EFFECT OF *HIBISCUS CANNABINUS* (KENAF) METHANOLIC LEAVE EXTRACT ON SOME BIOCHEMICAL PARAMETERS IN AN ANIMAL MODEL INDUCED WITH DIABETES

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ABSTRACT

Background: Medicinal plants have long been used traditionally in the management and control of diabetes in Nigeria with reduced burdens of unwanted side effects associated with the use of synthetic anti-diabetic drugs in the market.

Aim: The present study was carried out to evaluate antidiabetic and hypolipidemic activities of *Hibiscus cannabinus* kenaf methanolic Leave extract in Alloxan induced diabetic rat by administering 2000mg of extract for 21 days.

Methodology: Body weight and glucose levels of an animal were measured on day 0, 7, 14, and 21.The blood glucose was determine using Glucose Oxidase method (Cheesbrough 2006). ELISA method was used to assess the thyoid hormones.

Results: Body weight and glucose levels was measured on day 0, 7, 14, and 21. The extract showed antidiabetic activity but not to a significant level and also decrease in body weight. Administration of extract for 21 days also resulted in reduction of serum cholesterol, triglycerides and HDL cholesterol level was found to be also decreased but not to a significant level ($p>0.05$) as compared to diabetic control group. The effect was compared with diabetic control and normal control. The determination of blood glucose level by Glucose Oxidase method. The result shows the methanolic extract of *Hibiscus cannabinus* kenaf leaves lowered the blood glucose of hyperglycemic rats and Lipid profile level but does not showed a significant level. From the toxicity study it was observed that methanolic extract of *Hibiscus cannabinus* kenaf was nontoxic up to 4000mg/kg body weight.

Conclusion: It is concluded that *Hibiscus cannabinus* leaves extract does not has a significant antidiabetic activity, which lowered the fasting blood glucose level in Alloxan induced diabetic rat.

Keywords:, Alloxan, *Hibiscus cannabinus* ,Glucose, Lipids and Thyroid Hormones

INTRODUCTION

According to the World Health Organization (WHO) there are about 350 million people suffering from diabetes mellitus (DM) and by 2030, diabetes will become the seventh leading cause of death worldwide with diabetes deaths expected to raise by 50% during the next 10 years. (WHO 2012). The number of diabetic persons is increasing in every country, 4 out of 5 people with diabetes live in low and middle income countries and half of diabetics don't know they suffer from this disease (International Diabetes Federation, 2012).

This global epidemic could be largely attributed to the rapid increase in the rates of overweight, obesity and physical inactivity (WHO 2012). There are described two common types of diabetes mellitus: type 1, type 2 and gestational diabetes mellitus, triggered by a complex interaction between environmental and genetic factors and sharing hyperglycemia as a common characteristic (Fauci et al., 2008). Type 1 diabetes results from the complete or near-complete lack of insulin production whereas type 2 diabetes results from insulin resistance, impaired insulin secretion and increased glucose production which could take place in various degrees (Fauci et al., 2008). Gestational diabetes occurs in nearly 4% of pregnancies in United States and, even though in most cases the glucose tolerance is back to normal, these women face an increased risk of developing Diabetes Mellitus later in life (Fauci et al., 2008). Diabetes Mellitus is diagnosed by measuring the plasma glucose.

Diabetes mellitus is a syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion, insulin action or both result from impaired metabolism of glucose and other energy- yielding fuels such as lipids and protein (scheen, 1997). Hyperglycemia is involved in the etiology of development of diabetic complications, such as relinopathy, neuropathy, and peripheral vascular insuficiencies (Kamboj, 2000).

Hyperlipidemia is a condition, which characterized by elevated serum total cholesterol, triglyceride, and lipoproteins such as low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c) and decreased high density lipoprotein cholesterol (HDL-c) (Tilak *et al.,* 2001). These are the biomarkers for elevated risk of cardiovascular diseases such as atherosclerosis, coronary artery diseases and cerebral vascular diseases (Villanueva et al., 2011). Oxidative stress has been prescribed as the main mechanism responsible for cardiovascular diseases while hypercholesterolemia under oxidative stress could trigger the progression of atherosclerosis and abnormal lipid metabolism (Zulkhairi *et al.,* 2010).

