

## Effect of xylopic acid on alloxan-induced diabetic neuropathy in rats

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**Received:** 20 January 2021

**Accepted:** 02 March 2021

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### ABSTRACT

**Background:** Neuropathic pain is a very disturbing condition commonly found in diabetic patients. This study investigated xylopic acid (XA), the major constituent of *Xylopic aethiopia* in diabetic neuropathy as well as established possible toxicity of the compound on some selected tissues.

**Methods:** Diabetes was induced in six groups of male rats with 120 mg/kg alloxan monohydrate. Diabetes was confirmed as a blood glucose level >15 mmol/dl. Neuropathic pain was confirmed on day three post-diabetes induction and treatment with 10 mg/kg, 30 mg/kg or 100 mg/kg xylopic acid, 10 mg/kg glibenclamide, 10 mg/kg morphine, and 10 ml/kg normal saline were initiated and continued for the next 15 days. The effects of the treatments on cold allodynia (cold water at 4°C) and thermal hyperalgesia (hot water at 55 ± 1°C) were evaluated within the duration of treatments. Histology of the liver and kidney, as well as haematological, serum biochemical, and semen analyses, were done after the fifteenth day of the experiment.

**Results:** Xylopic acid produced significant anti-hyperglycaemic and analgesic effects in the cold allodynia and thermal hyperalgesia tests. Sperm motility, viability and count were significantly restored at 10 mg/kg XA as compared higher doses and negative control. The outcome of haematological analysis revealed a protective effect of XA although histological damage liver and kidney due to alloxan treatment was observable.

**Conclusions:** Xylopic acid ameliorates diabetic neuropathy in rats and does not exert detrimental effects at low doses.

**Keywords:** Xylopic acid, Neuropathic pain, Hyperglycaemia, Diabetes

### INTRODUCTION

Diabetes mellitus has been one of the common micro- and macrovascular metabolic disorders that result in substantial morbidity and mortality.<sup>1</sup> The International Diabetes Federation describes the existence of diabetes in adults to be 8.8% in 2017, which corresponds to 425 million people worldwide. The incidence is expected to

increase by 48%, and the number of people with diabetes will escalate to 629 million by 2045.<sup>2</sup> It is regarded to be one of the world's top five leading causes of death. In modern medicine, no agreeable effective therapy is still available to cure diabetes mellitus.<sup>3</sup> As the disease develops, serious diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications, and ulcerations result in tissue or vascular

injury.<sup>4</sup> Neuropathy is one of the many complications of diabetes mellitus, which may affect both the central and peripheral nerve tissues. Cerebral involvement in diabetic lesions can be seen as impairments in human and animal learning, comprehension, and memory.<sup>5</sup>

Neuropathic pain has been redefined by the Neuropathic Pain Special Interest Group (NeuPSIG) to relate to “pain initiated or caused by a primary lesion or dysfunction in the nervous system”.<sup>6</sup> Neuropathic pain is more common than normal, with population-based reports estimate that 7-10% of adults are affected.<sup>7</sup> It is an area of large unmet therapeutic need. The pharmacotherapy of neuropathic pain involves tricyclic antidepressants and other anticonvulsants, but these only provide clinically meaningful pain relief (>50% percent) in 40-60% percent of patients and are associated with many side effects.<sup>8</sup> Opioids, which are the cornerstone of long-standing pharmacological pain control, are often prohibited from being used in neuropathic pain conditions for concerns over ineffectiveness and the potential for the advancement of tolerance.<sup>9</sup> Quite recently, subchronic therapy with morphine was not only unsuccessful at reducing the effect but could have even intensified cold and mechanical allodynia in nerve-injured mice.<sup>10</sup> Apart from their ineffectiveness due to their toxicity, these drugs also have a longer duration of administration and alarming side effects. Thus, patients are unwilling to use these drugs this has necessitated the search for new therapies with minimum side effects, if possible, to manage neuropathic pain associated with diabetes. Given the increased understanding of neuropathic pain pathophysiology and the development of animal models, the effectiveness of various types of medications is now being tested.<sup>11</sup>

Xylopic acid (XA) is a pure crystalline compound derived from the petroleum ether extract of *Xylopic aethiopicum*. The fruit of *Xylopic aethiopicum* is commonly used as a cough cure, as a carminative, postpartum tonic, as an antimalarial agent, and for the treatment of uterine fibroids and also amenorrhoea.<sup>12</sup> XA has shown to have anti-androgenic activity in orchietomized rats.<sup>13</sup> Neuropharmacological effects of an ethanolic fruit extract of *X. aethiopicum* and XA, a kaurene diterpene isolate, in mice has also been established.<sup>14</sup> Research undertaken by Woode et al indicated that XA has reversible spermatotoxic, antifertility and anti-androgenic properties, the mechanism of which could involve a direct effect on the germ cells and other cells of the testes with a potential involvement of the hypothalamic, pituitary and gonadal axis.

