Effects of Petroleum ether and n-Hexane Extracts of Globemetula braunii on glucose, Lipids and Some Biochemical Parameters of Diabetic Rats

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Abstract- *Globimetula braunii* is a parasitic plant (mistletoe) used to treat diabetes and hypertension by Nupe speaking people of Niger Sate, Nigeria.Extracts of petroleum ether and n-hexane showedthe presence of steroids, terpenes,and phenols.Rats of both sexes, weighing (135-244)g were randomly allotted to five groups of four rats each. Rats in group one (control) were the normoglycaemic (administered 10 ml distilled water daily),while those in groups two, three, four, and five were rendered diabetic by the administration of 100mg/kg bodyweight of alloxan monohydrate. Group five rats were treated with 500mg/kg bodyweight of standard drug (metformin), groups three and four rats were respectively treated with 500mg/kg bodyweight of petroleum ether and n-hexane extracts for two weeks, while the group two rats were untreated(negative control). Blood glucose was checked after every two days using glucometer. Blood glucose concentration of rats in the extracts (n-hexane and petroleum ether)treated groups decreased significantly (217.16±4.33 – 84.33±2.33), with n-hexane extract having the highest (84.33±2.33).The animals were anaesthesized under chloroform at the end of the treatment andblood samples were collected by jugular puncture and used for the analysis of biochemical parameters. Serum cholesterol of the two extract treated groups reduced significantly((162.43±1.03)and (147.93±3.40) for petroleum ether and n-hexane groups respectively). There was a similar reduction inserum triglyceride levels of extract groups, with petroleum ether group having the highest (152.91±2.15) activity. Total protein decreased (5.57±0.03) in n-hexanetreated group while alkaline phosphatase increased in all the groups with the standard drug treated group having the highest (32.2±0.35) value. Activity of alanine aminotransferase in n-hexane treated group increased (47.7±12.4) while that of aspartate transaminases in groups increased with the petroleum ether group having the highest activity (84.7±2.96).Urea in all groups increased, except in the n-hexane treated group. Creatinine levels decreased in petroleum ether and standard drug treated groups. Na^+ levels reduce in all groups except the standard drug treated group, K^+ levels increased in petroleum ether group, Cl^- values increased in all groups and HCO_3 decreased in petroleum ether and standard drug treated groups.The extracts have hypoglycaemic and hypolipidemic properties, but may be hepatotoxic.

Index Terms- Globemetula braunii, hypoglycaemia, hypolipedemia

I. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia, because of defects in insulin secretion, insulin action or both (American Diabetic Association (ADA) (2010). It is one of the most prevalent chronic metabolic disorders affecting nearly 10% of the world population and its incidence is increasing rapidly. The disease may occur as a result of pancreatic β-cells impairment, leading to reduction in insulin secretion. It could also occur when the insulin receptors are resistant to the functions of circulating insulin. Recurrent or persistent hyperglycemia during diabetes causes glycation of body proteins, which in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries. Another major factor, besides hyperglycaemia, which complicates diabetic state and results in death is hyperlipidaemia (Nagappa *et al*., 2003). Diabetes- induced hyperlipidemia is attributable toexcess mobilization of fat from adipose tissues due to underutilization of glucose and has been implicated in the etiology of atherosclerosis (Abolaji *et al.*, 2007).

There is a significant disturbance in water and electrolyte homeostasis by this disease (ADA, 2010). There exist a complex association between blood glucose and serum electrolytes and can be related to certain factors such as age and associated conditions. Elevated blood glucose mainly results in serum electrolyte imbalance in type 1 diabetes (Bukonla *et al*., 2012). Water and electrolyte loss are due to increased urination, and as with hyperglycemia, the body's urinary output increases in an attempt to get rid of the excess blood glucose. This balance is especially disturbed between sodium and potassium. In a disease state, the mechanism for maintaining sodium balance may be disturbed causing sodium deficiency or excessive sodium retention with consequent oedema and other adverse clinical conditions (Andrew *et al*., 2013).

The liver plays a central role in metabolism of drug and xenobiotics, protein synthesis and in maintaining biological equilibrium of organisms. Due to these important roles, liver enzymes are used as markers in assessment of drug or plant extract safety or toxicity (Satyapal *et al*., 2008). The transaminases are involved in intermediary metabolism and are

thus present in high concentration in the liver. They are rapidly released into the serum in cases of acute destruction of tissues as in myocardiac infarction or hepatocellular necrosis.

