The Effect of *Bridelia ferruginea* and *Senna alata* on Plasma Glucose Concentration in Normoglycemic and Glucose Induced Hyperglycemic Rats

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Abstract

A total of 30 female albino rats were used in this study. The plasma glucose levels in these subjects were estimated after administration of methanol extracts of *Bridelia ferruginea* and *Senna alata*. The mean ages of the rats were 8 weeks old and mean weights were 165g.Phytochemical screening of the methanol bark extract of *Bridelia ferruginea and* methanol leaves extract of *Senna alata* revealed the presence of tannins, polyphenols, steroids, triterpenes, and alkaloids. Methanol extract of Bridelia *ferruginea* bark achieved statistical significant difference in the normoglycemic rats and glucose induced hyperglycemic rats (P<0.05). There was no statistical significant difference observed in the control rats (P<0.05). However, *Senna alata* did not achieve any significant difference statistically in normoglycemic, hyperglycemic and control rats (P>0.05). The effect of *Bridelia ferruginea* and *Senna alata* methanol extracts on plasma glucose concentration in normoglycemic and glucose induce hyperglycemic rats is therefore discussed.
Key Words: *Bridelia ferruginea, Senna alata,* normoglycemic, hyperglycemic.

Introduction

The pharmacological management of diabetes mellitus has changed dramatically in the past few years with the introduction of many new medications, including a-glucosidase inhibitors, a biguanide, the thizolidinediones, insulin analogs, maglitinides and D-phenylalanine derivatives. These new agents have dramatically increased the number of options available to providers and patients (Unwin et al., 1999). Combination therapy has become common place for the management of hyperglycemia in patients with type II diabetes (Yki-Jarvinan, 2001).

Man since time immemorial has been using herbs or plant products as medicine for developing immunity or resistance against cold, joint pains fever and so on. Scientific data on a good number of medicinal plants investigated has been well documented (Gupta, 1994). However, only very few drugs of plant origin could reach clinical use and the National Formulatory could not adopt

even a dozen of plant for medicines. For this reason, a special effort is needed for the development of herbal drugs having therapeutic utility (Gupta, 1994). Plant products investigated for anti-diabetic effect have been exhaustively reviewed (Irobi & Daramola, 1994). There are 1,200 species of plants representing 725 genera in 183 families extending from the marine algae and fungi with anti-diabetic activity. The mechanisms of action of most anti-diabetics herbs are not clear, although a few have been documented. Diasulin is a polyherbal drug, which control glucose level by increasing glycolysis and decreasing gluconeogenesis with a lower demand of pancreatic insulin. It also regulates the activities of hepatic glucose metabolic enzymes (Pan et al., 2001).

In India, herbal preparations have been used in the treatment of diabetes. A review carried out in April 2004 showed that a few herbs have been scientifically tested to have anti-diabetic activity. These includes; *Mamordrica charantia, Pterocarpus marsupium* and *Trigonella foenumgreacum*. Mechanisms such as the stimulating or regenerating effect on B-cells or extrapancreatic effects are proposed for the hypoglycemic action of these herbs (Saxera & Vikram, 2004).

Natural compounds with anti-diabetic activity in descending frequency of occurrence includes, complex carbon hydrates, alkaloids, glycopeptides, terpenoids, peptides, amines, steroids, flavonoids, lipids, coumarins, sulphur compounds and inorganic ions. The anti-diabetic mechanisms involved in hypoglycemic activity are numerous, including direct competitive antagonism with insulin, stimulation of insulin secretion, stimulation of glycogenosis and hepatic glycolysis, Pancreatic beta cell potassium channel blockers, cAMP (Cyclic adenosine monophosphate) stimulation, modulation of glucose absorption from the gut among others (Marles, 1996).

Bridelia ferruginea is the commonest savannah *Bridelia*. It is usually a gnarled shrub, which sometimes reaches the size of a tree in suitable condition, and the down curved tip of the leave is destructive. Its common names are Kirni, kizni (Hausa), Marehi (Fulani), Iralodan (Yoruba), Ola (Igbo). The bark is dark grey, rough and often markedly scaly (Rashid et al., 2002). The bark extract of the plant has been used for milk coagulation and also lime juice, for the formulation of traditional gargle "Ogun efu" (Orafidiya et al., 1990). It is also used as purgative and a vermifuge (*Cimanga* et al., 1997). Iwu, 1984 also showed that the plant has molluscidal activity. Adeoye et al., (1988) reported that the bark extract of the plant has anti-microbial activity against microorganisms' known to cause enteric and secondary upper respiratory tract infections.