A thorough literature search revealed that no study has been done on the analgesic properties of XA on alloxan-induced neuropathic pain in rats despite an array of pharmacological properties. Though XA has been reported to have an analgesic effect in cancer chemotherapy, it still relevant to assess its effects in diabetes-induced neuropathic pain because of the variance in the etiology of neuropathic pain.

## METHODS

### *Drugs and chemicals*

Morphine hydrochloride (Phyto-Riker, Accra, Ghana); glibenclamide (Denk Pharma GmbH and Co. KG., Germany), Alloxan monohydrate (BDH Chemicals, England), cremophor (Sigma Aldrich, St. Louis, USA).

### *Extraction of Xylopic acid (XA) from fruits of Xylopic aethiopicum*

In the month of December, 2019, dried fruits of *Xylopic aethiopicum* (Dunal) A. Rich (Annonaceae) were collected and authenticated by the University of Cape Coast Herbarium against a voucher specimen (No. FP/09/77). It is called “Hwentia” in Akan language. Extraction and purification of XA was as described earlier.<sup>15</sup> The name of the plant was checked with <http://www.theplantlist.org>

### *Animals*

Forty (40) laboratory-bred male Wistar Rats were obtained from the Centre for Medicinal Plants Research, Akuapem Mampong, Ghana, and transported to the animal house of the Department of Biomedical Sciences, School of Biological Sciences, University of Cape Coast. The animals were conveyed in cages to ensure less or no exhaust fume pollution during their transportation. They were fed with normal rodent chow. The animals were housed under standard conditions (25°C±0.2), 12 h light/12 h dark cycle). Water and rat chow were available ad libitum preceding the experimental session. All animal protocols were per the National Institute of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

### *Induction of diabetes*

Healthy male rats weighing 150-200 g were divided into seven (7) groups. Six groups of the rats were injected intraperitoneally (i.p.) with alloxan monohydrate dissolved in distilled water at a dose of 140 mg/kg i.p. whereas the naive control animals received only vehicle. Rats were fasted 14 h before and 12 h after injection of alloxan. The blood glucose levels were estimated by enzymatic glucose oxidase method using a commercial glucometer (Accu-Chek®). Rats with moderate hyperglycaemia (blood glucose level above 15 mmol/dl) were used for the experiments, 5 days post-induction.<sup>16</sup>

### *Effect of XA on hyperglycaemia*

Six (6) groups of rats (n=5) were used for this experiment. Five (5) diabetic groups received the following oral treatment schedule; Group 1 control rats received vehicle (10 ml/kg) p.o., Group 2 diabetic rats received 10 mg/kg glibenclamide p.o., Groups 3-5 diabetic rats received 10-100 mg/kg XA p.o. daily from day 5 after induction. Group 6, sham rats were not induced with diabetes and received

vehicle (10 ml/kg). Blood glucose levels were estimated using 10  $\mu$ l of blood drawn from the tail vein on days 0, 5, 7, and 13.<sup>16</sup>

### **Bodyweight assessment**

The body weights of the animals were monitored and recorded on days 1, 2, 3, 7, 9, 11, and 13 post-induction using a Sartorius top loading balance (CPA3202S, Goettingen, Germany). These were calculated as a percentage change in body weight and plotted.

### **Pain assessment**

The tail immersion and cold allodynia methods were used to assess pain in the experimental rats.<sup>16</sup>

### **Cold allodynia test**

Diabetic animals used in this model were placed in 5 groups (n=5). Group 1 diabetic rats received 10 ml/kg saline p.o., Group 2 received morphine 10 mg/kg p.o. while Groups 3 – 5 received 10-100 mg/kg XA p.o. from day 5 post-induction. A sixth group, Group 6 included animals in which diabetes was not induced and animals were treated with 10 ml/kg saline. All treatments commenced from day 5 post-induction. The temperature of the cold water was set (4°C) as previously described and allowed to stabilize for 5 minutes.<sup>16</sup> The left hind paws of the animal were then placed into the cold water and the time taken for the first brisk lift or stamp of the ipsilateral hind paw to occur was recorded with a digital timer with a cut-off of 20 s. Pain measurements were taken on days 0, 3, 7, 9, and 13 during the drug treatment period.

### **Tail immersion test**

This experiment was done using the hot water method where the temperature of the water was raised and maintained at 55°C $\pm$ 1 using a water bath (Precision Water Bath Model 283, U.S.A.). Animals induced with diabetes were randomly placed in groups (n=5). Rats received orally either saline 10 ml/kg, morphine 10 mg/kg, or XA 10-100 mg/kg while sham control animals were not induced with diabetes and received saline 10 ml/kg from day 5 post-induction. The tail of the rat was immersed into the hot water 5 cm from the tip with the rats held vertically above the water bath. The time taken for the first rapid flick or withdrawal from hot water to occur was recorded with a digital timer with a cut-off of 10 s. Tail withdrawal latencies were taken on days 0, 3, 7, 9, and 13.<sup>16</sup>

### **Blood sample collection and analysis**

Blood samples were collected from animals after treatment ended on day 15. These were collected by cardiac puncture from groups treated with either saline, glibenclamide, XA, or sham control animals. Rats were euthanized using diethyl ether and blood collected into MediPlus vacutainer K3 EDTA tubes (Sunphoria Co. Ltd., Taiwan) or serum

separator tubes (SST) (BD Vacutainer® Blood Collection Tube Product, USA) for haematological or biochemical analysis respectively.

