Nigerian Journal of Biochemistry and Molecular Biology 2013; 28(1&2): 69-77

0189-4757/96 $3.0 + 0.00
Printed in Nigeria

Available online at http://www.nsbmb.org/

NJBMB/026/13

Biochemical Evaluation of Anti-hyperglycemic Effects of Petroleum Ether Extract of *Meliponula ferruginea* propolis in Alloxan-induced Diabetic Rats

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ABSTRACT: Petroleum ether extract of *Meliponula ferruginea* propolis at 50, 100 and 200 mg/kg body weight was investigated for antihyperglcemic activity and its effect on some biochemical indices of alloxan-induced diabetic rats. Twenty four albino rats were randomized into six groups (A-F) of four animals. Group A (normal control) were treated orally with 0.5 ml of normal saline solution while animals in groups B, C, D, E and F were made diabetic with single injection of alloxan monohydrate (100 mg/kg body weight, p.i.). The fasting blood glucose of the alloxanized rats after 48 hours ranged from 130.05-139.05 mg/dl. The animals were thereafter treated with 0.5ml of paraffin oil, 2.5 mg/kg body weight glibenclamide, 50, 100 and 200 mg/kg body weight, p.o. of the extract dissolved in paraffin oil respectively. During the seven days of treatment, the persistently high fasting blood glucose in the alloxanized rats treated with extracts progressively reduced. The progressive decrease compared favourably with the glibenclamide treated diabetic rats. The high serum activity of alkaline phosphatase, acid phosphatase, aspartate transaminase as well as urea and creatinine levels in the diabetic control rats were significantly ($p<0.05$) reduced in all the extract treated diabetic treated rats. Also, the reduced serum concentration of albumin and bilirubin in the diabetic control rats significantly ($p<0.05$) increased in the animals treated with extract at all doses. However, there was no significant difference in the serum activity of alanine transaminase of the extract treated animals when compared with the diabetic control animals. The results suggest that petroleum ether extract of *Meliponula ferruginea* propolis possess anti-hyperglycemic activities and may not pose any deleterious to the biological systems.

KEYWORDS: antihyperglcemic, *Meliponula ferruginea,* propolis, glibenclamide, diabetic**.**

1.0 Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (ADA, 2011). It is a major cause of disability and hospitalization and result in significant financial burden (Nagappa *et al*., 2003). The number of people suffering from the disease worldwide is increasing at an alarming rate with a projected 366 million people likely to be diabetic by 2030 as against 191 million estimated in 2000 (Wild *et al.*, 2004). Shaw *et al.*, 2010 projected that between 2010 and 2030; there will be a 69% increase in the number of adults with diabetes in developing countries and a 20% increase in developed countries while WHO, 2011 projects that diabetes will be the 7th leading cause of death in 2030. Nigeria presents a difficult picture for its 3.1 million people with diabetes which is the highest in Africa Diabetes-related deaths in Nigeria in 2011 accounted for 63,340 people (Oputa and Chinenye, 2012). People with diabetes have an increased risk of developing a number of serious health problems. Consistently high blood glucose levels can lead to serious diseases like nephropathy, retinopathy, neuropathy and cardiovascular problem like stroke and coronary heart diseases **(**International Diabetes Federation, 2013**)**. According to the WHO, over 150 plants are known to be used for

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the treatment of diabetes mellitus in folklore medicine. Thus, study of plants with hypoglycemic claims is encouraged (Marles and Farnsworth, 1995). This is because herbal drugs are believed to have lesser or no side effects are less expensive as compared to synthetic drugs. Therefore identification and isolation of antihyperglycemic compounds from plants and plant-derived substances have become important (Akanksha *et al.,* 2010).

Propolis or bee glue, as it is commonly named, is a natural resinous mixture produced by honeybees from substances collected from parts of plants, buds and exudates (Ghisalberti, 1979). Normally it is dark brown in colour, but it can be found in green, red, black, and white hues, depending on the sources of resin found in the particular hive area (Mesquita and Franciscon, 1995). Due to its waxy nature and mechanical properties, bees use propolis in the construction and repair of their hives - for sealing openings and cracks and smooth out the internal walls (Bankova *et al.,* 2000) and as a protective barrier against external invaders or against weathering threats like wind and rain.

