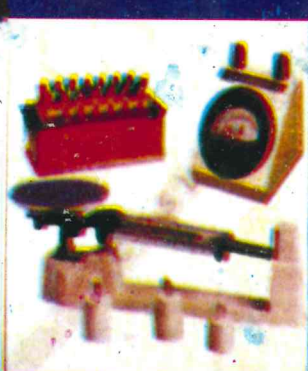


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Hypoglycemic property of *Ximenia americana* stem bark extracts, and it's effects on serum enzymes and lipid profile.

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ABSTRACT

Phytochemical screening of aqueous and methanolic extracts demonstrated the presence of alkaloids, saponins, tannins, flavonoids, phenols, volatile oils, reduced compound sugar and carbohydrate. The hypoglycaemic property of the aqueous and methanolic stem bark extracts of *Ximenia americana* was investigated in alloxan induced diabetic mice. Mice in group one were used as negative control while those in groups two, three, four and five were rendered diabetic by the administration of 305 mg/kg of alloxan monohydrate. Group one mice were treated with 10 ml/kg of normal saline, while groups two, three and four mice were treated with 500 mg/kg of standard drug (Metformin), 500 mg/kg of aqueous extract and 500 mg/kg of methanolic extract respectively. Group five mice were not treated. Aqueous and methanolic extracts produced significant ($P < 0.05$) reductions in the blood glucose concentrations of diabetic mice in descending order within 1 to 8 days of treatment while the blood glucose of diabetic untreated mice shows an increase within the same period. The hypoglycemic potential of both extracts compared favourably to that of metformin. Biochemical analyses of serum shows that, though *X. Americana* has hypoglycaemic potential, it may have hepatotoxic effect (especially the methanolic extract) but with no nephrotoxic effect. The two extracts showed a reduction in the lipid profile of treated mice and can therefore help to reduce the risk associated with increased serum lipid profile of diabetic patients.

Keywords: *Ximenia americana*, metformin, diabetes mellitus, alloxan, hypoglycemic effect.

Introduction

Traditional medicine as described by World Health Organization (WHO, 1978) is the total combination of knowledge whether explicable or inexplicable, used in diagnosing, preventing or eliminating a physical, mental or social disease. This may rely exclusively on past experience handed down from generation to generation. Treatment and control of disease by the use of medicinal plants continue to play a very significant role in the medical and dental primary health care implementation in Africa and other developing countries of the world. The advantages of traditional medicine include low cost, affordability, availability, acceptability, and to some extent, low toxicity (Sofowora, 2006).

The plant *Ximenia americana* "Wild plum or Spony Plum" in English or locally called "Tsada" in Hausa and "Chabbuli" in Fulani is a short shrub or small tree from Africa that is bushy and spiny, 4 – 5 m high with an open crown. The fruits are green but turn golden yellow or red when ripe. The fruit when eaten is refreshing and has an almond acid taste. The plant is found from Senegal to Cameroon including Northern parts of Nigeria (Arbonnier, 2004). It has been in use for centuries in many countries. It is used for many herbal preparations in Nigeria. The plant is extensively used among the Hausa/Fulani communities as herbal remedies in treating malaria, leprostatic ulcers and skin infections of mixed origin (Ogunleye and Ibitoye, 2003). The preparation of branched leaf, bark, and root is used for headaches, toothaches, mumps and conjunctivitis in frontal applications (Von May Dell, 1986). The roots are used for treating abdominal pains, dysentery, inflamed joints and mouth ulcers (Ake and Guinko, 1991). The extracts of the plant was found to be active against test organisms including *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* (Arora and Kaur, 1999). Chemical constituents of the plant extract include tannins, flavonoids, saponins, anthraquinones, alkaloids, starch, general glycosides (including cyanogenic glycosides) and bitter principles. Arbonnier (2004) reported that the fruits contain hydrocyanic acid which is toxic.

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Diabetes is a chronic disorder in the metabolism of carbohydrate, protein, and fat due to absolute or relative deficiency of insulin secretion with/without varying degree of insulin resistance (Barar, 2000). Also, it may be defined as a disease where the body either produces little insulin/ceases to produce insulin, or becomes progressively resistant to its action (Ranjan and Ramanujam, 2002). There are lots of chemical agents available to control and to treat diabetic patients, but total recovery from diabetes has not been reported up to this date (Torben, 2009). Alternative to these synthetic agents, plants provide a potential source of hypoglycemic drugs and are widely used in several traditional systems of medicine to prevent diabetes. Since *Ximenia americana* has been reported to be a wide spectrum medicinal plant among the local folks, and it is the aim of this research work to scientifically determine the efficacy of this plant as a wide spectrum medicinal plant, especially on diabetes, with a view to providing a pharmacological rationale for the folkloric use of the stem bark of *X. americana* by the Hausa and Fulani people of Northern Nigeria.