The genus *Senna* (Leguminosae) comprises of 750-800 tropical and sub-tropical species. They are mostly trees with typical leaf form bipinnate with numerous leaflets and small scaly strip (Airy, 1973). The plant *Senna alata* is an ornamental shrub, which grows all year round, and flowers during November to January. It grows well in the forest areas of West Africa. In Tanzania, Mustasa et al., (1990) conducted an investigation on the root bark of *Senna alata* gel which is used in local herbal medicine against convulsions, gonorrhoea, bilhazia, heart-burn, stomach ache, constipation, wounds and Snake bites. In the light of all these, the bark extracts of "Ganna Ganna" tree (*Senna spp*) so called in the kpelle language in Liberia folk medicine was investigated and the effect of some bark extracts *in vivo* and *in vitro* on microfilaria were examined and found effective (Kilian et al., 1990).

Moreover, the leaves have also been reported to be efficacious in the treatment of ringworm

and eczema. The bark was found to be useful for various skin diseases (Marshall, 1951). This study therefore intend to examine the effects of the bark extract of *Bridelia ferruginea* and leaves extract of *Senna alata* on plasma glucose concentration in normoglycemic and glucose induced hyperglycemic rats.

Materials and Methods

Subject selection

A total number of 30 female albino rats weighing between 150-180g were obtained from the animal breeding unit of the Department of Veterinary Physiology, University of Ibadan. These rats were kept in well-ventilated cages at the animal House of the virology Department, University college hospital, Ibadan.

Breeding and feeding

The animals were fed with pellets bought from Ladokun feeds, Mokola, Ibadan and adequate clean water was provided. After about 8 weeks of breeding to the desired weight (the average of which was 165g), the rats were divided into groups.

Grouping

The rats were divided into 2 major groups' based on the extracts to be administered. Each major group was then subdivided into 3 subgroups each, based on their glycaemic states (i.e. normoglycemic, glucose induced hyperglycemic) and the third group, the control. Each major group consists of 15 female albino rats while each subgroup consists of 5 female albino rats.

Sub-group

Normoglycemic group consists of rats fed with the normal diet, water and extracts administered intraperitoneally. Glucose induced hyperglycemic group consists of rats fed with the normal diet with a glucose load. This was done by dissolving 50g of D-glucose in 100mls of water, which they drank throughout the experiment such that at every point in time, there was significant increase in the glucose compared with the control. This group was then administered with the extracts intraperitoneally after hyperglycemia had been induced in the rats. The control group consists of rats fed with the normal diet and given clean water. No extracts were administered to these rats.

Extracts administration

The animals were administered with 250mg/kg body weight of the leaf extract of *Senna alata* and bark extract of *Bridelia ferruginea* respectively for period of 21 days. The extracts were administered intraperitoneally (Kuma et al., 2000)

Specimen collection

Blood samples were collected from the rats through intracardiac puncture using tuberculin

syringes. Samples were collected into specimen bottles containing fluoride oxalate as anticoagulant, which inhibits glycolysis as well as preserves the samples. Samples were collected at intervals of 7 days over a period of 21 days from both the test and the control rats.

Samples were later centrifuge at 3,000rpm for 10 minutes using a MSE centrifuge (Centaur 2). The plasma was extracted and dispensed intro serum bottles.

Basal samples were collected first before the administration of the extracts (i.e. at day 7, 14 and 21) daily.

Determination of blood glucose

The determination of blood glucose is adapted from the Glucose oxidase method of Trinder et al., 1972.

Collection of plants

The leaves of *Senna alata* and the bark of *Bridelia ferruginea* benth were collected within and outside of Ilorin Township. A voucher sample was deposited in the herbarium of the Department of Plant Biology, University of Ilorin, Nigeria.

Extraction procedure

On the basis of information obtained on the mode of usage from traditional healers, laboratory extraction of the leaves of Senna *alata* and the bark of *Bridelia ferruginea* was done using the solvent methanol at ambient temperature (Kim et al., 1999)

Bridelia bark pieces were collected from the tree of *Bridelia ferruginea* Benth, Linn. (Euphorbiaceae). The bark pieces were cleaned and the epidermal layer and pith removed, while the wine coloured portion of the bark pieces was then exposed. The bark pieces were cut into small pieces and dried in an oven (Gallenkamp Oven BS size two) at 40^0C for 48 hours. The dried pieces pulverized using the Laboratory Mill (Christy and Norris limited, Machine type 8). Two kilograms of the powder were exhaustively extracted with one liter of absolute methanol over a period of six days at room temperature. The suspension was then decanted and filtered through whatsman paper No.1. The residue obtained was then concentrated to reddish-brown gummy material by evaporation to dryness at 45^0C in a rotary evaporator (Buchi-rotavapor).

