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Determination of the Hypoglycaemic Effect of the Ethanol Stem Bark Extract of *Vitellaria paradoxa* on Hyperglycemic Wistar Rats

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Abstract

This study investigates the hypoglycaemic effects of the ethanol stembark extract of *Vitellaria paradoxa* on hyperglycemic rats. It also investigates the phytochemical content of ethanol stem bark extract of *Vitellaria paradoxa* using the method of Soforh. The result shows that the ethano| stem/bark of the plant contains Tannins 3.92/mg/100g, Alkaloid 7.12/mg/100g, Flavonoids 1.03/gm/100g, phenolics 12.72/mg/100g, Glycosides 4.13/gm/100g, saponins 2.15/mg/100g respectively. The hypoglycemic properties of ethanol stem bark extract of *Vitellaria paradoxa* were investigated using Fifteen albino Wister rats weighing between 200g to 230g were induced with alloxan monohydrate to make them diabetic. The rats were divided into five groups of three rats. The first group were not induced, tagged normal. The second groups were induced sstandard drug glibenclamide throughout the study period. The fourth groups were induced and treated with 250/mg/kg body weight of the ethanol stem/bar extract of *Vitellaria paradoxa* throughout the study period. The fifth groups were induced and treated with 500/mg/kg body weight of the extract. The fasting blood glucose level of the rats were taken every three days' interval for 21 days. At the end of 21 days, the rats treated

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with 250/mg/kg body weight which started with elevated blood glucose level after induction of 207/mg/dl reduces to 124/mg/dl, similarly the treated with 500/mg/kg body weight of the extract with initial elevated blood glucose level after the induction with diabetic of 221/mg/dl got reduced to 145/me/dl. This indicates that ethanol bark tract of *Vitellaria paradoxa* showed hypoglycemic properties

Keywords: Hypoglycaemic effects, Ethanol stembark extract, Vitellaria paradoxa, Phytochemical content, Hyperglycemic rats, Alloxan monohydrate

INTRODUCTION

Diabetes mellitus (DM), often simply referred to as diabetes is a group of metabolic diseases in which a patient has high blood sugar level, either because the body does not produce enough insulin or because the cells do not respond to the insulin that is produce. (Akah *et al*., 2007). These high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). It is characterized by hyperglycaemia due to defective insulin action, insulin secretion or both. Several medicinal plants are used in the management of diabetes mellitus (Akah *et al*., 2007). Currently in the united states, up to lin 3 new cases of diabetes mellitus diagnosed in youth younger than 18 years is type 2 diabetes mellitus with a disproportionate representation in ethnic minorities occurring most commonly among youth between 10 to 19 years of age (Akah *et al*., 2007). This trend is not limited to the united states but is occurring internationally; it is projected that by the year 2030, an estimated 366 million people worldwide will have diabetes mellitus (Copeland *et al*., 2011).

Indian have the highest number of diabetic patients in the world, this sugar disease is posing an enormous health problem in the country. Calling Indian, the diabetes capital of the world, the international journal of diabetes in developing countries says that there is alarming rise in diabetes in Indian. An estimated 3.4 million death occur due to consequence of high blood sugar. World Health Organization also estimate that 80% of diabetes death occur in low- and middle-income countries and it is projected that such diabetes will double by 2030 (Kumar *et al*., 2015**)**. Despite great advances in diabetes care, type 1 diabetes is still linked with considerable premature mortality resulting from both acute and chronic complication of diabetes (Harjutsalo *et al*., 2005). Chronic

hyperglycaemia in diabetes mellitus is the course of many late complications characterized by kidney disease, blindness and the risk of hg cardiovascular disease. (Zimmet *et al*., 2001).

Available drugs often leave behind side effects. However, herbal treatment is an alternative in the treatment of this pathophysiology in as much as plants are used to cured diabetes. (Marles and Fransworth, 1995). The active ingredients present in some medicinal plants have been reported to possess pancreatic beta cells regenerating potentials, insulin releasing and fighting the problem of insulin resistance (Kavishantar *et al*., 2011).

Ethno-pharmacological surveys indicate that more than 1,200 plants are used in traditional medicine systems following claims of their hypoglycemic properties (Dey *et al*.,2002). The hypoglycemic activity of a large number of plant products have been evaluated and confirmed in animal models (Gupta *et al*., 2005), as well as in human beings (Musabayane, 2012). In some cases, the bioactive principles of the medical plants have been isolated and identified (Kesari *et al*.,2005). Nevertheless, the mechanisms of action of most of these anti-diabetic bioactive ingredients are not well defined and remain largely speculative. However, reports suggest that the array of anti-diabetic bioactive principles in medicinal plants may act in synergy to exert glycemic control (Ojiako *et al*., 2015); through interference with one or more processes involved in glucose metabolism and homeostasis (Bnouham *et al*., 2006).

