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Therapeutic Effects of Nettle Leaf Extract on Alloxan-induced Diabetic Wistar Rats

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Abstract: **WHO had drawn the attention of research investigation into herbal medicine to attest to their efficacy of plants with bioactive healing property that could be of great clinical importance in the health management. Objective of this study was to investigate the effectiveness of Nettle plant extract in controlling the blood glucose level. Sixty (60) male Wistar rats (4-6) week old, weighing (90-180)g were purchased from the Animal Research Center, Babcock University. The rats were housed in colony cages of seven (8) rats per cage, at 23◦C, relative humidity of (30-70) %, under light/dark cycle of 12hrs for 2 week acclimatization. All rats had equal access to free food and water. On 15th day the rats were separated into 4 experimental groups: Positive control, Negative control, Chemotherapeutic and Chemopreventive groups. Fasting blood glucose test was done to determine the blood glucose level. Oral glucose Tolerance Test was performed using glucometer to determine glucose tolerance level in the rats. The oxidative stress test was done to determine the hepatic enzyme activity. Results showed the means of fast glucose level ranged from (84.63) mg/dl in Positive control group, (97.63) mg/dl in Negative control group, (87.0) mg/dl in Chemotherapeutic group and (75.50) mg/dl for Chemo-preventive group control. Blood glucose levels in Chemotherapeutic and Chemopreventive treatments were within the safe limits of blood glucose levels. Such reduction in blood glucose was attributed to Nettle plant extract effectiveness to lower blood glucose level. Means of oxidative test results indicated that hepatic Superoxide Dismutase, Catalase and Glutathione inhibited the free radical activity efficiently and were improved by Nettle plant extract. In conclusion pathological tests indicated that Nettle plant extract was effectiveness in controlling and prevention of diabetes in rats.**

Keywords: **Stinging Nettle plant, Diabetes Mellitus, Fast blood glucose, Hepatic Glutathione enzyme activity, Wistar rats.**

1. INTRODUCTION

Diabetes mellitus is a common endocrine metabolic disorder experienced in all the nations of the world (Okolie, *et al*., 2008). It is a syndrome associated with lack of hormonal insulin, or insufficient insulin secretion or insulin resistance by receptor cells that consequently lead to hyperglycemia and glycosuria disorders (Modu, *et al*., 2013). Diabetes mellitus constitutes a public health risk factor to many patients due to its economic burden on the health care system (Sunday, et l., 2012). The World Health Organization (WHO) in (2006), predicted that diabetes patients in Africa alone would increase to 15 million people by the year (2025). Prevalence of diabetes mellitus in Nigeria was unknown in 20th century (Mbanya, et al., 2005). However, as a result of Nigeria's economic and industrialization most Nigeria populace began to adopt foreign food diet lifestyle, consequently there were emergence of chronic diseases attributed to such foreign diet lifestyle. The WHO (2000), indicated that Nigeria has the greatest number of people living with diabetes in Africa. Though the exact prevalence of diabetes mellitus, is likely to be in the region of (8-10)%, (Oghera and Ekpebegh, 2014). It is unfortunate that there is no cure for diabetes disorder. Okolie, et al., (2008), commented on the economic burden of

managing diabetes disorder as unsatisfactory, the medical approach of using insulin and oral drugs to control the diabetes disorder is costly and inadequate, boring and lack compliance, thus the patient's exposure to long term complication remains a risk and a challenge to the medical community. The objective of this study is to investigate the effectiveness of Stinging Nettle Plant Extract potential in managing diabetes disorder in experimental diabetic rat animals. Also this attempt would be in line with (WHO) request to carry out research investigation into herbal medicinal remedy perhaps this can be of great importance to the health of the individual (Adebanjo, et al., 2006). There are some wild herbs and botanical species that are effective and non-toxic and have substantial evidence to their efficacy in diabetes management (Okeke, 1998).

2. MATERIALS AND METHODS

The Study Area

The study was conducted in the Department of Nutrition and Dietetics of Babcock University as well as in the medical laboratory of Olabisi Onabanjo University Teaching Hospital, Shagamu in Ogun, State Nigeria.

Animal Acclimatization

Sixty (60) male Wistar rats (4-6) week old, weighing (90-180) g were purchased from the Animal Research Center in Babcock University. The rats were housed in colony cages of seven (8) rats per cage, at room temperature of 23◦C at relative humidity (30-70) percent under the light/dark cycle of 12 hours for at least two weeks of acclimatization. All animal rats had equal access to free food and free pure water. The rats were randomly selected into 4 experimental groups:

3. METHODOLOGY

Table 1. Experimental design for Type 1 Diabetes

Groups	Experimental Treatments	Rationale
Positive Control Group	Normal control. Rats were fed with feed pellet (6weeks).	Control without treatment
Negative Control Group	Application of Alloxan to induce Type 1 diabetes.	To induce Type 2 diabetes
Chemotherapeutic Group For Type 1 diabetes treatment with Nettle plant extract	Alloxan diabetic rats treated with 250 mg/kg of Nettle leaf extract.	To show the therapeutic effects of Nettle leaf extract to control diabetes by lowering blood glucose.
Chemo-preventive Group (Type 1 diabetes	Application (250 mg/kg **Nettle leaf extract**, plus Alloxan and water).	To show the prevention potential of Nettle leaf extract on diabetes mellitus.