Propolis contains at least 200 compounds that have been identified in different type of propolis, with more than 100 being present in any given type. These include fatty and phenolic acids and esters, substituted phenolic esters, flavonoids, terpenes, β-steroids, aromatic aldehydes and alcohols, and derivatives of sesquiterpenes, naphthalene and stilbenes (Marcucci *et al*., 1996). Propolis possesses a broad spectrum of biological activities. The flavonoids in propolis (mainly pinocembrin) are considered to be responsible for its inhibitory effect on bacterial and fungus (Tomás-Barberán *et al.,* 1993). It has been reported that propolis enhances immune system activities (Orsolic and Basic, 2003 and 2006), oxygen radical scavenging (Chen *et al*., 2004), antimicrobial, anti-inflammatory (Mizoeva and Calder, 1996) and antitumor activities (Duarte *et al*., 2006). Thus, it is extensively used in health food, pharmaceutical preparations (Orsolic and Basic, 2006) and beverages.

In folk medicine, propolis has been reported to possess therapeutic or preventive effects against inflammation, heart disease, diabetes mellitus, infectious microbes, hepatoxicity and cancer. In the Nigeria folk medicine, especially in the South Western part of the country, bees' propolis are for the management of diseases like diabetes, malarial and pain. There has not been adequate scientific report on the various uses attributed to it. Hence, this study was designed to investigate the acclaimed antihyperglycemic effect of *Meliponula ferruginea* propolis and to study any biochemical effects it may have in alloxan-induced diabetic rats.

2.0 Materials and Methods

2.1 Collection and Identification of Plant Materials

Meliporula ferruginae propolis was obtained from Apiary Unit, University of Ilorin, Ilorin, Nigeria and was authenticated at the Faculty of Agriculture, University of Ilorin, Nigeria.

2.2. Glucometer and Assay Kits

Accue™ blood glucose meter kit and Accue™ glucose strip were products of Roche Diagnostics GmbH, Mannheim, Germany. Assay kit for albumin, creatinine, urea, aspartate transaminase and alanine transaminase were products of Randox Laboratory, Co-Antrim, UK.

2.3 Drugs and Chemicals

Alloxan monohydrate was a product of Sigma-Aldrich, St. Louis, USA while Glibenclamide was a product of HOVID Bhd., Ipoh, Malaysia. All other reagents used were of analytical grade and were obtained from Sigma-Aldrich, St. Louis, USA.

2.4 Laboratory Animals

White albino rats (*Rattus norvegicus*) of average weight of 180.50 ± 10.00g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Nigeria. The animals were housed in clean wooden cages that provided with rat

pellets (Grand Cereals Limited, Jos, Nigeria) and tap water *ad libitum.*

2.5 Preparation of Extract

The propolis was cut into small pieces and air-dried in the laboratory at room temperature for two weeks. A known weight (50g) was extracted in 1 litre petroleum ether with Soxhlet extractor for 3 hours at 60^0C. It was extracted further then evaporated in vacuum oven at 40^0C.

2.6 Animal Grouping and Extract Administration

A total of twenty-four (24) albino rats (*Rattus novergicus*) were used. They were randomly divided into 6 groups (A-F) of four rats each. Group A (normal control) were given saline solution. Animals in groups B, C, D, E and F were made diabetic with 100 mg/kg b. w., of alloxan p.i.. They were orally treated with paraffin oil, glibenclamide (2.5 mg/kg b. w.), 50, 100 and 200 mg/kg b. w. of the extract in paraffin oil respectively. The animals were handled humanely in accordance with the guidelines of European Convention for the Protection of Vertebrate Animals and Other Scientific Purposes (European Treaty Series- No. 123, 1986).

2.7 Induction of Diabetes and Determination of Blood Glucose

The animals were made diabetic by single intraperitonal injection of 100 mg/kg b. w. of alloxan monohydrate in sterile physiological saline. After a six hours fast (without food, but water), blood samples (drops) were drawn from the tail vein and the fasting blood glucose levels were determined to confirm the induction of diabetes using the ACCU-CHEK™ test strips and blood glucose meter according to the instructions outlined in the User Guide. The animals were then allowed access to pellet and distilled water. Forty-eight (48) hours post induction; the fasting blood glucose was checked again as described earlier to confirm induction of diabetes in the animals. Only animals with blood glucose level higher than 126mg/dl were used for the study (WHO, 1999). The fasting blood glucose levels of the animals were also determined on the day 3, 5 and 7 after the confirmation of diabetes using the same procedure.