Materials and methods

Plant sample collection and extraction

Fresh stem bark of *X. americana* was collected from Sokoto town. The plant was identified and authenticated at the herbarium of Usmanu Danfodiyo University, Sokoto, Nigeria. The stem bark was air dried and made into fine powder using mortar and pestle, and sieved with 5 mm brass sieve. 50 g of the powdered sample was exhaustively refluxed with 300 ml of methanol and another 50 g was also refluxed with 300 ml of distilled water for two hours. The solutions obtained were filtered with muslin cloth and the extracts were evaporated to dryness over a water bath at 40 °C. The semi solid extracts obtained were transferred into appropriately labeled sterile air tight containers and stored in the refrigerator at 4°C until required for use. The methanolic extract yielded 11.28 g (22.56 %) while the aqueous extract yielded 9.35 g (18.7 %)

Experimental Animals

Swiss Albino mice with average weight of 20 – 30 g were purchased from Ahmadu Bello University (ABU), Zaria, Kaduna State, Nigeria. The mice were allowed to acclimatize to laboratory condition for two weeks. They were housed throughout the experimental period in plastic cages. Water and commercial grower feed were available to the animals *ad libitum*.

Grouping

The design consisted of 20 mice (16 diabetic mice and 4 non-diabetic mice), divided into five groups, with each group having 4 mice. Group 1 consists of non-diabetic mice treated with normal saline, group 2 consists of diabetic mice treated with standard drug (metformin). Groups 3 and 4 consist of diabetic mice respectively treated with aqueous and methanolic extracts. Group 5 consists of diabetic untreated mice.

Phytochemical screening of the stem bark extracts

Phytochemical tests for various constituents of were carried out by the method of Trease and Evans (1983). The extracts were screened for the presence of alkaloids, flavonoids, saponins, glycosides, tannins, reduced sugar, carbohydrates, amino acids, steroids, phenols, volatile oils and protein.

Induction of diabetes

0.3 g of crystalline powdered alloxan was dissolved in 10 ml of normal saline to yield a concentration of 30 mg/ml. 305mg/kg body weight of alloxan was administered intraperitoneally (i.p) (Lenzen, 2008) to 16 of the mice in the diabetic groups, after an overnight fast (with access to only water) of 12 hours making them more susceptible to developing diabetes (Rother, 1992). Diabetes was confirmed in the alloxan treated mice with a fasting blood sugar concentration > 300 mg/dl. This was estimated by using one touch Glucometer with blood obtained from the tail vein of the mice.

Treatment with extracts

Oral administration of normal saline (10 ml/kg), extracts (500 mg/kg) and standard drug (500 mg/kg) was carried out twice a day in a 12 hr cycle (6: am and 6: pm) for 8 days. Blood glucose was recorded before and 4

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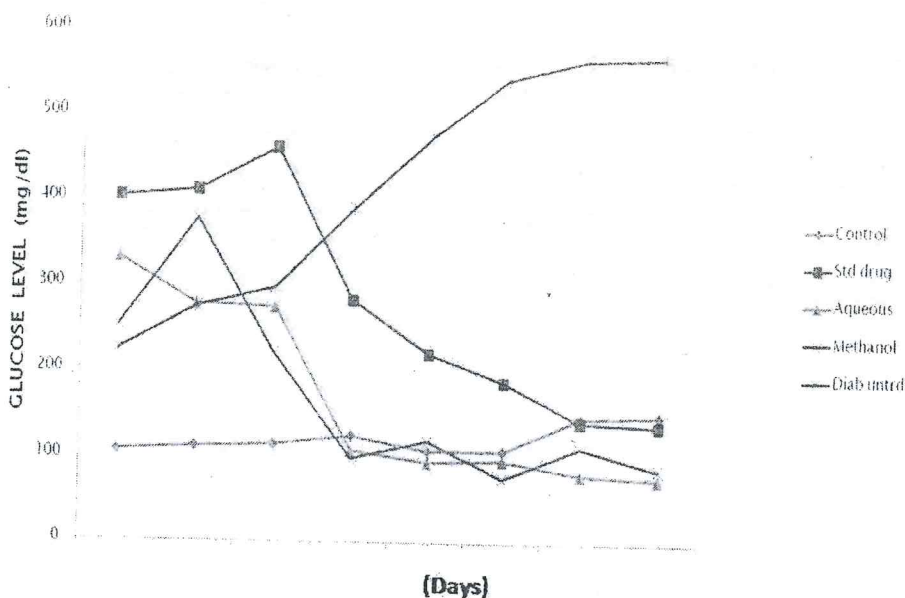