### **Haematological test**

Samples collected into EDTA tubes were analysed for haematological parameters using a haematology analyser (Sysmex XP - 300TM Automated Haematology Analyzer, USA).

### **Biochemical tests**

The samples collected in the tubes were allowed to clot and then centrifuged. The serum obtained was analysed for kidney and biochemical function tests using a clinical chemistry analyser (Vital Scientific N. V, Netherlands).

### **Histopathological assessment**

The liver, kidneys, and pancreas were harvested from euthanized rats post-treatment with XA and glibenclamide. The samples were fixed in 10% phosphate-buffered formalin and processed using standard techniques. Microscopy slides were obtained after tissue processing at Komfo Anokye Teaching Hospital in Kumasi, Ghana, and obtained slides observed under an Olympus trinocular light microscope (Jenoptik, Germany) at  $\times$ 100 magnification power. Images were captured by a microscope camera with LC Micro software (Olympus Soft Imaging Solutions GmbH, Germany) connected to the third eyepiece of the microscope.

### **Semen analysis**

A method described by Zemjanis was used to determine the Sperm motility.<sup>17</sup> Within 2-4 minutes of semen collection. Briefly, a drop of semen was collected from the caudal epididymis for the XA-treated and the controls onto a glass slide. Sodium citrate buffer (2.9%) was added to the semen and mixed until the desired dilution was obtained. The percentage motility of semen was evaluated microscopically. The total spermatozoa in the cauda epididymis sperm sample were counted from five large squares (volume: 0.5 mm<sup>3</sup>) using the improved Neubauer haemocytometer (depth 0.1 mm, area: 1/400 mm<sup>2</sup>; Yancheng Cordial Lab Glassware Co. Ltd., Jiangsu, China (Mainland). Morphological abnormalities of the sperm and viability were evaluated. This was done from a total count of 400 spermatozoa in smears obtained with Wells and Awa stains (0.2 g of Eosin and 0.6 g of fast green dissolved water and ethanol in ratio 2:1). Sperm viability was determined using 1% eosin and 5% nigrosine in a 3% sodium citrate dehydration solution.

### **Statistical analysis**

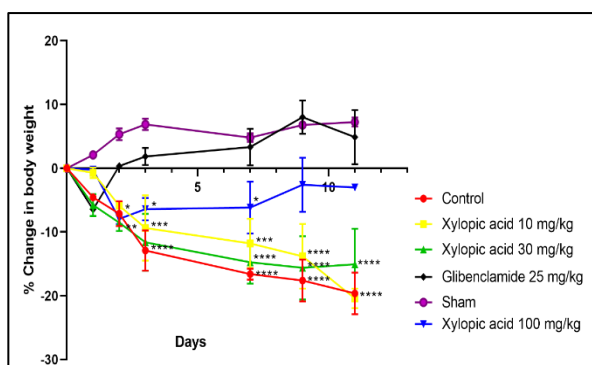
GraphPad Prism Version 7 was used for data analyses. Data are presented as mean  $\pm$  S.E.M. The time-course curves were subjected to two-way (treatment  $\times$  time)

repeated measures analysis of variance (ANOVA) with Bonferroni's post hoc test. The total nociceptive score for each treatment was calculated in the arbitrary unit as the area under the curve (AUC). Differences in AUCs were analysed with one-way ANOVA with drug treatment as a between-subjects factor. Further comparisons between vehicle and drug-treated groups were performed using Tukey's post hoc test.

## RESULTS

### Effect of xylopic acid, morphine, and glibenclamide on body weight of rats

Weight variations were observed over the period and calculated as a percentage change in body weight. Generally, the sham-treated animals, where no hyperglycaemia was induced, increased in body weight throughout the study. However, groups in which diabetes was induced had a reduction in body weight on the first-day post-induction but this was not statistically significant. Most glibenclamide-treated animals, however, had an increase in body weight throughout the rest of the study. XA 100 mg/kg group had a lower significant weight loss ( $p < 0.05$ ) on day 3 compared to other groups. XA 10 and 30 mg/kg together with the negative control group's weight loss was significant at  $p < 0.001$ . On day 7, XA 100 mg/kg treated rats recorded a change in weight loss significant at ( $p < 0.05$ ) while XA 10 mg/kg weight loss was significant at ( $p < 0.001$ ). The percentage weight loss for both the negative control group and XA 30 mg/kg was significant ( $p < 0.0001$ ). The negative control group, XA 10 and 30 mg/kg all recorded significant percentage weight losses on days 9 and 11 at  $p < 0.0001$ .

