**PHYTOCHEMICAL CONSTITUENTS AND HYPOGLYCEMIC EFFECT OF METHANOL EXTRACT OF *Vitellaria paradoxa* STEM BARK IN ALLOXAN INDUCED DIABETIC RATS**

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**ABSTRACT**

*Vitellaria paradoxa***,** commonly known as Shea butter tree is a highly medicinal plant which is locally abundant in Nigeria. This study aims to investigate the phytochemical constituents and hypoglycemic activity of methanol extract of *Vitellaria paradoxa* stem bark in alloxan induced diabetic rats. The phytochemical screening was carried out using standard procedures and diabetes was induced by a single intraperitoneal dose of 120mg/kg body weight of alloxan and 6hrs later, the animals were kept on 20% glucose for 24hrs.The result showed these phytoconstituents; alkaloids, flavonoids, anthraquinones, saponins, tannins, phlobatanins and cardiac glycosides were present. The oral administration of extract at a dose of 500mg/kg body weight showed a significant reduction (P<0.05) in the blood glucose level of diabetic rats from 168.75±10.61 - 95.25±1.65 while the standard drug (Glibenclamide 0.5mg/kg body weight) decreased Fasting blood glucose levels from 162.25±5.36 - 86.25±3.40. There was a significant increased (P>0.05) in blood glucose level of induced diabetic but not treated rats from 192.50±8.86 to 195.25±12.96 compared to non-induced but treated with extracts from 101.25±3.73 to 81.00±5.98. These results suggest that the extract may possess some anti-hyperglycemic potentials.

**KEYWORDS:** *Vitellaria paradoxa,* Alloxan*,* Phytochemical constituents, Hypoglycemic.

**INTRODUCTION**

World Health Organization (WHO) defined diabetes mellitus as a health disorder resulting from metabolic disorders of numerous etiology which is associated with chronic hyperglycemia with disturbances in the metabolism of carbohydrates, fats and proteins owing to the defects in insulin secretion or action, or combination of the two. Long-term damage, dysfunction and failure of several organs are effects associated with diabetes mellitus. Symptoms of diabetes mellitus may include thirst, polyuria, blear vision and loss of body weight (Rakesh *et al.,* 2015). Diabetes mellitus (DM) is the most common endocrine disorder affecting approximately 5% of the world population. In the year 2000, the estimated occurrence of diabetes in Nigeria was 1.707 million. It is projected by WHO that by 2030, the number of diabetics would be 4.835 million. Hyperglycemia is the principal cause of complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration. The effective blood glucose control is the key to improving the quality of life in patients with diabetes (Defronzo *et al.,* 1992; Laha and Paul, 201). Insulin therapy and oral hypoglycemic drugs have been used for the treatment of diabetes mellitus. The drawbacks in the use of the insulin therapy and hypoglycemic drugs are the adverse side effects exerted by these synthetic anti-diabetic agents on human body (Cinmay *et al.,* 2015). Since time immemorial, varieties of plants and their produce have been used by human as foods or drugs in treatment of disease (Sodipo *et al.,* 2001). Medicinal plant extracts have been valuable antidiabetic agents and may involve one or more active component responsible for blood glucose reduction (Grover, 2002). Based on the WHO recommendations hypoglycemic agents of plants origin used in traditional medicine are important. There are many literature reviews by different authors on the plants with hypoglycemic effect, but most informative is the review of Atta-Ar-Rahman who has documented over 300 plant species for their antihyperglycemia activity. WHO listed 21,000 plants which are used for pharmacological purposes (Cinmay *et al.,* 2015). Abdel *et al.,* (2014) reported that natural products are the major source of raw materials for the development of modern drugs. Herbal preparations are used for managing health conditions mostly by rural area dwellers due to its availability, low cost and lesser side effects.

*Vitellaria paradoxa* belongs to the family Sapotaceae. It is the only species in the genus which is of Africa origin (Lovett and Hag, 2000). It is a plant that is locally abundant in Nigeria in the derived savannah Zones, particularly near thousands of villages (Adegoke *et al.,* 2007). It is rich in oil and replaces oil palm as source of edible oil in Northern Nigeria. Shea butter is the fat extracted from the kernel of Shea fruits. It is becoming increasingly popular as a component of cosmetic formulation or addition to its long standing use as cocoa butter substitute in the chocolate industry (Adgidizi *et al.,* 2008). Shea butter contains high level of uv-absorbing triterpenes esters which include cinnamic acid, tocopherols (vitamin A), and phytosterols (Wiesman *et al.,* 2003). Shea butter oil is thoroughly used as cooking fat in Africa and serves as a suitable base for tropical medicine used locally to relieve inflammation of the nostril, skin burn, dermatitis, rheumatic and joint pains. There are no reports of allergic reaction owing to consumption of shea butter nuts or its produce (Watson, 2005). The consumption of plants and their produce have been associated with reduced risk of disease like Alzheimer disease, cancer, lipid peroxidation and other free radical driven disorder as well as malaria. Medicinal potentials of locally available plants and their produce was from the claim of some herbalists, native and ancient that some plants and their produce have medical value but they lack enough empirical proofs to justify these claims. Scientific evaluation of medicinal plant is important in the discovery of novel drugs and also helps to assess risk associated with the use of conventional drugs of herbal origin (Mroeh, 2008).