2.8 Preparation of Serum

Under diethyl ether anaesthesia, the neck areas of the animals were quickly cleared of fur and skin to expose the jugular veins. The animals were then made to bleed through their cut jugular vein into a clean, dry centrifuge tube which was allowed to clot for 30 min. The blood samples were centrifuged at 224 g x 10 min using Uniscope Laboratory Centrifuge (Model SM800B, Surgifriend Medicals, Essex, England). The sera were thereafter aspirated with Pasteur pipette into clean, dry, sample bottles and kept frozen overnight before being used for the assays (Yakubu *et al.*, 2010).

2.9 Determination of Biochemical Parameters

The biochemical parameters were determined in the serum using standard methods described for albumin (Grant and Kacchman, 1987), creatinine (Blass *et al.*, 1974), urea (Veniamin and Varkirtzi, 1970), bilirubin (Evelyn and Malloy, 1938), aspartate transaminase, alanine transaminase (Schmidt, and Schmidt, 1963), alkaline phosphatase acid phosphatase and (Wright *et al*., 1972a and b).

2.10 Statistical Analysis

All data were expressed as mean of four replicates ± S.E.M. and analyzed using one-way analysis of variance (ANOVA), followed by Duncan's Post Hoc test for multiple comparison. Differences between groups were considered statistically significant at $P < 0.05$.

3.0 Results

The anti-hyperglycemic effect of the extracts on alloxan-induced diabetic rats is illustrated in Table 1. Administration of alloxan (100 mg/kg, p.i.) lead to elevation of fasting blood glucose levels, which was reduced significantly ($P<0.05$) and progressively in the glibenclamide and

extract-treated animals. The effect of alloxan significantly ($p<0.05$) increased serum biochemical parameters such as alkaline phosphatase, acid phosphatase, aspartate transaminase, creatinine and urea but glibenclamide (2.5mg/kg) and extracts of *Meliponula ferruginea* reversed the alloxan-induced changes as shown in Table 2. Similarly, there was significant ($p<0.05$) increase in the concentration of serum bilirubin of the treated groups when compared with the diabetic control group. Likewise, administration of petroleum ether extract of *Meliponula ferruginea* propolis significantly increased ($p<0.05$) alanine transaminase activity and serum albumin concentration at all doses of extract when compared with the diabetic control group.

Table1: Effect of administration of petroleum ether extract of *Meliponula ferruginea* propolis on blood glucose level (mg/dL) of alloxanized diabetic rats

	Days after administration of alloxan		
Treatment groups	**Day 0**	**Day 3**	**Day 7**
Control	104.00±2.00[a]	120.33±0.88[a]	115.67±1.76[a]
Diabetic + Untreated	139.50±2.50[b]	124.50±0.50[a]	123.00±0.00[a]
Diabetic + Reference drug	134.50±4.50[b]	111.00±3.00[b]	80.00±6.00[c]
Diabetic + 50 mg/kg body weight of the extract	131.50±5.50[b]	121.50±0.50[a]	104.33±6.36[b]
Diabetic + 100 mg/kg body weight of the extract	133.00±2.00[b]	107.50±15.5[b]	102.00±5.00[b]
Diabetic + 200mg/kg body weight of the extract	130.50±2.50[b]	108.00±4.00[b]	119.33±1.20[a]

Values are mean of four (4) determinations ± SEM.
Values with different superscripts are significantly different from the control

Table 2: Effect of administration of petroleum ether extract of *Meliponula ferruginea* on selected biomolecules of alloxanized diabetic rat serum