Traditional medicine has developed in Nigeria in response to the health needs of the people. A large proportion of the population relies on traditional healers and their medicinal plants for the treatment of many diseases and about 75% of Nigerians solve their health problems consulting traditional practitioners. Even with the existence of modern medicine, herbal medicines are well known for their historical and cultural purpose. Such medicines have become more widely available commercially, especially in the developingcountries. *Globimetula braunii* is a hemi-parasitic plant that grows on deciduous trees preferring those with soft bark, like guava, coffee, cocoa, citrus etc. The Nupe speaking people of Niger state, Nigeria use *G. braunii* leaves for the folkoric treatment of diabetes, and hypertension.In African, many indigenous cultures used herbs in their healing rituals, due to believe that the allopathic medicines are toxic and have severe side effects.

The leaf extracts of the plantexhibit lipid lowering and antioxidative activities (Okpuzor *et al.,* 2009) and is effective in the management of high blood pressure. The roots attaching to the host plant are used for other therapeutic uses like ulcer and cancer treatment. The chemical components help in the treatment of diabetes by opposing autoimmune response, and helping the beta cells to produce insulin (Schmutterer, 2002). Research on the antidiabetic activity of this plant is very limited.

Other phytoconstituents such as tannins, terpenoids and alkaloids have been shown further in various pharmacological activities including antibacterial and antidiabetic properties of plants (Uzochukwu and Osadebe, 2007).

The research isaimed at investigating the hypoglycemic and hypolipidemic properties of*Globimetula braunii* leafextracts and its effects on activity of liver enzymes, and kidneys of diabetic rats.

II. MATERIALS AND METHODS

Plant Materials

The leaves of *Globimetulla braunii*were collected between in May 2013, from College of Education Minna, Niger State, Nigeria. They were identified and authenticated at the herbarium, Biological Sciences, Ahmadu Bello University, Zaria, Nigeria with voucher specimens v/no: 2829 for future reference. The leaves of *Globimetulla braunii* were properly rinsed and air dried in Departmental laboratory, according to Rocha *et al*., 2011. The leaves were pulverized under aseptic condition into fine powder with mortar and pestle and thereafter with electric blender.

Preparation of Plant Extracts

Three hundred and twenty grams (320g)of the dried powdered leaves were weighed into two beakers and 2400ml of n-hexaneand petroleum ether were respectively added and extracted using soxhlet extraction method at temperatures of 68ºC and 65ºC. The n-hexane extract yielded 16.68g while the petroleum ether extracts yielded 19.35g.

Phytochemical Screening

Qualitative phytochemical tests were carried out on the extracts according to the standard procedure described by Trease and Evans, (1989) and Sofowora(1993).

Experimental Animals Model

Swiss albino rats of both sexes weighing between (135-244) g were purchased from the animal house center, College of Health Sciences, Benue State University Markurdi, Nigeria. They were kept under standard environmental conditions in the Department of Biochemistry Laboratory to acclimatize for two weeks. They wereallowed access to gro6rtttwers feed mesh and water (ad libitum) in compliance with internationally accepted principles for human and laboratory animals in the Canadian Council of Animal Care guidelines and Protocol Review (CCAC, 1997).

Experimental Design

A total of 20 rats divided into five groups of 4 rats each. Group 1 is the normoglycaemic rats, labeled: (NMG), Group 2: Diabetic untreated rats, labeled (DU),Group 3: Diabeticrats treated with 500 mg/kg bodyweight petroleum ether extract labeled (PEETR),Group 4:Diabetic rats treated with 500 mg/kg bodyweight n-hexane extract labeled (NHETR), and Group 5: Diabetic rats treated with 500 mg/kg bodyweight standard drug (metformin), labeled (STD).

Induction of Animals models with Alloxan for Diabetes Mellitus.

The rats were fasted overnight with but allowedaccess to water. 0.5g of alloxan monohydrate was dissolved in 10ml of normal saline. 100 mg/kg bodyweight of alloxan monohydrate was administered intraperitonially to rats in the respective groups and fasting blood glucose was checked after 72 hours. Rats with blood glucose $\geq$ 200 mg/dl were considered diabetic and used for research.The animals were afterwards allowed free access to water and food and maintained at room temperature in plastic cages.

