HGB in groups D, F, G and H that received 600mg/kg of *C. papaya*, 600mg/kg of *N. laevis*, 200mg/kg of *C. papaya* + 200mg/kg of *N. laevis*, and 300mg/kg of *C. papaya* + 300mg/kg of *N. laevis* respectively when compared with the control group A. However, there was no significant difference (P>0.05) on HGB in groups C and E that received 400mg/kg of *C. papaya* and 400mg/kg *N. laevis* respectively when compared with the control group A.

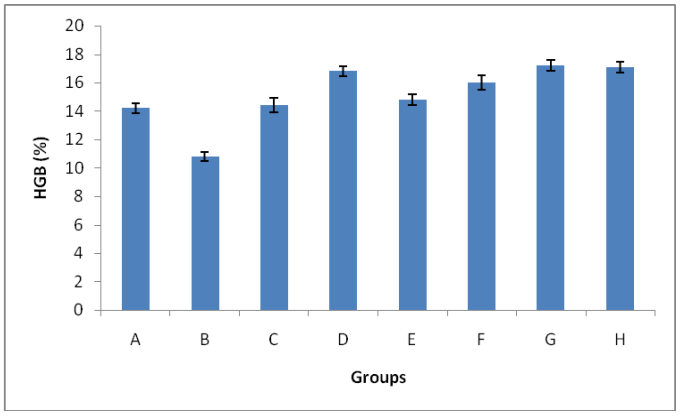

Figure 3.3: The effect of ethanolic leaf extracts of *C. papaya* and *N. laevis* on hemoglobulin (HGB) of alloxan induced diabetic wistar rats.

TABLE 3.4: A table showing the effect of ethanolic leaf extracts of C. papaya and N. laevis on packed cell volume (PCV) of alloxan induced diabetic wistar rats.

Groups	Dosage of ethanolic leaf extracts	PCV (%)
A	Control (Distilled water)	38.70 ± 0.86
B	Diabetic (Distilled water)	31.92 ± 1.25*
C	400mg/kg C. papaya	36.52 ± 1.96
D	600mg/kg C. papaya	44.78 ± 0.86*
E	400mg/kg N. laevis	40.35 ± 1.63
F	600mg/kg N. laevis	45.40 ± 1.08*
G	200mg/kg C. papaya + 200mg/kg N. laevis	46.01 ± 0.86*
H	300mg/kg C. papaya + 300mg/kg N. laevis	45.83 ± 0.66*

Table 3.4 showed a significant decrease (P<0.05) on PCV in group B that received no treatment when compared to the control group A; and a significant increase (P<0.05) on PCV in groups D, F, G and H that received 600mg/kg of *C. papaya*, 600mg/kg of *N. laevis*, 200mg/kg of *C. papaya* + 200mg/kg of *N. laevis*, and 300mg/kg of *C. papaya* + 300mg/kg of *N. laevis* respectively when compared with the control group A. However, there was no significant difference (P>0.05) on PCV in groups C and E that received 400mg/kg of *C. papaya* and 400mg/kg *N. laevis* respectively when compared with the control group A.

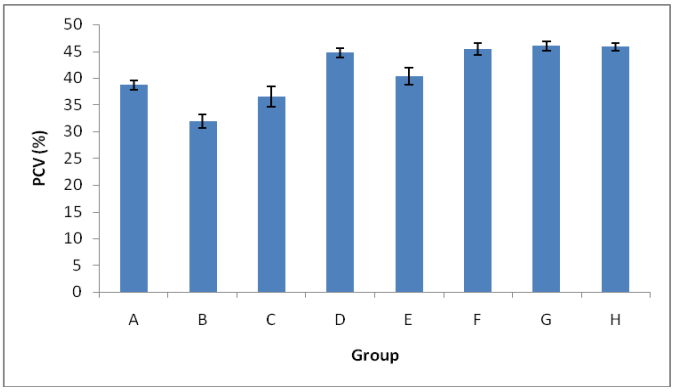

Figure 3.4: The effect of ethanolic leaf extracts of *C. papaya* and *N. laevis* on packed cell volume (PCV) of alloxan induced diabetic wistar rats.

TABLE 3.5: A table showing the effect of ethanolic leaf extracts of C. papaya and N. laevis on sperm motility of alloxan induced diabetic wistar rats.