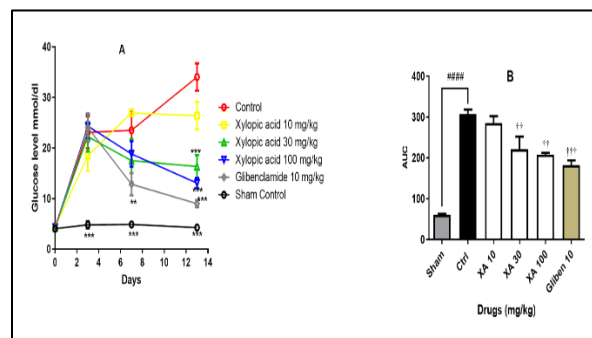


**Figure 1: Effect of xylopic acid (10, 30, and 100 mg/kg, morphine 10 mg/kg and glibenclamide (10 mg/kg) on body weight of animals pre-treated with alloxan. Data are presented as mean  $\pm$  S.E.M. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$  compared to sham group (Two-way ANOVA followed by Bonferroni's post hoc test).**

### Effects of xylopic acid treatment on hyperglycaemia

Hyperglycaemia was observed in animals employed in the experiment after i.p injection of alloxan with mean glucose

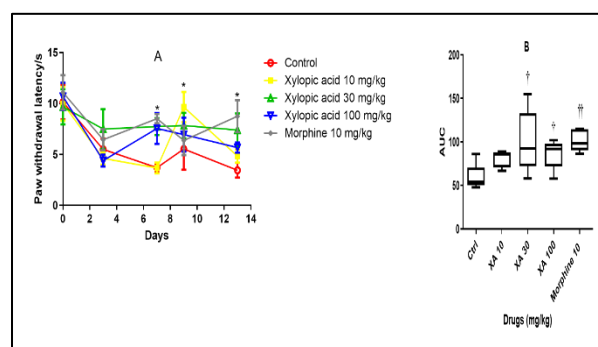
levels ranging between 18.5 mmol/dL – 24.6 mmol/dL (fig. 2A). Treatment of hyperglycaemic rats with different dose of XA produced a significant ( $p < 0.001$ ) anti-hyperglycaemic effect at the doses of 30 and 100 mg/kg but no such effects was seen at the 10 mg/kg of XA dose level as shown in Figure 2B. The reference oral hypoglycaemic drug, glibenclamide, produced significant ( $p < 0.001$ ) antihyperglycaemic effects in the treated rats as expected.



**Figure 2: Effect of xylopic acid (10, 30, 100) mg/kg and glibenclamide (10 mg/kg) on (a) the time course curve of alloxan-induced hyperglycemia and (b) the total hyperglycemia (AUC). Data are presented as mean  $\pm$  S.E.M. \*\*\* $p < 0.001$ ; \*\* $p < 0.01$  compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni's post hoc test); †† $P < 0.01$ , ††† $P < 0.001$  compared to vehicle-treated group (One-way ANOVA followed by Tukey's post hoc test); ####  $P < 0.0001$  compared to the sham control (One-way ANOVA followed by Tukey's post hoc test).**

### Effect of XA on cold allodynia

XA produced a significant inhibition of the cold allodynia and this observed anti-allodynic effect was dose-dependent as shown in Figure 3.



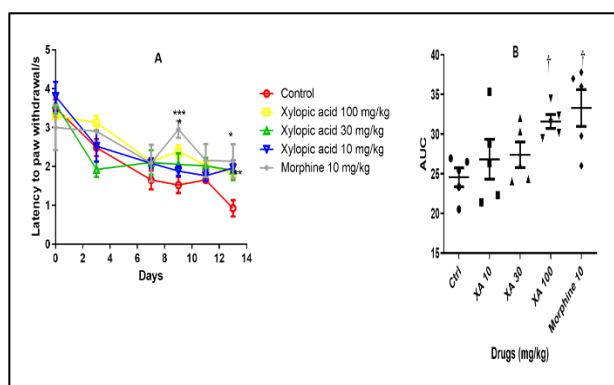
**Figure 3: Effect of (a) xylopic acid (10, 30, 100) mg/kg and morphine (10 mg/kg) on the time-course curve of cold allodynia and the AUC (b). Data are presented as mean  $\pm$  S.E.M. \* $P < 0.05$  compared to the vehicle-treated group (Two-way ANOVA followed by Bonferroni's post hoc test); † $P < 0.05$ , †† $P < 0.01$  compared to the vehicle-treated group (One-way ANOVA followed by Tukey's post hoc test).**



The 30 and 100 mg/kg doses produced a significant anti-allodynic effect ( $p < 0.05$ ) while no such effect was seen at the 10 mg/kg treated dose as shown in Figure 3b. The reference analgesic drug morphine was observed to produce a significant ( $p < 0.01$ ) anti-allodynic effect in the rats.

### Effects of XA treatment on thermal hyperalgesia

Thermal hyperalgesia was present in all the animals used in the experiment after the injection of alloxan (Figure 4a). Treatment with XA (10, 30, 100) mg/kg increased the latency to paw withdrawal. Observations during the experiment revealed a dose-dependent activity of XA in the thermal hyperalgesia model with 100 mg/kg displaying an increased latency to paw withdrawal as presented in fig 4b. However, no such effects were observed in the animals treated with XA doses of 10 and 30 mg/kg. The standard analgesic drug (10 mg/kg morphine) exhibited a significant ( $p < 0.001$ ) reduction in thermal hyperalgesia as expected.