Vitellaria paradoxa is also known in English as sheanut tree. The leaves of *Vitellaria paradoxa* is used traditionally for the treatment of some form of ulcer, and the bark for the treatment of malaria, dental pain and neurologic treatments. It has also been reveals that it has potential antidiabetic effects (Coulibaly *et al*., 2007). *Vitellaria paradoxa* (C.F. Gaertn) is an indigenous fruit tree belonging to the family *Sapotaceae*, known locally as Shea butter tree sometimes comparatively assessed for their phytochemical constituents.

MATERIALS AND METHODS

Freshly harvested stem bark of *Vitellaria paradoxa* were harvested along Katsina-Ala road near Federal University Wukari, Taraba State. The taxonomic authentication of the plant was conducted at Forestry and Wildlife Management Department, Federal University Wukari.

The chemical and reagents used for the experimental findings were product of Sigma Chemical Company, USA. All the reagents were freshly prepared. Distilled water was also used throughout the experiment. Normal saline was equally used.

The apparatus used for this study include the following: test tubes, beakers, conical flasks, burettes, round bottom flasks, sample tubes, measuring cylinder, masking tape, volumetric flasks, separating funnels whatmann filter paper, electric weighing balance, drying oven, water bath, foil mortar, pestle and electronic blending machine, mechanical shaker. All the glass wares were thoroughly washed and cleansed with liquid detergent rinsed with distilled water before been oven dried at 105°C.

Method

The dried bark/stem of the *Vitellaria paradoxa* were pulverized (100g), the crude extract was extracted with 400mL 70% ethanol using soxhlet apparatus at 60°C for three hours. Concentrates was done using water bath. The residues were then stored in a sterilized bottle and then refrigerated until ready for used.

The oral antidiabetic drug glibenclamidine was purchased at the pharmacy in Wukari metropolis. A tablet of the drug contains 5mg of glibenclamidine which was dissolved in 10mL of distilled water to make a concentration of 0.5/mg/ml.

Healthy Wistar rats of average weights 180-200g were purchased from Kwararafa University at the Animal and Life Sciences Department, Wukart Taraba State (animal house). The rats were kept in a clean aluminium cage and maintained under standard laboratory conditions. They were allowed to feed on purchased poultry feed pellets purchased at a location adjacent Gongola clinic, Wukari, Wukari Local Government. They were also allowed to acclimatize for two weeks before the commencement of the experiment.

Induction of Diabetes

A freshly prepared solution of alloxan monohydrate (120/mg/kg) was injected intraperitoneally. After 18 hours. The fasting blood sugar level of the animal was checked after three days to detect those animals that were hyperglycemic. Animals with marked hyperglycemia (120/mg/dl and above) were selected for the study (Etuk *et al.*, 2010).

Research Design

Fifteen rats were divided into five groups of three rats each and were administered the following;

Group A; Normal control rats.

Group B; Negative control rats given 2/mL/kg of normal saline after diabetic induction.

Group C; Diabetic rats given aqueous *Vitellaria paradoxa* extract 250/mg/kg once daily.

Group D; Diabetic rats given aqueous *Vitellaria paradoxa* extract 500/mg/kg once daily.

Group E; Diabetic rats given glibenclamide 0.5/mg/kg once daily.

All these treatments were administered orally once daily for twenty-one days

Estimation of body weight was taken.

Estimation of Body Weight

The weights of all the rats were taken prior to induction of diabetes using an electronic weighing balance. The weight of all the rats were also taken after diabetes induction and subsequently at three days' interval.

Estimation of Blood Glucose Level

The blood glucose level in mg/dl was determined during the period of treatment within three days' interval using glucometer which was done by collecting the blood through orbital puncture of the tail vein of the rats.

RESULTS

The data presented in table 4.1 depicts the phytochemical investigation reports for the ethanol stem bark extract of *Vitellaria paradoxa*, this result indicates that the ethanol extract from the stern bark of *Vitellaria paradoxa* was found to contain tannin, alkaloid, flavonoid, phenolic, glycosides, and saponins respectively. The phytochemicals composition is express in mg/100g as shown below.

Table 1: Result for Phytochemical Investigation

No	Phytochemical parameters	Concentration (mg/100g)
1	Tannins	3.92
2	Alkaloids	7.12
3	Flavonoids	1.03
4	Phenolics	12.72
5	Glycosides	4.13
6	Saponins	2.15

From the result presented in the table 1 above shows that *Vitellaria paradoxa* contain high amount of phenolic, follow by alkaloid, while glycoside and tannin are moderately present, saponin and flavonoids are very low respectively.