Groups	N	Actively motile (%)	Sluggishly motile (%)	Non-motile (%)
A	5	71.0 ± 2.25	15.0 ± 1.58	14.0 ± 1.87
B	5	48.0 ± 2.55*	28.0 ± 2.25*	24.0 ± 1.87*
C	5	59.0 ± 3.32	21.0 ± 1.87	20.0 ± 1.58
D	5	44.0 ± 3.39*	30.0 ± 2.92*	26.0 ± 2.0*
E	5	73.0 ± 3.39	14.0 ± 2.92	13.0 ± 2.0
F	5	89.0 ± 1.0*	6.0 ± 1.0*	5.0 ± 0.01*
G	5	91.0 ± 3.30*	5.0 ± 2.81*	4.0 ± 1.8*
H	5	88.0 ± 1.60*	7.0 ± 0.80*	5.0 ± 0.01*

Table 3.5 showed a significant decrease (P<0.05) on sperm motility in group B that received no treatment and group D that received 600mg/kg of *C. papaya* when compared to the control group A; and a significant increase (P<0.05) on sperm motility in groups F, G and H that received 600mg/kg of *N. laevis*, 200mg/kg of *C. papaya* + 200mg/kg of *N. laevis*, and 300mg/kg of *C. papaya* + 300mg/kg of *N. laevis* respectively when compared with the control group A. However, there was no significant difference (P>0.05) on sperm motility in groups C and E that received 400mg/kg of *C. papaya*, and 400mg/kg *N. laevis* respectively when compared with the control group A.

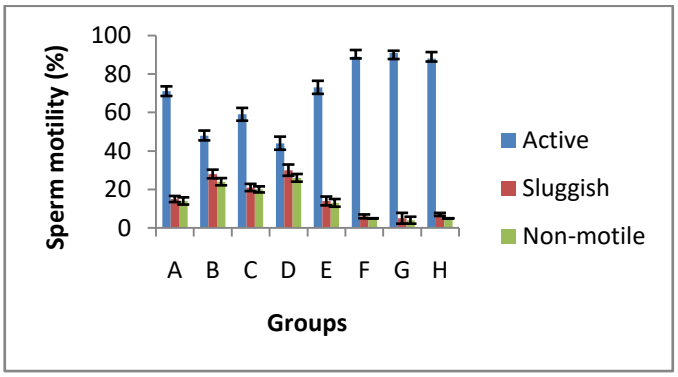

Figure 3.5: The effect of ethanolic leaf extracts of *C. papaya* and *N. laevis* on sperm motility of alloxan induced diabetic wistar rats.

TABLE 3.6: A table showing the effect of ethanolic leaf extracts of C. papaya and N. laevis on sperm count of alloxan induced diabetic wistar rats.

Groups	Dosage of ethanolic leaf extracts	Sperm count (%)
A	Control (Distilled water)	68.74 ± 2.30
B	Diabetic (Distilled water)	50.86 ± 3.18*
C	400mg/kg *C. papaya*	58.54 ± 4.71
D	600mg/kg *C. papaya*	42.44 ± 2.17*
E	400mg/kg *N. laevis*	67.64 ± 2.10
F	600mg/kg *N. laevis*	82.74 ± 3.19*
G	200mg/kg *C. papaya* + 200mg/kg *N. laevis*	88.78 ± 2.50*
H	300mg/kg *C. papaya* + 300mg/kg *N. laevis*	80.92 ± 0.26*

Table 3.6 showed a significant decrease (P<0.05) on sperm count in group B that received no treatment and group D that received 600mg/kg of *C. papaya* when compared to the control group A; and a significant increase (P<0.05) on sperm count in groups F, G and H that received 600mg/kg of *N. laevis*, 200mg/kg of *C. papaya* + 200mg/kg of *N. laevis*, and 300mg/kg of *C. papaya* + 300mg/kg of *N. laevis* respectively when compared with the control group A. However, there was no significant difference (P>0.05) on sperm count in groups C and E that received 400mg/kg of *C. papaya*, and 400mg/kg *N. laevis* respectively when compared with the control group A.