Animal Treatment

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 h cycle (6 am and 6 pm) for twelve days to monitor any change in blood glucose. During this period the *in vivo*measurement of blood glucose was carried out with blood obtained from tail vein of the mice using One Touch digital ® Glucometer (Accu-chek) and the unit expressed as mg/dl. The treatment was terminated after seven days.

Animal Sacrifice

Twelve hours after the final treatment, the rats were anaesthetized under chloroform and sacrificed. The blood sample was collected through jugular puncture. Theblood samples were centrifuge at 1000rpm for 10minutes to get the serum for the biochemical assays.

Determination of Biochemical Parameters

These were determined using assay kits (Randox Laboratories Limited kits, United Kingdom and Teco diagnostic kits) as follows:

Determination of Serum Cholesterol and Triglycerides

The serum level of total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample as described by method of Stein (1987), the serum triglyceride level was determined by method of Tietz (1995).

Determination of Liver Enzymes

Assay of Alanine aminotransaminase(ALT) and Aspartate aminotransaminase(AST) activities were carried out by the procedureoutlined in the diagnostic kit as described by Reitman

and Frankel (1963) and Alkaline phosphatase (ALP) was according to the method outlinedby Gesellschaft, 1972. Total protein was according toTiez, 1995 method.

Determination of Urea and Creatinine

Urea and creatinine were determined respectively by the methods of Weatherburn 1967, and Bartels *et al.*, 1972.

Determination of Serum Electrolytes

Sodium was determined based on the modification of those first described by Maruna and Trinder (1951). Potassium was determined using turbidimetric determination method by Terri *et al*., (1958). Chlorides were analyzed based on the method of (Tietz, 1976).

Statistical Analysis

Results were analyzed by t-test using SPSS version 16 software package. All the data are expressed as mean ± standard error of mean (SEM), (n=4) and differences between the groups considered significant at ($p< 0.05$).

III. RESULTS

Table 1: Phytochemical components of n-hexane and petroleum ether extracts of *Globimetula braunii* leaf

Components	n-hexane extract	Petroleum ether extract
Saponins	+++	+
Alkaloids	+	_
Phenols	++	+++
Tannins	+	+++
Cardiac glycosides	+++	_
Terpenes	++	++
Steroids	++	+++
Flavonoids	_	+++
Anthraquinones	_	_
Anthranoides	_	_
Phlobatanins	_	_

+++: Highly present
++: Moderately present
+: Mildly present
_ Not detected

Saponinsand cardiac glycosides are highly present in n-hexane extract, while phenols, tannins, steroids, flavonoids are highly present in petroleum ether extract.

The figure shows the mean blood glucose levels of rats in all groups. The fasting blood glucose levels of diabetic untreated rats significantly increased as opposed to that of the normoglycaemic, standard drug, and extract treated groups. Highest hypoglycaemic activity was recorded at the 12th day of the treatment.