Alloxan is a urea derivative compound experimentally used to induce Type 1 diabetes and in so doing, selectively destroys the pancreatic insulin producing β-cells (Ankur and Ali, 2012). Lifestyle and physical inactivity are some of the leading cause of diabetes mellitus affecting millions of people with high frequency rate worldwide. There are also evidences that genetic and environmental factors could lead to hyperglycemia, dyslipidemia and inflammation, resulting in β-cell dysfunction, thereby triggering the pathogenesis of diabetes (Fu *et al*., 2013).

Medicinal plants have long been used by traditional practitioners in management and control of diabetes in Nigeria with reduced burdens of unwanted side effects associated with the use of synthetic anti-diabetic drugs in the market.

Kenaf (*Hibiscus cannabinus*) is an annual herbaceous dicotyledonous plant, belongs to the Malvaceae family, is widely distributed in Asia and Africa, and grows mostly in temperate to tropical areas (Zhao *et al.,* 2014). Kenaf (leaves and seed) has many significant medicinal properties, including anticancer, antioxidants, analgesic, anti-inflammatory, aphrodisiacs, and hepatoprotective activities (Kubmarawa *et al.,* 2009; Monti and Alexopoulou, 2013). In traditional medicine, Kenaf is used to treat various diseases; for instance, a paste of the leaf and stem is used to treat Guinea worms disease and anemia in Africa (Monti and Alexopoulou, 2013). Moreover, in ayurvedic medicine, the leaves are used to treat various disorders, such as of the blood, diabetes, bilious, the throat, and coughs (Monti and Alexopoulou, 2013 and Jin *et al.,* 2013). Although, herbal remedies have promising potential and are widely used, many of them remain untested and their uses not monitored (Ekor, 2013). Traditional health care systems in many countries of the world, including those in Africa, have used

medicinal plants since ancient times and also this study is to determine the effect of Hibiscus cannabinus methanolic leave extract on blood glucose, lipid and thyroid hormone levels in an alloxan induced diabetic rats.

MATERIALS AND METHODS

Study area

The research was conducted in the Pharmacology Laboratory, Chemical pathology Aminu Kano Teaching Hospital (A.K.T.H), Kano State.

Collection and preparation plant extract

Fresh leaves of Hibiscus Cannabinus was collected from Aminu Kano TeachingHospital. 1he plant was identitied and authenticated at Botany department, Bayero Universi1yKano.

The leaves was dried under the shade and subsequently pulverised to obtain coarsepowder. The coarse powder (450g) was soaked in 1.5L methanol for 8 days with intermittent shaking and then filtered. The extract was then evaporated and stored in a bottle for use.

Laboratory animals

Healthy adult Wistar Albino rats (160-200g) of either sex between the ages of 2-3 month was purchased from the University of Jos and kept in standard polypropylene cages at room temperature (26±2°C, 12 hours light/dark cycle) and fed with commercially formulated feed (Vital Feed Nig. Ltd.) and clean water ad libitum.

All methods involving the use of laboratory animals in this study complied with the regulatory principles for research involving animals in the above-named institution and as enshrined by the declaration of Helsinki in the care and use of animals. Animals were acclimatized for two weeks prior to the beginning of experiments.

Induction of Diabetes

Diabetes was induced by intraperitoneal (i.p) injection of alloxan monohydrate (180 mg/kg body weight) in a total volume of 10mL (Luqnman et al., 2012). The animals were left for a period of four days prior to the commencement of the experiment.

Acute oral toxicity studies

Various doses (1000mg/kg to 4000mg/kg) of methanolic leaf extract of Hibiscus Cannbinus kenaf was administered orally to respective groups of rats. Normal saline was administered orally to the control group. Rats was observed closely for toxic symptoms and behavioural changes for the first 2 hours of administration and mortality (if any) recorded wthin 24hours. The animals was sacrificed after 24hours and the blood collected for liver enzyme tests (i.e. ALT, AST, & ALP) while the liver and kidneys was taken to histology lab for histological analysis.

Chronic toxicity studies

The animal was allowed free access to food and water for 4 weeks; every week, blood was withdrawn for blood glucose estimation monitored with a glucometer after subjecting the animals to fasting for 24 hours. The animals was divided into five groups of three rats each. Methanolic leaf extract of *Hibiscus cannabinus* was administered to groups I, II, III, and at dose levels of 4000mg/kg, 3000mg/kg, 2000mg/kg, and 1000mg/kg body weightrevely. Group V was served as control group and receive the normal saline only.

At the end of the third week, the animal was sacrificed. Blood samples was collected in flourides oxalates bottles and plain bottles for serum glucose, lipid profile and thyroid function test respectively.