Treatment groups	**Alkaline Phosphatase (nM/min/mg protein)**	**Acid Phosphatase (nM/min/mg protein)**	**Alanine Transaminase (nM/min/mg protein)**	**Aspatate Transaminase (nM/min/mg protein)**	**Urea (mg/dl)**	**Creatinine (mg/dl)**	**Bilirubin (mg/dl)**	**Albumin (mg/dl)**
Control	5.29±1.37[a]	3.62±0.64[a]	6.88±0.28[a]	3.33±1.11[a]	16.83±0.24[a]	2.18±0.12[a]	2.81±0.27[a]	4.03±0.34[a]
Diabetic + untreated	17.51±5.52[b]	4.47±40.30[b]	8.29±1.71[b]	7.63±1.60[b]	27.96±1.16[b]	4.61±1.30[b]	1.06±0.14[d]	3.27±0.06[b]
Diabetic + Glibenclamide	6.23±3.61[a]	2.66±0.22[a]	7.51±0.01[b]	3.99±0.34[a]	18.12±0.24[a]	3.95±0.11[a]	1.94±0.05[b]	4.16±0.06[a]
Diabetic + 50mg /kg extract	6.51±0.00[a]	2.38±0.19[b]	8.54±0.00[b]	3.54±0.00[a]	18.37±0.36[a]	2.79±0.50[a]	1.67±0.23[b]	4.13±0.15[a]
Diabetic + 100mg /kg extract	5.42±0.12[a]	2.09±0.31[c]	8.73±0.61[b]	3.31±0.07[a]	18.85±0.35[a]	3.35±0.27[a]	1.56±0.35[b]	4.52±0.08[a]
Diabetic + 200mg /kg extract	7.86±0.00[a]	2.24±0.42[b]	10.10±0.15[b]	3.90±0.97[a]	17.38±1.00[a]	2.85±1.08[a]	1.19±0.11[c]	4.75±0.05[a]

Values are mean of four (4) determinations ± SEM.
Values with different superscripts are significantly different from the control

4.0 Discussion

Management of diabetes with the agents devoid of any side effects is still a challenge to the medical system. This concern has led to an increased demand for natural products with antihyperglycaemic activity, having fewer side effects (Sharma and Kumar, 2011).One of the most potent methods to induce experimental diabetes mellitus is chemical induction by alloxan. Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas (Lenzen and Panten, 1988; Oberley, 1988). *In vitro* studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis (Jorns *et al.,* 1997; Ledoux *et al.,* 1986). The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells (Szkudelski, 2001).

Furthermore, glibenclamide, a reference anti-diabetic drug, commonly used in several studies to compare the efficacy of various hypoglycemic compounds acts by various mechanisms such as binding to the ATP-sensitive potassium channel and in the process, lowers glucose levels. Others include suppressing hepatic glucose production, increasing insulin sensitivity of extra-pancreatic tissues and fatty acid oxidation, enhancing peripheral glucose uptake, decreasing hepatic glycogenolysis and gluconeogenesis as well as absorption of glucose from the gastrointestinal tract (Yakubu *et al.,* 2010)

The present experimental studies reveal that the petroleum ether extracts of 100 mg/kg body weight, administered orally for 7 days produced a significant decrease in the blood glucose level in the model of (Table 1). The presence of phytochemicals like alkaloids, flavonoids, anthraquinones and terpenoids in *Meliponula ferruginea* propolis may be responsible for the hypoglycaemic activity obtained in treated alloxan-induced diabetes rats. Garzon de la Mora *et al.,* 2008 and Dineshkumar *et al.,* 2010 have independently reported that alkaloid extract of *Lupin exalyatus* and carbazole alkaloid from *Murraya koengii* reduced the elevated fasting blood sugar in diabetic rats. It has also been reported that flavonoids extracted from lotus (*Nelunbo nucifera* Gaertn) leaf at the doses 50 and 200 mg/kg body weight for 28days significantly decreased fasting blood sugar in alloxan-induced diabetic mice (Taoying *et al.,* 2009). Several plant-derived flavonoids, apart from possessing their common antioxidant activity, have been reported to inhibit aldose reductase activity and impart beneficial action in diabetic complications (Shin *et al.,* 1995; Iwata *et al.,* 1999; Aida *et al.,* 1990). Similarly, these phytochemicals may also contribute beneficially in mitigating glucose autoxidation (Larson, 1988), glycation, and act against the major contributors for increased free radicals generation in diabetic lens (Wolff and Dean, 1987; Srivastava *et al.,* 1996).

Alkaline phosphatase (ALP) is a liver marker enzyme often employed to assess the integrity of plasma membrane and endoplasmic reticulum (Akanji, 1993), while acid phosphatase (ACP) is an indicator of damage to the lysosomal membrane (Collins and Lewis, 1971). The transaminases (AST and ALT) are well-known enzymes used as biomarkers to predict possible toxicity to the liver (Rahman, 2001). The significant increased ($p<0.05$) activities obtained for ALP, ACP, ALT and AST in the untreated diabetic rats compared with normal control (Table 2) may be an indication of impairment of liver function. Yanpallewar *et al.* (2003) stated that high serum concentrations of ALT, AST, ACP and AST indicated cellular leakage due to disintegration of cell membrane in liver. Also elevation in serum activities of both transaminases (ALT and AST) obtained in diabetic control rats may suggest damage to the liver cells as well (Wolf *et al.,* 1972). Yakubu *et al.* (2003), in the same vein, has reported increased serum ALP and ACP activities as a confirmation of damage to the plasma membrane, with a consequential compromise of membrane integrity