Fig. 1: The Effect of the Stem Bark Extracts and Standard Drug on Blood Glucose Levels of the Treated Animals.

Liver and kidney are two important organs that perform vital functions for the healthy survival of the body. The liver primarily detoxifies harmful substances, secretes bile into intestine, synthesizes and stores important molecules among other functions (Saydah *et al.*, 2001). The kidney helps to maintain homeostasis of the body by reabsorbing important materials and excreting waste products (Victor, 1993).

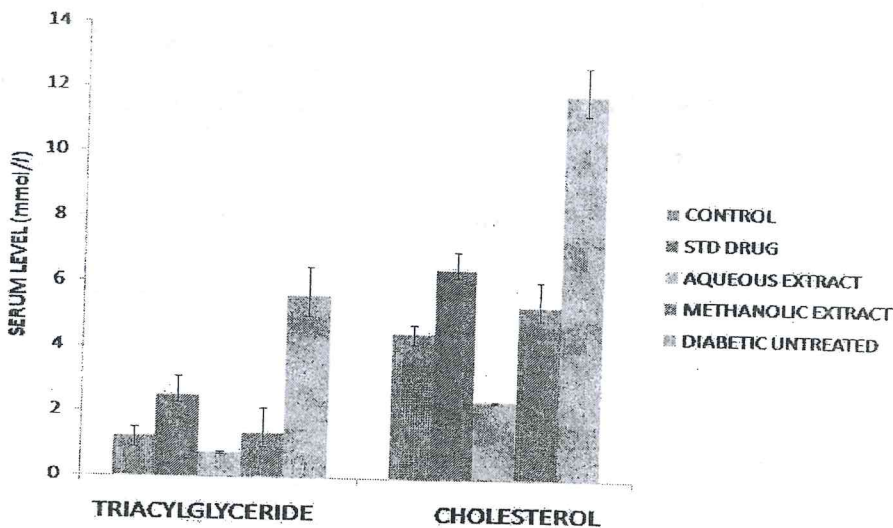


Fig. 2: Serum level of Triacylglyceride and Cholesterol (mmol/l)

Biochemical assay of the plant extracts on the animals revealed that both aqueous and methanolic extracts of *X.americana* may have hepatotoxic effect (especially the methanolic extract) because some liver enzymes were elevated (Figure 3). The extracts however have no nephrotoxic effect and both have the ability to lower lipid profile which can help to reduce the risk associated with high serum lipid levels in diabetic patients. Administration of the extracts to respective mice in the treated groups effectively reduced the concentration of triacylglyceride much more than those treated with standard drug (Figure 2). Triacylglyceride levels which are usually increased in diabetics are known to be reduced by some plant constituents. Such increase in

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triacylglyceride may be due to lack or insufficient insulin under diabetic condition, with insulin activating the enzyme lipoprotein lipase which hydrolyses triacylglyceride under normal conditions (Eisenbarth *et al.*, 2008). Mice in the treated groups showed lower concentration of the cholesterol with those in the stem bark aqueous treated group having the lowest values (Figure 2). The excess glucose associated with diabetes is converted to fat for storage, and further breakdown of fat will produce triacylglycerides and cholesterol (Ivanova *et al.*, 2009).