The above extraction procedures were repeated for the leaves of *Senna alata*. The residue obtained was then discarded and the filterate was then concentrated to greenish brown gummy material, by evaporation to dryness at 45^0C in a rotary evaporator (Buchi-rotavapor)

Phytochemical screening

Methanol extracts of *Bridelia ferruginea* and *Senna alata* were subjected to screening procedure as described by Trease & Evans, (1985).

Statistical analysis

All results are presented as mean $\pm$SEM. Data were analyzed by the student's T-test and F-

test. Groups for the pair of observations dependent upon each other. Results were considered statistically significant at P<0.05.

Results

Phytochemical screening of the methanol extract of *Bridelia ferruginea* revealed the presence of saponins, carbohydrates, tannins, polyphenols, steroids, triterpenes and alkaloids while methanol extract of *Senna alata* revealed the presence of flavonoids, carbohydrates, tannins, polyphenols, steroid triterpenes, and alkaloids. Thirty female albino rats were used in this study. The mean weight was 165±5.68g. The data was used for calculating and comparing significance of random plasma glucose before and after administration of extracts and the average data obtained from the determined concentration of plasma glucose, before and after extract administration. Comparison was made between day 7, and days 14 and 21 post extracts administration, as well as between the hyperglycemic and control groups.

The results in Table 2 showed comparison of random plasma glucose before and after administration of methanol extracts of *Bridelia ferruginea*. In normoglycemic group, the mean value was 142.20mg/dl at day 7; 121.00mg/dl at day 14 of experiment and 142.80mg/dl at day 21 posts extract administration. There was a statistically significant difference in glucose levels before and after extract administration (P<0.05). In the glucose induced hyperglycemic group, the value was 167.40mg/dl at day 7, 135.00mg/dl at day 14 and 166.00mg/dl at day 21 post extract administration. There was a statistically significant difference in glucose levels before and after administration (P<0.05). The results in Table 3 showed the comparison of random plasma glucose before and after administration of methanol extract of *Senna alata leaves.*

In the normoglycemic group, the mean value was 117.80mg/dl at day 7; 119.00mg/dl at day 14 and 116.80mg/dl at day 21 posts extract administration. There was no significant difference in glucose level between pre and post extract administration (P>0.05).

In the glucose induced hyperglycemic group, the mean value was 141.00mg/dl at day 7, 138.20mg/dl at day 14 and 139.00mg/dl at day 21 post extract administration. There was no significant difference in glucose levels between pre and post extract administration (P>0.05). In the control group, no extract was administered. The mean value was 115.60mg/dl at day 7, 114.00mg/dl at day 14 and 116.00mg/dl at day 21-post administration. There was no significant difference in plasma glucose levels (P>0.05). The results in Table 4 also showed a comparison between the glucose induced hyperglycemic and control groups, after administration of *Bridelia ferruginea* extracts. There was a statistically significant change in both groups as well; (P<0.05) for the normoglycemic-control comparison, and for glucose induced hyperglycemic-control comparison (P<0.05).

Discussion

Diabetes is a chronic illness that requires continuous monitoring and treatment. To prevent acute complications and to reduce the risk of long-term complications the cost of life long treatment is tremendously increasing and effective preventive measures will be most fruitful in controlling the physical and financial burden of this disease (Irobi & Daramola, 1994)

In a country like Nigeria, where the economy is depressed and average income can barely meet basic needs of life, compliance with treatment is poor (Iwu, 1993). The anti-diabetes activities of these plant extracts could be due to the presence of tannins, polyphenols, steroids, triterpenes and alkaloids as revealed in the phytochemical screening. This study showed that *Bridelia ferruginea* probably reduces plasma glucose levels, an effect which is more pronounced in hyperglycemic states than in normoglycemic states. This is in agreement with the report of Ampofo (1977) and Iwu (1983) that aqueous extract of the leaves of *Bridelia ferruginea* had been shown to possess hypoglycemic activity.