**MATERIALS AND METHODS**

The fresh stem bark of *vitellaria paradoxa* were collected in August 2018 from bosso secondary school Minna- Niger state, Nigeria. Authentication and identification was done in the department of Biological science, Federal University of Technology, Minna, Niger state. All chemical and reagents used were of analytical grade.

**SAMPLE PREPARATION AND EXTRACTION**

Fresh stem bark of *Vitellaria paradoxa* was washed, rinsed and air-dried in the laboratory room temperature for a period of two weeks and pre-crushed manually using mortar and pestle. It was further pounded into powder and kept in polythene bag. 200g of powdered stem bark of *vitellaria paradoxa* were divided into a four portion and each 50g was dissolved in 400ml of 70% methanol and was dispensed into a reflux apparatus for extraction at 60oC for 3hrs. The extract was filtered using filtered paper. The filtrate was concentrated using rotary evaporator and water bath. The extract was collected, weighed and stored in avail, and then kept in the refrigerator until required for used.

**PHYTOCHEMICAL SCREENING**

The methanol extract of *Vitellaria paradoxa* stem bark was screened for qualitative phytochemical properties by standard methods of (Trease and Evans, 2002; Sofowora *et al.,* 1982)

**SAPONINS**

To 0.5 mL of extract, 10mL of distilled water was added. Frothing which persist on warming of test confirms the preliminary evidence for the presence of Saponins (Trease and Evans, 2002).

**TANNINS**

About 3 mL of the extract was added to 5ml of distilled water and then heated in water bath. To this solution, iron (III) chloride (FeCL3) solution was added. A blue black or green precipitate indicated the presence of tannins (Trease and Evans, 2002).

**ALKALOIDS**

To 2 mL of the extract, an aliquot of 10 mL of 1 % HCL in water bath for 30minutes. The solution was then treated with few drops of Wagner’s reagent and colour change was observed. Presence of alkaloids was indicated with precipitate formation (Sofowora, 1982).

**CARDIAC GLYCOSIDES**

An aliquot (2 mL) of chloroform was used to dilute 0.5 mL of extract. Sulfuric acid was carefully added to the solution drop wise. At the sulfuric acid interphase, a reddish coloration indicated the presence of cardiac glycosides (Trease and Evans, 2002).

 **ANTHRAQUINONES**

To 5 mL of the extract, 10 mL of benzene, 5mL of 10 % aqueous ammonia was added and shaken. Presence of anthraquinones was indicated by a pink/violet colour in the ammonia phase at the bottom of test tube (Sofowora, 1981)

**PHLOBATANINS**

About 0.5g of the extract was left boiling with 5ml of 1 % HCL in a test-tube. A red precipitate indicated the presence of phlobatanins (Aktar *et al.,* 2015*)*.

**FLAVONOIDS**

To 4ml of extracts, 1.5ml of 50%% methanol was added. The solution was slightly heated and metal magnesium was added. Then 5 drops of concentrated hydrochloric acid to the solution and observed for red coloration.

**REDUCING SUGARS**

To 0.5ml of extract solution, 1ml of distilled water is added and about 6 drops of Fehling’s solution was prepared by mixing equal volumes of equal molar concentrations of A and B. The formation of brick red precipitates is an indication of the presence of reducing sugars.

 **PREPARATION OF STOCK SOLUTION**

The concentrated methanol extract of *Vitellaria paradoxa* stem bark (1 g) was dissolved in 10ml of distilled water and the prepared solution was stored in the refrigerator.

**EXPERIMENTAL ANIMALS**

Thirty Wistar rats of both sexes (8-11 week) old obtained from the animal house of Ahmadu Bello University, Zaria weighing between 100 -150g were used for the study. The rats were housed in well ventilated plastic cages, allowed free access to food, water and acclimatized for two weeks.

**INDUCTION OF DIABETES**

Prior to diabetic induction, the blood glucose concentration of the rats was taken. The rats were fasted for 12hrs and diabetes was induced by intraperitoneal injection of alloxan at a dose of 120mg/kg body weight and 6hrs later the animals were kept on 20% glucose for next 24hrs. After 72hrs (3days) of induction, the animals with blood glucose level above 150mg/dl were selected for the experiment.

**EXPERIMENTAL DESIGN**

 The rats were divided into the groups below with each group consisting of 6 rats.

 GROUP A; Alloxan induced diabetic rats treated with 500mg/kg body weight of extract

GROUP B; Alloxan induced diabetic rats treated with 0.5mg/kg body weight of standard

GROUP C; Alloxan induced diabetic rats but not treated

GROUP D; Non-induced rats but treated with 500mg/kg body weight of extract

GROUP E; Non- induced not treated (normal).