**Figure 4: Effect of (a) xylopic acid (10, 30, 100) mg/kg and morphine (10mg/kg) on the time-course of alloxan-induced neuropathic pain in rats (tail-flick test). Each point represents the mean  $\pm$  S.E.M (n = 8). \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$  compared to respective controls (two-way repeated-measures ANOVA followed by Bonferroni's post hoc); (b) the AUC (total response) for XA and morphine respectively. Each column in b and d represents the mean  $\pm$  S.E.M. † $P \leq 0.05$  (one-way ANOVA followed by Tukey's post hoc test). Graph and description should be on the same page.**

### Effect of xylopic acid on sperm motility, viability, and total cell count

The outcome of diabetes on sperm count, motility, and viability is shown in Table 1. Following the induction of diabetes with alloxan, there was a considerable fall ( $p < 0.0001$ ) in the percentage of sperm motility, percentage viability, and total sperm count in the diabetic group. The use of 10 mg/kg of XA however, exhibited significant ( $p < 0.01$ ) protective effect on sperm motility, live/dead ratio, and the total sperm count. At 30 and 100 mg/kg XA did not exhibit ameliorative effects but rather enhanced the

destruction activity of alloxan significantly on sperm motility, live/dead ratio, and the total sperm count.

**Table 1: Effects of xylopic acid treatment on sperm motility, viability and sperm count.**

Treatments (mg/kg)	Motility %	Viability %	Cell Count $\mu$ L
SHAM	99 $\pm$ 1.2	99 $\pm$ 0.6	23.2 $\times$ 10 <sup>9</sup>
Neg control	60 $\pm$ 1.4*** *	70 $\pm$ 5.5****	3.9 $\times$ 10 <sup>9</sup> ****
X6A 10	95 $\pm$ 2.1	97 $\pm$ 1.6	20.1 $\times$ 10 <sup>9</sup> ***
XA 30	10 $\pm$ 1.6*** *	35 $\pm$ 0.3****	0.1 $\times$ 10 <sup>9</sup> ****
XA 100	2 $\pm$ 0.8****	15 $\pm$ 2.3****	0.03 $\times$ 10 <sup>3</sup> ****

Values are expressed as mean  $\pm$  S.E.M. \*\*\* $P < 0.001$  \*\*\*\* $P < 0.0001$  compared to sham control.

### Effects of XA on haematological parameters and serum biochemistry

The effects of alloxan-induced diabetes on haematological parameters and blood biochemistry were investigated and compared with similar parameters of normal non-diabetic rats as shown in Tables 2 and 3, respectively. Following the induction of diabetes in the rats, most of the haematological parameters and blood biochemistry were altered in the negative control group. Nonetheless, alterations caused by XA were not statistically significant.

### Histopathological analysis

Examination of the liver sections from the naïve control group revealed the normal histological structure of hepatocytes; hepatocytes were polygonal in shape, tightly packed, containing basophilic central rounded nuclei separated by the hepatic sinusoids radiating from the central vein (CV), and with the presence of non-activated spindle-shaped Kupffer cells within the sinusoids, Plate 1A (1A). Liver sections from negative control diabetic rats showed oedematous tissue with activated Kupffer cells and marked dilatation of central vein and hepatic sinusoids (1B). Sections from XA 100 mg/kg animals were normal (1C) while those of XA 30 mg/kg were slightly oedematous with few highly basophilic nuclei of hepatocytes (1D). However, XA 10 mg/kg treated animals exhibited oedematous tissue sections with dilatation of central vein and sinusoids, in addition to highly basophilic nuclei of hepatocytes and activated Kupffer cells (1E). For glibenclamide (1F), sections appear to contain numerous highly basophilic nuclei which were more conspicuous as the ones in Plate 1B.

Kidney sections from negative control diabetic rats (Plate 2B), showed the diminished outline of renal corpuscles, the absence of capsular space, and with loss of the glomerular in some corpuscles from the cortex, which predicts

degeneration of cells in the kidney of this group. This was in contrast to the naïve control group (2A) which showed tubules and renal corpuscles (with well-defined capsular space). Sections from XA 10 mg/kg-treated animal (2C), had atrophied renal tubules and corpuscles with intact capsular space, predictive of regeneration of kidney tubules while XA 30 mg/kg animals (2D) had diminished outline of renal corpuscles and absence of capsular space. XA 100 mg/kg (2E) showed normal histology which reveals the regeneration of cells. Histological sections from group 2F

(glibenclamide) revealed semi-capsular space around the glomerulus of the corpuscles and loss of glomerulus.

Sections of the pancreas for naïve were largely normal (3A) whereas diabetic control rats had both islets of Langerhans displaying degenerative and necrotic changes (3B). Plate 3C Sections from XA 30 mg/kg-treated rats were consistent with naïve control (3C).