Analytical Biochemical Assay

At the end of the third week, the animal was sacrificed. Blood samples were collected in flourides oxalates bottles and plain bottles for serum glucose, lipid profile and thyroid function test borespectively. Serum was obtained by centrifugation at 5000rpm for 10 minutes. Serum samples were analysed using the prescribed kits for Glucose (cheesbrough 2006), Lipid and Thyroid hormone(wadker2008). All parameters were determined calorimetrically (Shuguichi et al., 1995).

Statistical analysis

All values were expressed as Mean± SD. Ihe differences between control and groups were tested for significance using ANOVA followed by Dunnet's test. P<0.05 were considered significant.

RESULT

The animal were placed individually and observed for any sign of toxicity, mobidity or motality during the first 12hrs. From the toxicity study it was observed that methanolic extract of *Hibisbiscus cannabinus* was nontoxic up to 400mg/kg body wight with special given attention during the first 4 hours and daily thereafter from a total of 21 days. Serum Liver enzyme analysis showed normal ALP levels with normal AST and ALT concentration in chromic toxicity study the methanolic extract of *Hibiscus cannabinus* did not produce lethality up to thedose level of 2000mg/kg.

Effect of the methanolic extract of *Hibiscus cannabinus* leaves on body weight (g)

In the antidiabetic activity, the effects of *Hibiscus cannabinnus* leaves extract on body weight is measured on initial, 14th and 21th day ot post induction and were compared with normal and diabetie control groups. Body weight was recorded every week and data below shown in Table 1. Normal control (negative control) and extract treated appeared to have weekly increase in weight but diabetic control (positive control) had reduction in weight at the end of the third week. Administration of learat the dose of 2000mg/kg does not show significant increase (P>0.05) in body weight of post imaucion when compared to untreated diabetic rats.

Table1: Body weight (g) at different day intervals (g) ± SEM

	Initial(mmol/L)	Day14	Day21
Normal control n=3	56.00±10. 5	71.33±11.57	85.00±14.00
Diabtic conrtol n=3	95.00±13.00	63.00±0.00	58.00±0.00
Extract treated HK	101.50±14.50	63.00+8.00	58.00±0.00
P-Value	p>0.005	p>0.05	p.0.05

Values expressed as Mean ± SEM. P>0.05, as compared with cotrol group

Effect of the methanolic extract of *Hibiscus cannabinus* leaves on blood glucose level (mmol/l)

The result shows the methanolic extract of *Hibiscus cannabinus* leaves lowered the blood glucose hyperglycemic rats but does not have significant decrease in serum glucose comparison to the normal control group. However, there was no significant decrease in blood glucose levels in diabetic treated rats compared to diabetic control (p>0.05) as shown in table 2 pressed as Mean ± SEM. P>0.05, as compared with cotrol group

Table 2 Effect of the methanolic extract of *Hibiscus cannabinus* leaves on blood glucose levels (mmol/L) ± SEM

	Initial(mmol/l)	Day7	Day14	Fasting
Normal control	6.47±0.37	7.77+0.28	7.13±0.03	6.80±.044
Diabetic conrtol	21.15±9.850	12.00±2.00	9.65±0.75	11.40±2.90
Extract treated	24.15±3.05	16.85±1.15	12.60+4.10	11.25+3.75
p-Value	p>0.05	p>0.05	p>0.05	p>0.05

Values expressed as Mean ± SEM. P>0.05, as compared with cotrol group

Effect of the methanolic extract of *Hibiscus cannabinus* leaves on lipid profile level (mmol/l)

The effect of the methanolic extract of *Hibiscus cannabinus* leaves on lipid profile level are shown in table 3. Total cholesterol levels in diabetic rats showed a rise in the values in non-diabetic control rats (2.86±0.44 vs. 1.774±0.24mmol/L) but it is not significant since (p> 0.05). Serum trigyceride levels were also decrcased in diabetic rats compared (1.820±29 vs 1.561**3** ±0.22) to non-diabetic animals. Low-density lipoprotein levels were decreased in extract treated group when compared to diabetic control group .