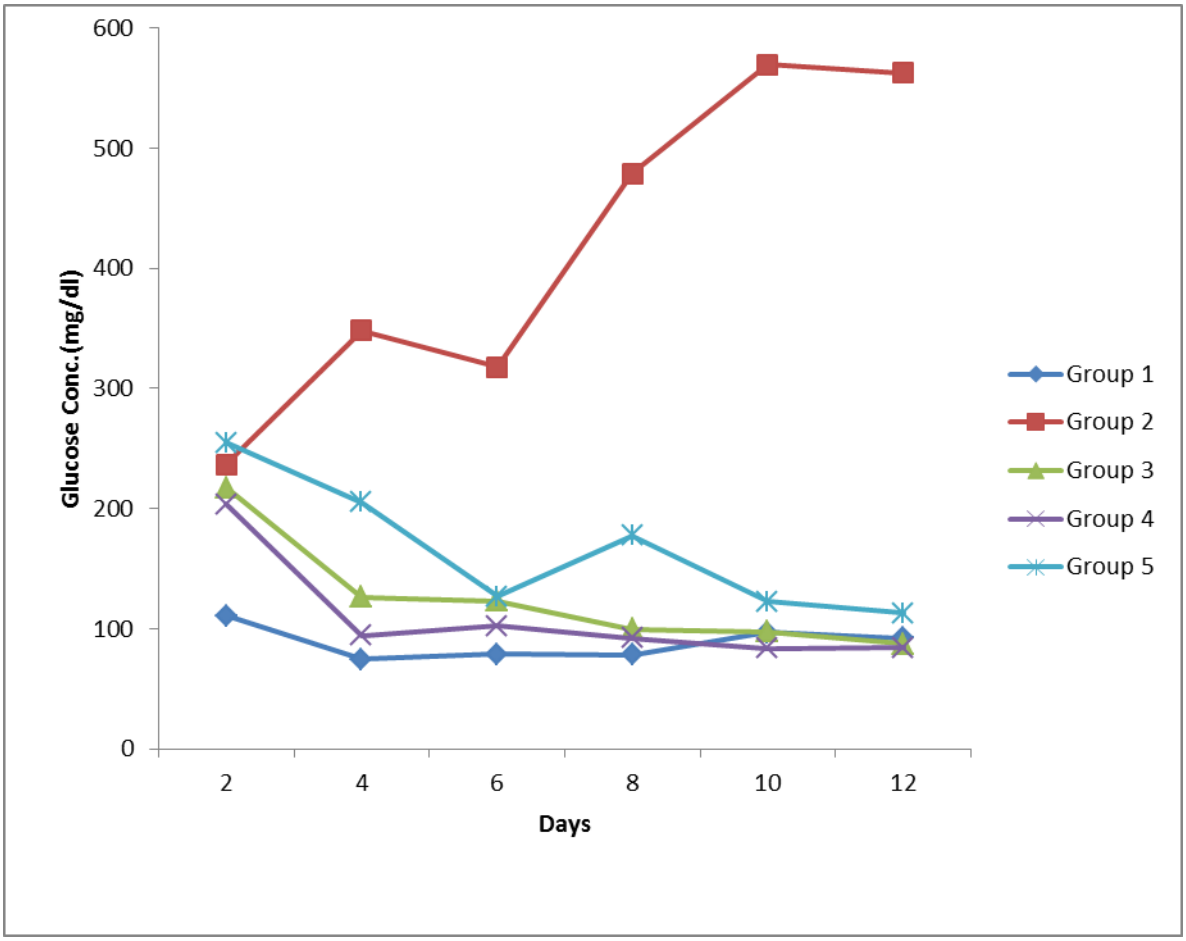

Figure 1: Blood Glucose Concentration of Treated and Untreated Rats.