**Table 2: Haematological parameters of alloxan-induced diabetic rats following various treatments for 15 days.**

Parameter	Sham	Neg control	GLIB	XA 10	XA 30	XA 100
<b>WBC (<math>\times 10^3/\mu\text{l}</math>)</b>	4.3 $\pm$ 1.4	3.6 $\pm$ 0.3	3.8 $\pm$ 0.2	4.1 $\pm$ 0.7	4.5 $\pm$ 0.8	4.8 $\pm$ 0.4
<b>Platelets (<math>\times 10^3/\mu\text{l}</math>)</b>	869 $\pm$ 5.0	860 $\pm$ 4.3	867 $\pm$ 2.4	861 $\pm$ 3.3	862 $\pm$ 2.0	867 $\pm$ 3.5
<b>RBC (<math>\times 10^6/\mu\text{l}</math>)</b>	6.8 $\pm$ 0.7	6.0 $\pm$ 0.9	6.3 $\pm$ 1.1	6.6 $\pm$ 0.5	7.2 $\pm$ 0.1	7.0 $\pm$ 0.5
<b>HCT (%)</b>	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.5 $\pm$ 0.2	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.2
<b>Hb (%)</b>	13.9 $\pm$ 1.2	12.3 $\pm$ 1.6	13.3 $\pm$ 2.7	13.4 $\pm$ 0.5	13.4 $\pm$ 0.3	13.8 $\pm$ 0.1
<b>MCV (<math>\mu\text{m}^3</math>)</b>	75.6 $\pm$ 0.8	71.3 $\pm$ 0.4	73.3 $\pm$ 1.4	73.4 $\pm$ 1.8	71.9 $\pm$ 1.5	73.7 $\pm$ 0.3

**Table 3: Chemical parameters of alloxan-induced diabetic rats following treatment with Xylopic acid for 15 days.**

Parameter	Sham	Neg Control	XA 10	XA30	XA 100
<b>CHO</b>	65.5 $\pm$ 2.5	67.5 $\pm$ 2.5	63.5 $\pm$ 3.5	70.5 $\pm$ 7.5	74.5 $\pm$ 1.5
<b>TGC</b>	35.0 $\pm$ 0.4	37.0 $\pm$ 0.7	36.0 $\pm$ 0.8	35.5 $\pm$ 1.5	34.5 $\pm$ 0.5
<b>HDL</b>	28 $\pm$ 0.5	23.0 $\pm$ 0.2	25.5 $\pm$ 0.5	27.5 $\pm$ 2.0	29.5 $\pm$ 1.5
<b>LDL</b>	25.5 $\pm$ 0.5	25.0 $\pm$ 0.1	26.0 $\pm$ 0.6	25.5 $\pm$ 0.2	24.0 $\pm$ 0.4
<b>VLDL</b>	10.0 $\pm$ 0.7	11.0 $\pm$ 0.3	9.0 $\pm$ 0.6	8.5 $\pm$ 0.5	11.0 $\pm$ 0.2
<b>UREA</b>	3.6 $\pm$ 0.3	3.8 $\pm$ 0.2	3.7 $\pm$ 0.1	3.2 $\pm$ 0.7	2.9 $\pm$ 0.3
<b>CREA</b>	2.4 $\pm$ 0.1	2.5 $\pm$ 0.01	2.3 $\pm$ 0.2	2.2 $\pm$ 0.1	2.4 $\pm$ 0.5
<b>T. BIL</b>	5.9 $\pm$ 0.6	5.5 $\pm$ 0.3	5.2 $\pm$ 0.3	5.5 $\pm$ 2.5	5.7 $\pm$ 1.10
<b>AST</b>	45.2 $\pm$ 0.5	47.0 $\pm$ 0.9	49.0 $\pm$ 0.3	45.1 $\pm$ 0.2	43.0 $\pm$ 0.1
<b>ALT</b>	34 $\pm$ 0.2	36.0 $\pm$ 0.8	34.3 $\pm$ 0.9	36.0 $\pm$ 0.7	35.0 $\pm$ 0.3
<b>GGT</b>	17 $\pm$ 0.2	16.0 $\pm$ 0.7	16.0 $\pm$ 0.4	18.0 $\pm$ 0.9	16.5 $\pm$ 0.5
<b>ALP</b>	11.5 $\pm$ 0.9	11.0 $\pm$ 0.2	10.5 $\pm$ 0.5	12.5 $\pm$ 1.5	10.0 $\pm$ 0.3
<b>PROT</b>	51 $\pm$ 0.9	54 $\pm$ 0.3	53 $\pm$ 0.8	52.5 $\pm$ 0.3	55.5 $\pm$ 0.5
<b>ALB</b>	37 $\pm$ 0.2	39 $\pm$ 0.2	38.0 $\pm$ 0.1	36.0 $\pm$ 0.7	35 $\pm$ 0.2
<b>GLOB</b>	16.0 $\pm$ 0.1	15.0 $\pm$ 0.4	16.0 $\pm$ 0.6	16.5 $\pm$ 0.5	17.5 $\pm$ 0.3