Table 2: Blood Glucose Level

Period (Days)	Extract concentrations (250/mg/kg b.w)	Extract concentrations (500/mg/kg b.w)	Standard drug Glibenclamide (5mg/kg b. w)	Normal control	Negative control
0	93.0±2.44	97.0±6.04	94.0±7.14	84.00±3.51	98.5±3.50
3	207±8.50	221±9.17	229±2.25	83.70±3.40	297±2.51
6	203±6.20	223±2.30	219±7.43	85.00±6.02	333±2.50
9	199±6.32	215±5.41	184±4.23	88.50±2.50	348±2.42
12	192±5.51	208±3.41	170±9.25	85.00±6.02	348±2.51
15	188±8.17	199±3.05	156±9.25	95.00±6.02	347±3.45
18	159±2.52	178±2.11	136±2.44	84.00±5.51	349±7.22
21	127±5.30	145±9.51	127±3.47	87.03±1.11	349±7.32

The values are expressed in mean ± standard error of mean followed by one-way ANOVA (analysis of variance).

Table 2 mean ± standard error of mean of ethanol stem bark extract of *Vitellaria paradoxa* on Diabetic Rats. (Blood Glucose level in mg/dl)

The normal blood glucose level of the rats ranges from 100 to 103/mg/dl in albino rats. However, all the rats used for this experimental research were hypoglycemic. Their blood glucose concentration ranges from 84/mg/dl to 98/mg/dl before induction of diabetes. However, when diabetes or hyperglycemia was induced, at the third day, all the rats used

for this experiment had been made diabetic as their blood glucose level rose significantly. This is an indication that all the rats have been induced with hyperglycemia. The rats used for control group are not induced at all, the average blood glucose concentration level was 84/mg/dl at the beginning of the experiment, it remains fairly within that range for the first nine days of the experiment, a concentration of 85/mg/dl. However, the blood glucose level rose from 85/mg/dl to 95mg/dl within the next 3 days from 12" days to the 15" days, sharp increase of 10/mg/dl. But from then the blood glucose level drop to within 85 to 88/mg/dl. Since the rats were fed constantly with normal rat's feeds, the little variation may be as a result of feeding habit or feeding schedules.

For those rats induced with diabetes and treated with standard drug glibenclamide. The blood glucose level before induction and commencement of treatment was 94/mg/dl. After the induction, the blood glucose level rose to 229/mg/dl, indicating a high hyperglycemic condition had been induced. However, with commencement of treatment with glibenclamide, the blood glucose level begins to decrease from 229/mg/dl to 219meg/dl after 6 days of treatment. Further treatment saw the glucose level decreases down to 170/mg/dl after 12 days and tol56/mg/dl after 15 days, and the down decline in blood glucose concentration continue throughout the period of experiment.

The rats treated with 250/mg/kg body weight extract after induction with alloxan monohydrate became diabetic and showed blood glucose level of 207/mg/dl from blood glucose concentration level of 93mg/dl before induction. However, with commencement of treatment with extract, (250/mg/kg b.w) there commence gradual decreases all throughout the period of the experiment. This extract (ethanol stem bark of *Vitellaria paradoxa*) shows a great promise of clearing hyperglycemia. This is because from day 3 to day 21 of treatment, there was a decrease in blood glucose concentration level all through.

The rats treated with the extract concentration 500/mg/dl body weight followed similar pattern. Before induction of diabetes, the blood glucose concentration of the experimental rats for this group was 97/mg/dl, after induction, the blood glucose rose sharply to 221/mg/dl. However, with the start of treatment, the blood glucose level began to drop all through the period of the treatment and by extension to the period of experiment. The blood glucose level reduced from 221/mg/dl to 215/mg/dl after 9th days. On the 12th days of treatment, a significant drop in blood glucose level concentration brought the blood

glucose level to 208/mg/dl, and in subsequent treatment, the down ward decrease in blood glucose level continued.

Those rats induced, but not treated with the extract or the standard drugs representing negative control. The blood glucose level concentration initially was 98/mg/dl, but immediately after induction, there was gradual and steady rise in blood glucose level all through the period of the experiment. This was incidentally followed by decrease in weight of the rats. The result for the effect of ethanol! stem bark extract of *Vitellaria paradoxa* on the body weight of the rats at every three days' interval for 21 days are depicted in the table below:

Table 3: Body Weight of the Rats N

Period (days)	250mg/kg b. w	500mg/kg b. w	Standard drug Glibenclamide (5mg/kg b. w)	Control not induced	Control induced not treated
0	214.00±2.60	211.60±6.30	219.50±6.41	217.00±2.80	231.00±2.05
3	175.40±3.61	163.50±4.00	171.00±6.45	219.00±3.00	199.00±4.00
6	177.00±2.52	168.00±4.32	172.60±5.51	186.00±4.51	174.00±3.51
9	180.50±1.29	165.00±5.09	184.40±2.53	196.00±2.50	167.50±4.51
12	184.60±1.45	173.60±5.44	186.00±3.22	199.00±7.37	165.00±3.21
15	185.00±2.04	178.50±6.21	198.00±1.43	171.00±4.45	159.60±6.52
18	187.00±5.51	184.00±5.54	203.50±7.01	184.00±6.20	161.00±2.50
21	205.50±3.05	210.00±4.43	212.00±7.36	191.00±4.32	150.00±3.45

The values are expressed in mean + standard error of mean Followed by one-way ANOVA (analysis of variance).