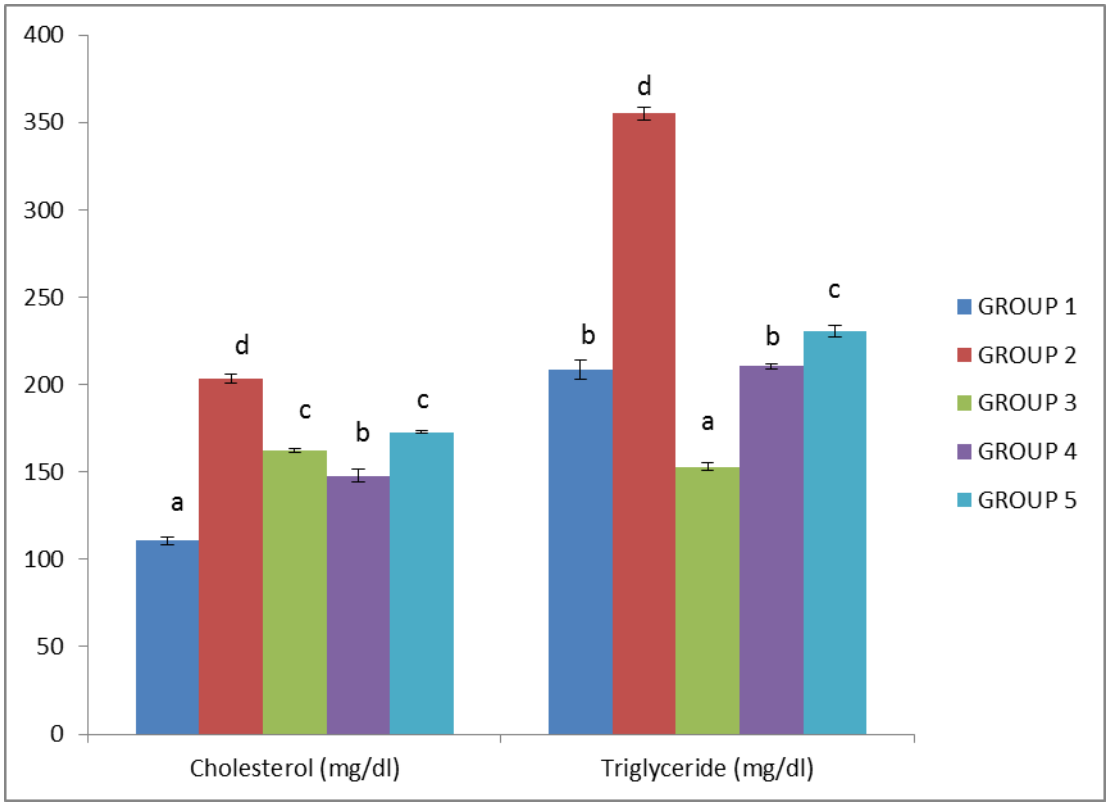

Figure 2: Serum Cholesterol and Triglyceride Concentrations

Group 1: Normoglycaemic rats
Group 2: Diabetes untreated rats
Group 3: Petroleum ether extract treated rats
Group 4: n-Hexane extract treated rats
Group 5 Standard drug (metformin) treated rats

Figure 2 shows the levels of total cholesteroland triglyceride. There was an increase in cholesterol concentration of diabetic control group as compared with the normal control group. There was a reduction in the serum total cholesterol of rats treated with petroleum ether and n-hexane leaf extracts of *G. braunii*, and that of the standard drug treated group when compared with the untreated rats. Triglyceride level decreased in n-hexane treated group and shows no significant difference in other groups except the diabetic control group that has elevated values.

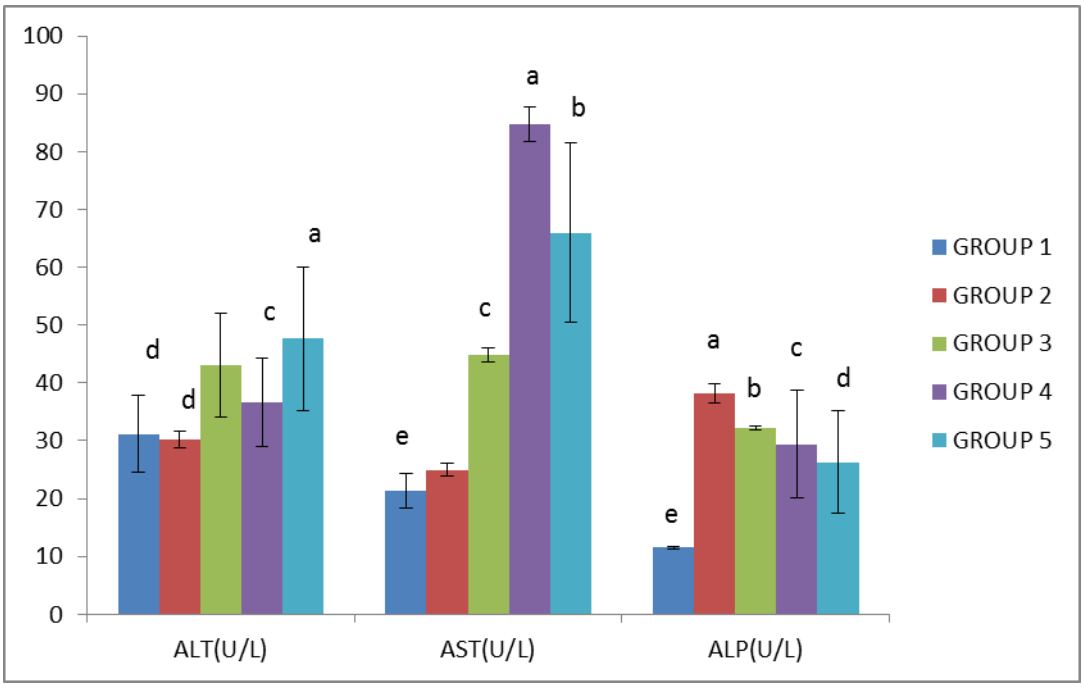

Figure 3: Serum Alanine aminotransferase (ALP), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP)