Values are expressed as mean  $\pm$  S.E.M. CHO – Cholesterol, TGC – Triglycerides, HDL – High Density Lipoproteins, LDL – Low Density Lipoproteins, VLDL – Very Low Density Lipoproteins, CREA – Creatinine, T. BIL – Total Bilirubin, AST – Aspartate Transaminase, ALT – Alanine Transaminase, GGT – Gamma-Glutamyl Transferase, ALP – Alkaline Phosphatase, PROT – Protein, ALB – Albumin Blood Test, GLOB - Globulin

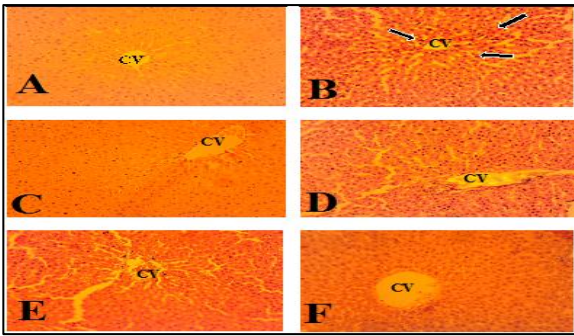
Signs of regeneration were observed marginally in XA 10 and 30 mg/kg-treated rats in the cells at the centre of the islets of Langerhans, with intact and uniformly arranged peripheral islet cells. Plate 3F shows a decrease in cells at the centre of the Islets of Langerhans, which shows a sign of no or little regeneration.

## DISCUSSION

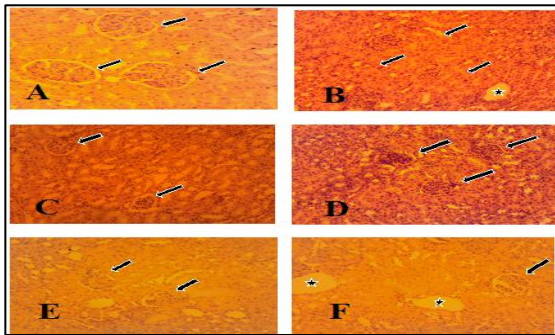
The current study has demonstrated that XA has antinociceptive effects in alloxan-induced diabetes neuropathy which may be mediated through the hypoglycaemic effect of XA. However, XA had destructive activities on the sperm cell in terms of its

motility, viability, and total cell count at higher doses but had a high restoration on these parameters at lower doses. XA did not prove to have any good significance recovery on the weight loss experienced by the diabetic rats.

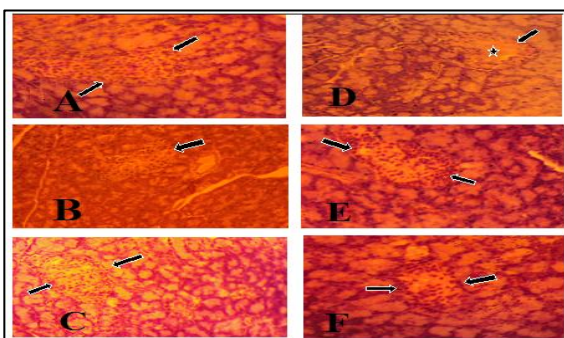
Experimental diabetes was induced by Alloxan due to the selective destruction of the insulin-producing pancreatic beta ( $\beta$ ) cells of the Islets. Alloxan induces a multiphasic blood glucose response when injected into an experimental animal, which is followed by corresponding inverse variations in the plasma insulin concentration followed by sequential ultra-structural  $\beta$ -cell changes, ultimately leading to necrotic cell death.<sup>18</sup>



**Figure 5: Photomicrograph of liver sections of xylopic acid and glibenclamide treated alloxan-induced diabetic rats. (A) Normal histology, (B) negative diabetic control, (C) XA 10 mg/kg (D) 30 mg/kg, (E) 100 mg/kg (F) glibenclamide (H and E stain X100). CV= central vein.**



**Figure 6: Photomicrograph of kidney sections of xylopic acid and glibenclamide treated alloxan-induced diabetic rats. (A) naïve control (B) negative diabetic control (C) XA 10 mg/kg, (D) XA 30 mg/kg, (E) XA 100 mg/kg and (F) glibenclamide (H and E stain 100X).**



**Figure 7: Photomicrograph of histological sections of the pancreas of sections of xylopic acid and glibenclamide treated alloxan-induced diabetic rats. (A) naïve control (B) negative diabetic control (C) XA 10 mg/kg, (D) XA 30 mg/kg, (E) XA 100 mg/kg and (F) glibenclamide (H and E stain 100X).**

Diabetic neuropathy is associated with axonal demyelination which manifests itself by the reduction in the amplitude of compound muscle action potential

(CMAP). This decrease is directly proportional to the reduction in the number of myelinated axons. The choice of drugs available for diabetic neuropathy is limited and the treatment is largely symptomatic.<sup>19</sup>