Lipid profiles kenaf	Normal control	Diabetic control	treated with
Total Cholesterol	1.77±0.24	2.86±0.44	2.73±0.03
Triglyceride n = 3	1.56±0.22	1.82±0.29	16.3±0.44
HDL Cholesterol n =3	12±10.02	2.17±0.39	1.60±0.21
LDL n =3	1.35±0.11	2.95+0.54	1.92+0.16
p-value	**p>0.05**	**p>0.05**	**p>0.05**

Value expressed as Mean ± SEM. p>0.05, as compared with control group, n=population size

Effect of the methanolic extract of *Hibiscus cannabinus* leaves on thyroid hormone level(ng/ml)

Thyroid hormone analysis showed decreased levels of total T3 and total T4 and an increased in level of TSH in the extract treated levels and there were no signiticant changess in the thyroid hormone analysis as shown in table below

Table 4 Effect of the methanolic extract of *Hibiscus cannabinus* leaves on Thyroid hormone level (ng/ml) ± SEM

Thyroid hormone treated	**Normal Co ntrol**	**Diabetic Control**	**Extract**
T3n = 3	0.95±0.03	0.54±0.31	0.38±0.10
T4 n = 3	0.92±0.04	.62±0.87	1.16±0.67
TSH n = 3	0.04±0.03	0.74±0.04	0.07±0.08
p-value	**p>0.05**	**p>0.05**	**p>0.05**

Value expressed as Mean ± SEM. p>0.05, as compared with control group

DISCUSSION

Diabetes is a chronic condition the control of which demands the combining efforts of the patients and a group of specialized care providers. The patient' participation, motivation and enthusiasm are critical for achieving optimal control of the disease. The successful management of diabetes requires more than just controlling the plasma glucose levels. It requires a multidisciplinary approach (Rothe *et al al.,* 2008; Fauci *et al.,* 2008).

Table 1 shows result of body weight as affected by *Hibiscus cannabinus.* There was a gradual deerease in body weight of alloxan diabetic group fed on *Hibiscus cannabinus*, the leaves showed a weight reducing effect on the test group (Group III) when compared with the induced controL (Group II) and normal control (Group I) but not to a signicant level P>0.05.The methanolic extract of *Hibiscus cannabinus* at a concentration of 2000mg/kgbody weight lowered blood glucose but did not produce any significant reduction in blood glucose level in the extract treated group (p<0,05). The fasting blood glucose in mmol/IL after 21days showl1.25+3.75, while that of the diabetic control was 11.40+2.90.

Diabetes is associated with a greater risk of mortality from cardiovascular disease (CVD) which is well known as dyslipidaemia, which is characterized by raised triglycerides, low high densitylipoprotein and high low density lipoprotein particles. It may be present at the diagnosis of type 2 Diabetes mellitus and is a component of the metabolic syndrome and the determination of the serum lipid levels in people with diabetes is now considered as a standard of the diabetes care (Miller, 1999). Table 3 shows the effect of *Hibiscus cannabinus* on lipid parameters. There was decrease on the LDL, TG and TC of extract treated (Group I1l) when compared with diabetic control (Group II) but the decrease is insignificant (P>0.05). However there is no significant difference between Group III and Group I for TC ,LDL and TG levels, but there is marked decrease in the level of LDL in extract treated (Group III) when compared to groups II and group I. *Hibiscus cannabinus* treatment to the rats posses a hypolipidemic activity.

The findings of this study is in accordance with the findimgs of Karthik and Gayathri (2013) where he reported a significant decrease in the serum cholesterol, triglycerides, LDL-C and also significant decrease in the levels of SGOT and SGPT activities when compared to cholesterol diet induced group. The groups treated with the extracts of *H .cannabinus* also showed decrease in body weights when compared to cholesterol induced group (Karthik and Gayathri, 2013).

All the lipid components, total cholesterol, LDL and triglycerides was reduced but not significant (p>0.05) for the test animals when compared to the diabetic control and normal control animals.

animals. The more prominent effect being reduction is LDL-C which is a knownering factor for coronary occlusion or its block. Serum Liver enzyme analysis showed normal ALP levels with normal AST and ALT concentrations. In chronic toxicity study the methanolic extract of *Hibiscus cannabinus* did not produce lethality up to the dose level of 2000mg/kg

Thyroid hormone analysis showed decreased levels of total T3 and total T4 and an increased level in TSH in the extract treated levels and there is no significant change in the thyroid hormone analysis.

CONCLUSION

In conlusion, the methanolic extract of *Hibiscus cannabinus* leaf shows general reduction in body weight, decreased blood glucose level but does not produce a significant hypoglycemic activity in an alloxan induced diabetic rats. The study also demonstrated the hypolipidemic effects of *Hibiscus cannabinus* by reducing the levels of TC, TG, and LDL. Serum lipid profile level was also decreased in extract treated, normal control but does not produce a significant differences and there are no significant changes in the thyroid hormone analysis.

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