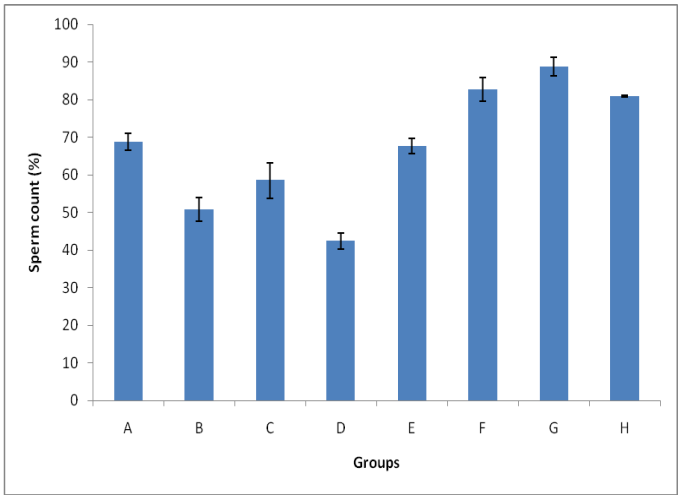

Figure 3.6: The effect of ethanolic leaf extracts of C. papaya and N. laevis on sperm count of alloxan induced diabetic wistar rats.

IV.DISCUSSION

Diabetes mellitus is a chronic, metabolic disease characterized by increased levels of blood glucose which lead to serious damage to the heart, blood vessels, eyes, kidneys, and nerves over time [89]. Long-term effects of DM include progressive development of specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, charcot joint, and features of autonomic dysfunction, including sexual dysfunction [90]. Also hematological changes due to diabetes may lead to complications such as anemia, and

a state of hypercoagulability, and contribute to cardiovascular disease (CVD) in diabetic patients [7]. Changes encountered in T2DM patients include changes in the function, structure, and metabolism of red blood cells (RBCs), white blood cells (WBCs), platelet (PLT) and the coagulation systems [9]. Waggiallah and Alzohairy, [10] revealed that these changes may manifest as immunological and coagulation problems, and anemia which is characterized by a decrease in the RBC count, hemoglobin (Hgb) and hematocrit (Hct) level as compared to non-diabetic individuals. Reproductive complications such as disruption of male fertility, impotence, retrograde ejaculation and hypogonadism due to damage to the beta cells of the pancreas which secret pancreatic insulin that regulate the male hypothalamic-pituitary-gonadal axis which is essential for fertility also occur due DM complication [15, 16, 17]. The onset of Type I diabetes is known to disrupt the HPG axis, resulting in impaired spermatogenesis and subsequent sub-fertility and disruptions in any part of the HPG axis impair fertility [91]. Thus this research work was undertaken to investigate the effect of ethanolic leaf extracts of *C. papaya* and *N. laevis* on the hematological parameters and sperm quality of alloxan-induced rats.

The result of tables (3.1, 3.2, 3.3 and 3.4)/figures (3.1, 3.2, 3.3 and 3.4) that showed significant decreases ($P<0.05$) on hematological parameters (WBC, RBC, HGB and PCV) in group B that received no treatment when compared to the control group A could be due to diabetic effect on those hematological parameters. Research has revealed that several hematological changes affecting the red blood cells (RBCs), white blood cells (WBCs), platelet and the coagulation factors are shown to be directly associated with DM [92, 93]. While the significant increases [(11.01 ± 0.28), (11.58 ± 0.50), (12.56 ± 0.41), (12.43 ± 0.58)], [(8.20 ± 0.26), (8.33 ± 0.28), (9.36 ± 0.28), (9.35 ± 0.25)], [(16.80 ± 0.37), (16.0 ± 0.51), (17.20. ± 0.37), (17.10 ± 0.37)], and [(44.78 ± 0.86), (45.40 ± 1.08), (46.01 ± 0.86), (45.83 ± 0.66)] ($P<0.05$) on hematological parameters (WBC, RBC, HGB and PCV) in groups D, F, G and H that received 600mg/kg of *C. papaya*, 600mg/kg of *N. laevis*, 200mg/kg of *C. papaya* + 200mg/kg of *N. laevis*, and 300mg/kg of *C. papaya* + 300mg/kg of *N. laevis* respectively when compared with the control group A could be due to the healing/ameliorating and anti-diabetic effects of the leaf extracts. Studies have revealed that *C. papaya* leaf extract has the ability to increase platelet and RBC counts thus it can be used as a medicine to boost haemopoiesis and thrombopoiesis when these have been suppressed by disease [53] because of its immunological effect [52]; and they possess and confer erythropoietic properties on pretreated rat groups as evident in the increased levels of Hb, PCV, RBC and lymphocytes [53]. Likewise, it has also be shown that leaf extract of *N. laevis* has low toxicity profile regarding hematological, blood biochemical and histological parameters [54]; and improves liver and kidney functions as well as improves antioxidant status are beneficial in the management of chronic diseases such as diabetes [55]. However, better result was obtained with the combined leaf extracts at lower dosages than using them individually.