At the point of induction of hyperglycemia on the rats, all the rats used had no significant difference in weight as shown in the table above. For the normal control (not induced) there was increase in body weight from the beginning of the experiment to third day of the experiment (217g to 219g). this was followed by a sharp decrease in body weight from 219g to 186g from day 3 to day 6 followed by an increase in weight from 186g to 199g from day 6 to day 12 of the experiment. This was followed again by a sharp drop in body weight to l71g from day15, and sharp increase again from 171g to 184g and 191g at day 18 and 21 respectively. This may be due to feeding habit.

For rats treated with standard drug glibenclamide, the average starting weight of the rats is 219g but on induction of diabetes, the weight decreased to 171g this therefore confirmed that diabetes can cause weight loss. However, on Commencement of treatment with glibenclamide, there 1s obviously a stead but Bradual increase in weight from 171g to 172 from day 3 to day 6 and 184g to 212g from day 9 to day 21. However, treatment with standard drug glibenclamide is an indication for treating hyperglycemia which indicate a gradual recovery from hyperglycemia with steady increase in weight of the rats from 171g to 212g from day 3 to day 21 respectively.

For those rats that were induced and treated with extract of 250/mg/kg body weight, the average starting weight of the rats was 214g, but after induction, on the 3rd day of the experiment, the weight drops to 175g, this shows an indication that diabetes can cause weight loss. As the rats were administered with the extract of the stem bark of *Vitellaria paradoxa* (250/mg/kg b.w), there is gradual weight recovering from 175g on the 3rd day to 177g on the 6th day and 180g on the 9th day followed by gradual increase throughout the period of the experiment.

The induced rats that are treated with 500/mg/kg body weight; the average weight of the rats before induction of diabetes was 211g. after induction, the weight of the rats dropped to 163g, again this show an indication that diabetes causes weight loss. As treatment begins, on the 6th day, the weight increases from 163g to 168g and gradually decreases back to 165g on the 9th day, on the 12 day the weight began to increases gradually again from 165g to 173g. and continue to increase throughout the period of the experiment.

For the rats that are used as negative control (induced with diabetes but not treated with extract nor standard drugs), has their average weight of 231g before induction of diabetes. However, after induction, there commence a gradual reduction in weight all throughout the period of the experiment. This is an indication that diabetes causes loss of weight.

DISCUSSION

The result obtained during the treatment of the animals were represented in tables. The data permit comparison of different trends of blood glucose values obtained from the plant extract and the standard drug glibenclamide. There was a significance reduction in the blood glucose level, when the alloxan induced animals were treated with these extract as well as glibenclamide. Treatment of animals with *Vitellaria paradoxa* (500/mg/kg) body

weight showed the best activity of all the concentration of the extract administered to the rats. We can therefore infer that the reduction of blood glucose level was associated with treatment given, since there was no corresponding blood glucose reduction in the animals in negative control group. The result was a prove that herbal treatment of hyperglycemia is a therapeutic option for diabetes mellitus. There was equally a significance increase in mean body weight of the diabetic rats when the standard drug and the extract were all administered. The result shows that there is a relationship between diabetes mellitus and change in the body weight. The weight of the animals began to reduce after induction with alloxan, but on treatment with extract, there was significance increase in the body weight of the animals. A survey of several medicinal plant research findings showed that the polysaccharides, sterols, terpenoids, alkaloids, saponins, flavonoids, amino acids and their derivatives are the most encountered bioactive ingredients that exhibited glycemic control in experimental animals. (Ivorra *et al*., 1989).

In this research finding, since the stem bark of *vitellaria paradoxa* contain bioactive ingredient such as alkaloid, saponins, flavonoids, yerpenoids, tannins and glycoside respectively. We can say that the stem park extract of *vitellaria paradoxa* exhibit glycemic control on albino cats.

CONCLUSION

According to the result obtained during the research finding, the data obtained shows that ethanol stem bark extract of *Vitellaria paradoxa* induced hypoglycemic activity in hyperglycemic albino wister rats at dose 250/mg/kg body weight and 500/mg/kg body weight compare to the standard drug glibenclamide. Therefore, we can conclude that, ethanol stem/bark extract of *Vitellaria paradoxa* shows an alternative herbal therapeutic remedy for diabetes mellitus as compared to the standard drug glibenclamide. This also shows that the bioactive substances present are responsible for activity of hypoglycemic control.

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