Possible indications for the reduced hyperglycaemia followed by XA treatment may be as a result of the antagonistic activity to glucokinase inhibition action of alloxan, which allows XA to be transported into the cytosol by the glucose transporter (GLUT2) in the plasma membrane of  $\beta$ -cell.<sup>20</sup> This proposed mechanism may further lead to effective prevention of redox cycling and generation of ROS thereby preventing pancreatic  $\beta$ -cell death and counteract the development of alloxan diabetes in vivo or it may be as a result of direct action on the redox action of alloxan pancreatic beta cells.<sup>21</sup>

Neuropathic pain induced with alloxan resulted in significant cold allodynia and thermal hyperalgesia. XA and morphine inhibited the hyperalgesia associated with thermal stimulation as well as the cold allodynia associated with cold water stimulus. Pharmacokinetically, paclitaxel distributes in the central and peripheral nervous system in rats following its administration.<sup>22</sup> The administration of XA was able to reduce thermal hyperalgesia. The tail immersion test measures the pain of spinal and supraspinal origin.<sup>16</sup> Xylopic acid produced analgesia in this pain model centrally. Again, glutamate excitotoxicity and increased transmission have been implicated in this pain model. XA has been reported to inhibit the glutamatergic nociceptive pathway. The inhibitory effect of XA on glutamatergic neurotransmission may account partly for the observed analgesic effect of XA.<sup>15</sup> In addition, morphine, an opioid agonist blocked nociception in this model. XA has been reported to act on opioid receptors and this could partly account for its antinociception in this model.<sup>23</sup>

Increased glutamate release leads to the amplification of neuronal firing rate and depolarization resulting in the sensitization of the nerves to produce pain and this effect may however be reversed by the XA treatment which may be capable of reducing the gradual depolarization and phosphorylation of the NMDA glutamate receptor in the post-synaptic surface which is followed by the increase in calcium concentration and cAMP subsequently activates the protein kinases C (PKC).<sup>24</sup>

Xylopic acid increased the paw withdrawal latencies in the cold allodynia test. It is not clear, however, how XA exerts anti-nociception in this model but it may do so by inhibiting calcium channels, TRPV4, CB2 sigma-1 nociceptive pathways that are activated in the course of neuropathy and it has been demonstrated that XA inhibits calcium channels as an antagonist of  $\alpha_2$ - $\delta_1$  Ca<sup>2+</sup> channel subunit of N-type voltage-dependent calcium channels.<sup>15</sup>

In the case of male infertility, lower levels of oxidative damage to the spermatozoa may hold their capacity for fertilization while carrying a significant level of oxidative



damage in their DNA and diabetes mediated oxidative stress has proven to damage the nuclear components of host cells, and it is considered to be the vital cause of mutation in both somatic and germ cells.<sup>25</sup> In concurrence with previous reports, there was a decrease in the sperm motility and count as well as the percentage live/dead ratio while the number of abnormal spermatozoa also increased considerably following the induction of diabetes with alloxan.<sup>16</sup> The lowest dose of XA exhibited increased in sperm count, motility, and viability indicating that the damage caused by alloxan on the sperm in terms of their motility, viability, and total cell count was reversed by this dose while the higher doses of XA did not exhibit this rescuing effect. It has been reported that high doses of XA possess reversible antifertility, spermatotoxic, and anti-androgenic properties. The mechanism of which may involve a direct effect on germ cells and other cells in the testes and a possible hypothalamic-pituitary-gonadal axis involvement.<sup>14</sup>

Alloxan not only destroyed the pancreatic  $\beta$ -cells but caused kidney damage, which is however reversible, while streptozotocin selectively destroys pancreatic insulin secreting  $\beta$ -cells. The chief function of the kidney is to process blood plasma and excrete urine. These functions are important because they play a vital role in the clearance and excretion of xenobiotics including drugs and drug-product, from the body.<sup>26</sup>

However, the present study reveals no pathological effects of XA on the various tissues used in the histopathological analysis which was confirmed in the biochemical analysis where all the parameters analysed were within the normal range. Analysis of the liver, spleen, and kidney revealed that tissue morphology remains intact after treatment with XA. However, it was observed that the pancreatic beta cells destroyed by alloxan injection were not fully restored by the treatment. The estimation of the histological effect on the kidney tissues and the determination of some waste metabolic products excreted exclusively via the kidneys provide useful information about the health status of the kidneys; such metabolites include urea and creatinine.<sup>27</sup>

## CONCLUSION

Xylopic acid ameliorates alloxan-induced diabetic neuropathy and also proved to have anti-hyperglycemic properties and restored to some extent the cell architecture of the pancreas. However, restoration in damage sperm motility, viability, and cell count were found to be present at low doses while higher doses exacerbated these conditions.

## ACKNOWLEDGEMENTS

We wish to acknowledge staff of the School of Pharmacy and Pharmaceutical Science, UCC; Silas Osei, Maame Yaa Adomaa Aboagye, Nana Ama Asantewaa, Selorm Kpandey for their support during the study.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee*

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**Cite this article as:** Ameyaw EO, Obese E, Asante DB, Biney RP, Karikari AA, Adakudugu EA et al. Effect of xylopic acid on alloxan-induced diabetic neuropathy in rats. *Int J Basic Clin Pharmacol* 2021;10:333-41.