Key

Group 1: Normoglycaemic rats
Group 2: Diabetic untreated rat
Group 3: Petroleum ether extract treated rats
Group 4: n-Hexane extract treated rats
Group 5: Standard drug (metformin) treated rats

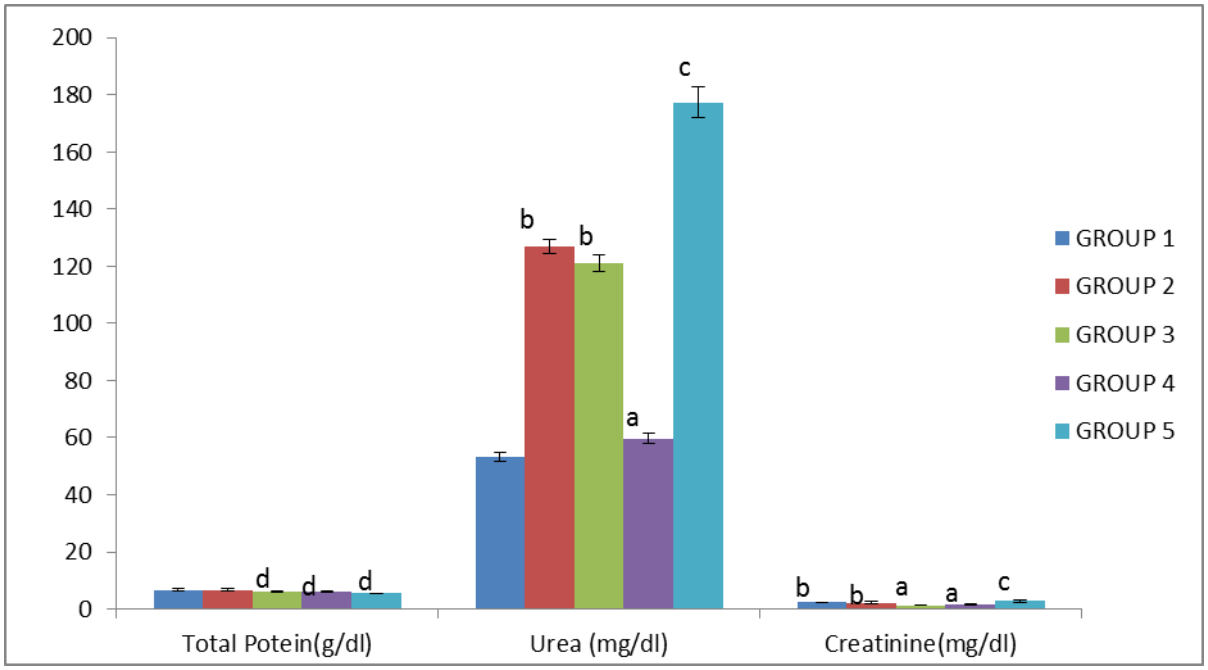

Figure 4: Serum Urea and Creatinine

Values are Mean ± standard error of mean (SEM),not significantly different at ($p < 0.05$).
Group 1: Normoglycaemic rats
Group 2: Diabetic untreated rats
Group 3: Petroleum ether extract treated rats
Group 4: n-hexane extract treated rats
Group 5: Standard drug (metformin) treated rats

Total protein and creatinine levels were not significantly affected by the extracts.Petroleum ether extract did affect the urea level as much as did the effect of diabetic, with the standard drug showing the most significant effect on urea