The plausible mechanism of action of these herbs are still unclear but may be thought to control blood glucose level by increasing glycolysis, and decreasing gluconeogenesis with a lower demand of pancreatic insulin. This is in consonance with the work done by Pari & Saravanan, (2004). Mechanisms such as the stimulating or regenerating effect of B-cells on extra pancreatic effects are proposed for the hypoglycemic action of *Bridelia ferruginea.* This finding is also in consonance with the work done by Saxena & Vikram, (2004). *Senna alata* was not found to possess hypoglycemic activity. There was no significant difference in glucose concentration before and after administration of the extract in all groups of rats.

Conclusion and recommendation

The results of this study suggest that *Bridelia ferruginea* bark extract achieved a reduction in plasma glucose levels especially in glucose induced hyperglycemic rats. This implies that the methanol extract has anti-diabetic properties and may thus be useful in the management and treatment of diabetes mellitus.

Further study can be made by observing the effect of the extracts for a longer period of time after achieving alloxan-induced hyperglycemia. Also, the effects on other organs in the rat should be investigated through histological examination to eliminate adverse side effects or toxicity.

Acknowledgement

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Table 1. Phytochemical screening of the methanol extracts (ME) of *Senna alata* and *Bridelia ferruginea*.

Compound tested	Test	Observation	*Senna alata* Inference	*Bridelia ferruginea*
Flavonoids	Shibata's reaction.	Orange-pink colouration	flavonoids present	Absent
	Lead acetate test.		”	”
I.	Sodium hydroxide test.	Whitish-yellow	”	”
	Mineral acid test.	colouration	”	”
II.	Boric acid test		”	”
	Frothing test	Yellowish colouration	saponins absent	Moderately present
III.	Borntrager's test		anthraquinones absent	Absent
	Universal reagent	Orange-yellow	Lipid absent	Absent
IV.	Molisch's test	colouration	Carbohydrates present	Present
	Barfoed's test	Yellowish colouration	Reducing sugars absent	Absent
V.	Fehling's solution A and B		”	”
Saponins	Ferric chloride test.	Frothing on warming		Present
Anthraquinones	Salkowski test	No reddish colouration	”	Moderately present
Lipids	Liebermann-Burchard's	No red colouration	Tannins and polyphenols faintly present	Moderately present
Carbohydrates	Dragendorff's reagent	Reddish brown colouration at the interface of the two liquids.	Steroids present	Present
Reducing sugar	Mayer's reagent	No brick red precipitates	Steroids/triterpenes present	Present
I.	Wagner's reagent	No reddish precipitate	Alkaloids moderately present	Present
II.		Bluish-green colouration	Alkaloids moderately present	
Tannins/Polyphenols		Reddish-brown at the interface of the two liquids.	”	
Steroids I.		Reddish-		
Steroids/triterpenes II.				
Alkaloids I.				
Alkaloids II.				
Alkaloids III.				

		brown ring with bluish-green upper layer. Reddish-brown Precipitate Yellowish-white precipitate Reddish-brown colouration		

Table 2. Random plasma glucose of rats at pre and post administration of methanol extract of *Bridelia ferruginea* bark.

Parameters	Day 7	Day 14	Day 21	f	P
Normoglycemic	142.00±6.22	121.00±8.00	142.80±5.81	16.96	0.00 (s)
Glucose induced hyperglycemic	167.00±5.68	135.00±10.59	166.00±4.69	30.26	0.00 (s)
Control	121.60±2.41	120.60±3.13	124.40±3.29	2.21	0.51 (ns)

Key: s-significant, ns-not significant

Table 3. Random plasma glucose of rats at pre and post administration of methanol extract of *Senna alata* leaves.

Parameters	Day 7	Day 14	Day 21	f	P
Normoglycemic	117.80±11.83	119.00±11.92	116.80±9.34	0.05	0.95
Glucose induced hyperglycemic	141.00±4.95	138.20±3.27	139.00±2.35	0.77	0.99
Control	115.60±10.60	114.00±8.22	116.00±8.72	0.07	0.07

Table 4. Comparison of significance between normoglycemic and control rats, at post administration of *Bridelia ferruginea* extract.

Parameters	X	S.D	t	P
Normoglycemic Control	13.13	12.08	4.21	0.00
Glucose induced hyperglycemic Control	33.93	15.82	8.31	0.00

X = Mean $P < 0.05$: - Significant at 5% confidence
S.D = Standard deviation interval
t = T test $P > 0.05$:- Not significant.
f = F test