**DETERMINATION OF BLOOD GLUCOSE LEVEL**

The blood glucose level was determined using Accu-check glucometer and glucose strips. The rats were fasted for 12 hours before the tail vein was punctured and blood from the tail was dropped on the tip of glucose strip placed in a glucometer, and blood glucose concentration was recorded in mg/dl

**DETERMINATION OF BODYWEIGHT**

The bodyweight of rats was determined using dry cell battery weighing balance. The weight was monitored before induction, after induction and during period of treatment weekly.

 **ORAL ADMINISTRATION OF EXTRACTS**

Methanol crude extract (500 mg/kg bodyweight) was administered orally to group A and D while 0.5mg/kg body weight of standard drug (glibenclamide) was administered to group B

**STASTICAL ANALYSIS**

The data obtained were subjected to Analysis of Variance (one- way Anova) using SPSS statistical package. Means were separated using Duncan’s Multiple Range Test (DMRT). The data was given as mean +SEM (Standard error mean). Differences were considered significant at *P* < 0.05.

**RESULTS**

**Table: 1 Phytoconstituents of the methanol extract of *Vitellaria paradoxa* stem bark**

|  |  |
| --- | --- |
| **Phytochemical** | **Inference** |
| Alkaloids | **+** |
| Saponins | **+** |
| Reducing sugar | **-** |
| Flavonoids | **+** |
| Anthraquinones | **+** |
| Cardiac glycosides | **+** |
| Phlobatannins | **+** |
| Tannins | **+** |

Key: + = present, ­ = absent

**Figure 1**: **Effect of methanol extract of *Vitellaria paradoxa* stem bark in alloxan induced diabetic rats**

Figure 2: **Effect of methanol extract of *Vitellaria paradoxa* stem bark on body weight of alloxan induced diabetic rats**.

**Key**:

Grp1-induced treated with 500mg/kg body weight of extract

Grp2-induced treated with 5mg/kg body weight of standard drug (glibenclamide)

Grp3-induced not treated

Grp4-no- induced but treated with extract

Grp5-non- induced not treated (normal

**DISCUSSION**

This study has revealed the presence of seven phytoconstituents in the methanol extract of the stem bark of *vitellaria paradoxa* (Table 1). The phytocomposition includes; alkaloids, flavonoids, anthraquinones, saponins, tannins, phlobatanins and cardiac glycosides. This result has been similarly reported by Onwuliri, 2004 and Falana *et al.,* 2016. The insignificant difference (P>0.05) hypoglycemic potentials observed in alloxan-induced diabetic rats treated with 500mg/kg body of methanol extract of *vitellaria paradoxa* stem bark when compared with the standard drug glibenclamide administered at 0.5mg/kg bw (Figure 1) showed some promising hypoglycemic activity. This result correlates with the work of Abubakar et al., 2019 that the Crude Methanol extract of *Vitellaria paradoxa* leaf had significant hypoglycemic and antioxidant activity. The hypoglycemic activity of the plant under study may be linked to the individual or synergistic action of of phytoconstituents present. Flavonoids have been reported by many authors to mediate it antihyperglycemic activity by potentiating the insulin effect either by increasing the pancreatic insulin secretion by the cells of islet of langerhan or it enhancing it release from bound insulin (Pari and Amarnath, 2004; Saidu et al., 2014: Abubakar et al., 2014). Alkaloids are also known to exert a wide range of antidiabetic activities through different mechanisms such as inhibiting intestinal glucose uptake or stimulating insulin secreation (Chattopadhyay, 1999). Saponins are potential hypoglycemic agents which acts by increasing glucose uptake and subsequent glycogen synthesis or as an insulin secretaguogue (Naik et al., 199; Santos, 2012). Tannins displayed beneficial effects in the prevention and treatment of type 2 diabetes mellitus, possibly by inhibiting alpha-amylase and alpha-glucosidase activities (Palanisamy *et al*., 2011). According to the report by Jouzier and Berké (2012), tannic substances extracted from *Eucalyptus* *globulus* (*Myrtaceae*) and fruits of *Terminalia catappa* (Combretaceae) possess valuable hypoglycemic properties and exhibit antidiabetic activity

Alloxan selectively destroys the pancreatic β-cells secreting insulin leaving the less active cell thus resulting in diabetic state. (Szkudelski *et al.,* 2001). Insulin deficiency or insensitivity causes increase in endogenous glucose concentration due to non-utilization of glucose by the cells. This causes increased in demand for gluconeogenic sources, there by leading to excessive breakdown of fats and protein as the alternative source of energy. The significant increase (P<0.05) in the body weight of diabetic rats treated with 500mg/kg of methanol extract of *vitellaria paradoxa* stem bark compared with the untreated rats showed that the weight improvement might be due to proper maintenance of glucose homeostasis while the decreased in body weight of the untreated rats might be due to excessive breaking down of tissue protein and lipid cause by insulin insufficiency (Ramachandran *et al.,* 2012).

**CONCLUSSION**

This study reveals that methanol extract of *Vitellaria paradoxa* stem bark possess some important phytochemical constituent that has hypoglycemic potentials

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