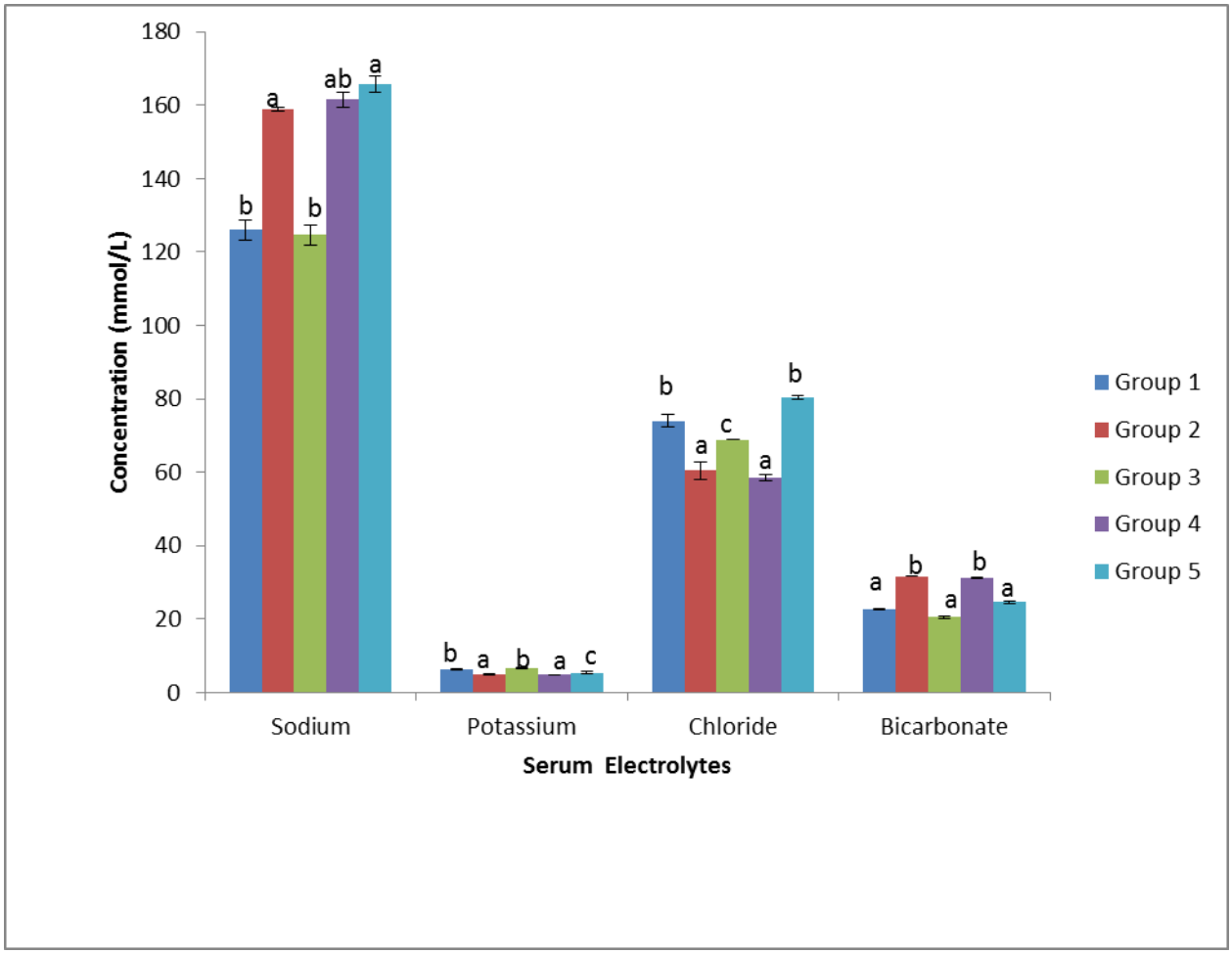

Figure 5: Effect of Extracts on Serum Electrolytes

Values are Mean ± standard error of mean (SEM),not significantly different at ($p < 0.05$).
Group 1: Normoglycaemic rats
Group 2: Diabetic untreated rats
Group 3: Petroleum ether extract treated rats
Group 4: n-Hexane extract treated rats
Group 5: Standard drug (metformin) treated rats

Sodium levels increased in diabetic untreated, n-hexane, and standard drug treated groups, while chloride decreased in diabetic untreated and n-hexane groups. Bicarbonate also increased in diabetic control and n-hexane treated groups.