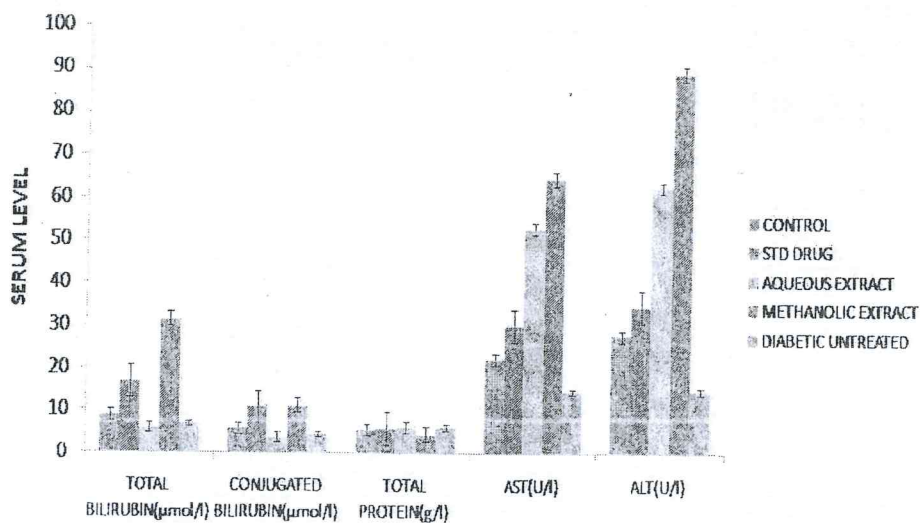


Fig. 3: Serum Level of Liver Enzymes of the Animals Treated with Extracts of *X. americana*

The methanolic extract of the stem bark induced the highest increase in serum levels of total bilirubin, conjugated bilirubin, aspartate transaminase (aspartate aminotransferase), alanine transaminase, alkaline phosphatase, urea, and creatinine (Figures 3 and 4). This result is in line with the work reported by Brown *et al.*, (2007), where chronic kava beverage consumption was associated with elevated ALT. The rise in the level of ALT is usually accompanied by elevation in the levels of AST which plays a role in the conversion of amino acids to keto acids. Both ALT and AST are excellent markers of liver damage caused by exposure to toxic substances (Ranjna, 1999). ALT is the more liver specific enzyme for diagnostic use when the integrity of the hepatocellular membrane is compromised, because there is extrusion of the enzyme into the plasma. The standard drug (metformin), however have little effect on the creatinine, and urea levels.

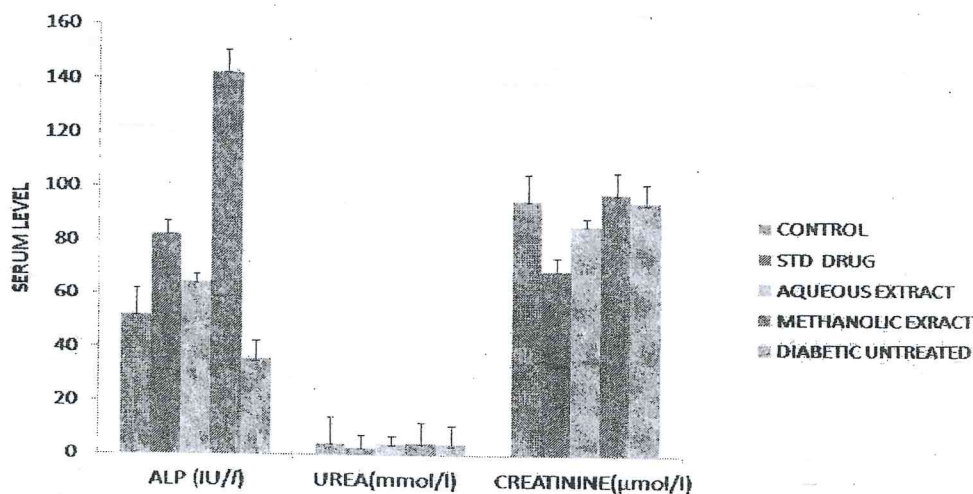


Fig. 4: Serum Levels of Alkaline Phosphatase, Urea and Creatinine

Conclusion

The stem bark extracts of *X. americana* has hypoglycemic property with no nephrotoxic effects. It can therefore be suggested that the plant extracts may in part stimulate insulin production and glucose utilization, like metformin. This brings about the hypoglycemic effects in the mammalian experimental model used. This property may be attributed to the presence of flavonoids and alkaloids in the plant as these two chemical components possess antidiabetic potentials. Although the present findings suggest the presence of hypoglycemic property in stem extracts of *X. americana*, the precise mechanism of its hypoglycemic action is still speculative and therefore requires further studies for appropriate elucidation.

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