In Table 3.5/figure 3.5, the significant decrease ($P<0.05$) on sperm motility in group B that received no treatment when compared to the control group A could be due diabetes which was induced by the alloxan. Research has shown that DM reduced sperm parameters in affected males [91], and has negative effects on semen quality (i.e., sperm volume, sperm concentration, total sperm motility, progressive sperm motility, normal sperm morphology, and sperm DNA fragmentation) [94]. Also, Sperm parameters are altered in patients or animals with DM, and the possible mechanisms involved in the onset of these alterations are hormonal changes, presence of neuropathy, and increased oxidative stress aspects present in patients with DM [95]. While the significant decrease ($P<0.05$) on sperm motility in group D that received 600mg/kg of *C. papaya* when compared to the control group A could be due to anti-fertility effect of the leaf extract at that dosage. According to Udeh and Nwaehujor, [96] *C. papaya* leaf possesses anti-fertility properties and may be used to control birth rate and aqueous extract of *C. papaya* leaf has been shown to cause reduction in mean values of andrological parameters as a result of lesion of the seminiferous tubule epithelium [97]. Then the significant increase ($P<0.05$) on sperm motility in groups F, G and H that received 600mg/kg of *N. laevis*, 200mg/kg of *C. papaya* + 200mg/kg of *N. laevis*, and 300mg/kg of *C. papaya* + 300mg/kg of *N. laevis* respectively when compared with the control group A could be due to the anti-diabetic and ameliorating/healing effects of the leaf extracts with better result when combined at lower dosage since both leaf extracts have anti-diabetic effects. However, there was no significant difference ($P>0.05$) on sperm motility in groups C and E that received 400mg/kg of *C. papaya*, and 400mg/kg *N. laevis* respectively when compared with the control group A despite the anti-diabetic and ameliorative effects of the leaf extracts at that dosage.

The result of table 3.6/figure 3.6 which showed a significant decrease ($P<0.05$) on sperm count in group B when compared to the control group A could be due to diabetes resulting from the induced alloxan. Research studies have showed that around 1 in 4 men with type 2 diabetes have low testosterone levels (hypogonadism), and low testosterone levels can lead to problems which can reduce fertility such as low sperm count, erectile dysfunction and decreased sex drive [98]. While the significant decrease ($P<0.05$) on sperm count in group D that received 600mg/kg of *C. papaya* when compared to the control group A could be due to anti-fertility effect of *C. papaya* at that given dosage as *C. papaya* leaf possesses anti-fertility properties and may be used to control birth rate [98]. Then, the significant increase ($P<0.05$) on sperm count in groups F, G and H that received 600mg/kg of *N. laevis*, 200mg/kg of *C. papaya* + 200mg/kg of *N. laevis*, and 300mg/kg of *C. papaya* + 300mg/kg of *N. laevis* respectively when compared with the control group A could

due to anti-diabetic and ameliorating/healing effect of the leaf extracts with better result at combined low dosage of the leaf extracts. Research has shown that leaf extracts of *C. papaya* and *N. laevis* have ameliorative effects on the histology of liver of alloxan-induced wistar rats [46] and improve liver and kidney functions as well as improves antioxidant status which is beneficial in the management of chronic diseases such as diabetes [99].

V. CONCLUSION

C. papaya and *N. laevis* leaf extracts have ameliorating effects on diabetes, thereby leading to increase on the serum levels of hematological parameters and sperm quality; and the ameliorating effects of the combined doses to the diabetic rats were better at lower dosages than when the individual leaf extracts were administered. Thus, the use of the leaf extracts of *C. papaya* and *N. laevis* in the treatment of diabetes should be encouraged, especially in the combined form because of their ameliorating effect to DM and complications associated with it.

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