However, *Meliponula ferruginea* propolis treatment significantly ($p<0.05$) decreased the serum activities of AST, ACP, and ALP except ALT (Table 2) towards the respective normal control. This may imply stabilization of plasma membrane as well as repairment of hepatic tissue damage that can be considered as an

expression of the functional improvement of the hepatocytes (Nirala and Bhadauria, 2008). This result is also in agreement with the findings that propolis induced reduction of the increased activity of AST in plasma of rats treated with alcohol (Kolankaya *et al.*, 2002) or galactoseamine (Nirala *et al.*, 2008).

Albumin is the most abundant plasma protein, accounting for 55–60% of the measured serum protein (Gosling, 1995). Albumin, which is manufactured in the liver, is a major carrier protein that circulates in the bloodstream (Tietz, 1986). Low serum albumin concentration suggests chronic damage to the liver as a result of infection (Naganna, 1999). Therefore, the reduction in serum albumin level in the untreated diabetic rats is an indication of diminished synthetic function of the liver (Sunmonu and Afolayan, 2013). Oral administration of petroleum ether extract of *Meliponula ferruginea* propolis, however, restored the albumin level towards the respective control value (Table 2)

Bilirubin is the major product that results from the breakdown and destruction of old red blood cells. It is an important metabolic breakdown product of blood with biological and diagnostic values (Tietz, 1986). It is removed from the body by the liver; hence, it is a good indication of the health status of the liver. Decreased serum level of total bilirubin in diabetic control rats as observed in the present study may be a result of inability of the liver to perform its synthetic function adequately. Treatment with *Meliponula ferruginea* propolis extract was able to reverse this condition in diabetic treated rats, to a level comparable to the values obtained for glibeclamide treated rats. All the data obtained with respect to liver function indices indicated absence of any significant liver damage as a result of treatment with petroleum ether extract of *Meliponula ferruginea* propolis in diabetic rats.

The increase in serum levels of urea and creatinine in the untreated diabetic control rats as observed in the present study is expected. Deficiency of insulin and consequent inability of glucose to reach the extra-hepatic tissues stimulate gluconeogenesis as an alternative route of glucose supply (Robinson and Johnston, 1997). This has been linked with increased proteolysis which releases free glucogenic amino acids into the plasma that are deaminated in the liver with the consequence of increased urea in the blood.

Creatinine is a metabolite of muscle creatine, and the concentration in serum is proportional to the body muscle mass. The amount of creatinine is usually constant; hence, elevated levels indicate diminished renal function only, since it is easily excreted by the kidneys (Loeb, 1991). In the same vein, Alder *et al.* (2003) reported that high creatinine levels in diabetes may indicate a pre-renal problem such as volume depletion and may also be due to impaired function of the nephrons as reported by Judkay (2007).

The administration of petroleum ether extract of *Meliponula ferruginea* propolis which significantly reduced the levels of these two metabolites, confer protection against impairment due to diabetes. Thus, indicating that the extract can effectively avert the incidences of diabetic nephropathy. Diabetic nephropathy is mainly associated with excess urinary albumin excretion, abnormal renal function as represented by an abnormality in serum creatinine and urea (Rao and Nammi, 2006) in diabetic control animals. Similar observations have been reported using *Picrorhiza kurroa* and *Vernonia amygdalina* extracts in diabetic rats (Joy, 1999; Atangwho *et al.*, 2007). Of particular interest is the fact that the effect of *Meliponula ferruginea* propolis at the dosage of 100 mg/kg body weight compared favourably well with the normal control.

From this study, the oral administration of petroleum ether *Meliponula ferruginea* propolis extract has beneficial effects on blood glucose levels. It can therefore, be concluded that the petroleum ether extract of *Meliponula ferruginea* propolis, besides its antihyperglycemic action, could protect the liver, kidney and blood against impairment due to diabetes. The anti-hyperglycemic activity of the extract of *Meliponula ferruginea* propolis was similar to the reference drug, glibenclamide, with the best activity at 100 mg/kg b. w. of the extract. Further investigation is necessary to determine the exact phytoconstituents

responsible for antidiabetic effect.

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