IV. DISCUSSION

The study has revealed the presence ofimportant medicinal phytochemicals in both petroleum ether and n-hexane leaf extracts of *Globimetula braunii*.Flavonoids have been reported for their antimutagenic and anticarcinogenic potentials due to their antioxidant and antiinflamatory properties (Parajuli *et al*., 2012). Saponins are used as adjuvant in the production of vaccines (Asl and Hossein, 2008), while phenols are known for their hypoglycemic properties (Nikrote *et al*., 2011). Phenols possessantiviral, antibacterial (Akiyma *et al*., 2007), and antiparasitic effects (Koladziel and Kiderlen, 2005).Alkaloid has been used as central nervous system stimulant, topical anesthetic in ophthalmology, powerful painkillers and in the production of certain drugs e.g cocaine, caffeine, and quinine. Tannins have also been shown to be potential antiviral, antibacterial and antiparasitic agents. Absence of anthranoid,anthraquinone and phlobatannin in both extracts and the absence of cardiac glycoside in petroleum ether extract (but present in n-hexane extract) (Table 1) is due to the fact that different parts of the plant have different ingredient profile and different extraction procedure. The solvent usedfor extraction may also yield different active components(Shan *et al*.,2007). The hypoglycemic effect (figure 1) of these extracts may be linked to their phytoconstituents specially the flavonoid and phenol because of their antioxidant properties (acting by potentiating the insulin effect) either by increasing the pancreatic secretion of insulin of the β-cells of islet of langerhanes or it releases the bound insulin (Pari and Armanath 2004).

According to Eleazu *et al*., 2010, alloxan is known to destroy beta cells of the islets of the pancreas that function in the regulation of insulin secretion which then leads to an increase in the blood concentration of glucose and type 1 diabetes mellitus ensues. Alloxan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycemia.

The oral administration of 500mg/kg bodyweight of n-hexane and petroleum ether leaf oil extracts of *G.braunii* and 0.5g/kg bodyweight standard drug on rats caused a significant ($p<0.05$) reduction in the blood glucose concentration of diabetic rats. Similar observation has been reported in rats following the administration of *Azadirachta indica* leaf extract and*Rubus ellipticus* fruit extract (Sharma, *etal*., 2011). This may be due to greater presence of phytochemical in n- hexane extract (Table 1).Metformin was used as a standard drug to compare the efficacy of the extracts. It acts by increasing fatty acid oxidation, decreasing hepatic glucose production and intestinal absorption, increasing peripheral glucose uptake and insulin sensitivity (Kirpichnikov*et al*., 2002). Administration of petroleum ether and n-hexane leaf extracts of *Globimetula braunii* has led to the reduction in the levels of cholesterol and triglyceride.

Activities of alanine aminotransferase (ALT), and aspartate aminotransferase (AST) increased in extract treated groups (figure 3). Increases in ALT and AST are also seen in drug hepatitis (cholestatic). ALT and AST are good marker enzymes that are found in the liver and kidney. According to Gressner *et al.,* 2007, the cellular rupture of the mitochondrial allows the enzymes to escape into the blood. An increase in the levels of these enzymes (ALT and AST) often means that liver disease such as cirrhosis, liver necrosis, hepatitis or exposure of the liver to toxic substance might have occurred. Elevated level of ALT is usually followed by increase in the level of AST (Shahraki, *et al*., 2007).

Alkaline phosphatase (ALP) is a protein present in all body tissues with high amount in the liver. The serum levels of ALP significantly increases in both extract treated groups compared to the normoglycemic. This agrees with the result obtained by Brown *et al.,* (2007). Higher than normal values of ALP may be due to biliary obstruction and hepatitis,while lower than normal values are due to malnutrition and protein deficiency, although it is very rare. Increased in the activity of ALP may not be due to hepatic cell disruption, nor to a failure of clearance, but rather to increased synthesis of hepatic ALP. Blood level of ALP is a good indicator of the rate of bone formation.

The total protein is a biochemical parameter for measuring the total amount of protein in blood serum. Total protein consists of albumin and globulin. The liver synthesizes large amount of albumin and some fraction of globulin. Low level of total protein was observed in n-hexane extract treated group. This is in line with the work of Alimat *et al*., (2012) who reported decreased level of total protein upon administration of neem gold extracts to laboratory animals.This observed low level of total protein may be due to low protein synthesis by the liver or damage to the liversuch as cirrhosis. It might lead to nephritic syndrome, where it is lost through the urine.

Hyperglycemia leads to the over production of free radicals and the non-enzymatic glycation of proteins. These changes are

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