Table 2. Experimental design for Type 2 Diabetes

Groups	Experimental Treatments	Rationale
Positive Control Group	Normal control.	Control without treatment
Negative Control Group	Application of 30% fructose and Streptocotozin for Type 2 induction	To induce Type 2 diabetes
Chemotherapeutic Group For Type 2 diabetes treatment with Nettle plant extract	Application of 30% fructose and Streptocotozin for Type 2 induction, (initial treatment). Later treat by 250mg/kg of Nettle plant extract plus water to control diabetes	To show therapeutic effect of Nettle leaf extract to control diabetes.
Chemo-preventive Group Diabetic Type 2 rat treated with **Metformin drug** for prevention.	Application of 30% fructose and Streptocotozin for Type 2 induction, (initial treatment). Treatetment using 100mg/kg of **Metformin** for prevention	To compare the effectiveness of **Metformin drug** to that of **Nettle leaf extract** on diabetes.

Preparation of stinging Nettle plant extract

Dried leaves of stinging Nettle plant were purchased from Relish Company in Ghana. The leaves were pulverized using a Panasonic industrial blender. The leaves were soaked in (100%) methanol at 1:3 ratio. The leaves were macerated for 72 hr. at room temperature. The mixture was filtered through (5µm) Whatman filter paper, while the filtrate was placed in a rotatory evaporator to remove the solvent. The concentrate was kept at 4◦C until it was needed for use.

Induction of Diabetes

Type 1 Diabetes

Twenty (24) four male of Wistar (90-180) g rats were induced. Diabetes mellitus was induced by intraperitoneal injection of 150mg/kg body weight of Alloxan monohydrate that was suspended in Normal saline solution according to the procedures of (Szkudelski, 2001; Yanarday and Colac, 1998). After 3 days, rats with blood glucose level ≥ 126 mg/dl was considered diabetic and was selected for the study.

Type 2 diabetes

Twenty (24) four male Wistar rats were fed 30% fructose in water for a week and later induced through intraperitoneal injection with 15 mg/ml concentration of Streptocotozin (STZ) was dissolved in Citrate buffer at 40 mg/kg according to the procedure of Racheal and Shahidul, 2012). After 5 days of induction diabetes mellitus was confirmed using calibrated glucometer to conduct an oral glucose tolerance test. The blood analysis was done in the Chemical Pathology and Immunology laboratories of Onabanjo University Teaching Hospital in Shagamu. The blood sample was drawn out from the tail vein and tested for glucose level using glucose strips and glucometer.

Fasting Blood Sugar

Blood samples from rat group treatments were withdrawn from their tail vein and tested using glucose strips and glucometer after overnight fast (Egedigwe, 2010).

Oral Glucose Tolerance Test

Negative control group rats (24) of them, were on 10 hr fast for five days. The rats were fed with 30% mixture of fructose and Streptocotozin (STZ) which was injected intraperitoneal . A 10% glucose solution was prepared and 2g/kg of the glucose solution was administered orally. The blood glucose level was taken from the tail vein at 30 min, 1hr, 2 and 3hrs intervals according to Atangwho, et l., (2009)

Oxidative stress study

Organs of interest (kidney and liver) were harvested from the experimental rat group and their blood media subjected to pathological tests following Igile, et al., (1994) procedure.

Statistical Analysis

Data collected were subjected to Analysis of Variance (ANOVA) using (SAS, 2004) and significantly ($P<0.05$) different means were compared using Duncan Multiple Range Test (DMRT) obtained in the same statistical package. Statistical values were reported as means of triplicates.

4. RESULTS AND DISCUSSION

Means of fast blood glucose levels in Type 1 induced diabetic rats are presented in **Table 3.** The Table classified the data information under: Positive control, Negative control, Chemopreventive and Chemotherapeutic groups. Under positive control group the blood glucose level was (84.63)mg/dl for normal rat control group without treatment. Under Negative control group the means of fast blood glucose level was (97.63)mg/dl, after diabetes induction using Alloxan. Under Chemothrapeutic group treatment the means of blood glucose level was (78.00)mg/dl after diabetes induction using alloxan and followed by treatment with 250mg/kg of Nettle leaf extract. Under the Chemopreventive treatment the means of fast blood glucose level was (75.50)mg/dl by giving 250mg/kg of Nettle leaf extract followed by Alloxan to determine the preventive level of Nettle extract against diabetes induction.

The result indicated a sharp increase in the means blood glucose level under Negative group treatment when compared to other experiment groups. This increase in the blood glucose level showed that Alloxan had the potency to initiate diabetes

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disorder in the rats. Alloxan was effective in suppressing insulin secretion from the Islet of Longerham organ in the pancreas and possibly damaged the pancreas. However, under Chemotherapeutic control group the means of fast blood glucose level reduced from (97.63 to 78.00)mg/dl after treatment with Nettle plant extract. This reduction in blood glucose level suggested that Nettle leaf extract had the effectiveness in lowering blood glucose level in the rats. Under Chemo-preventive control group the means of blood glucose level further decline from (97.63 to 75.50)mg/dl. This reduction in the means of fast blood glucose level indicted that Nettle plant extract could prevent Type 1 diabetes disorder efficiently.

Table 3. Means of Fast blood glucose level in Type 1 induced diabetic rats

Groups	Means	Standard Deviation	Standard error
Positive Control	84.63 ± 9.34	97.60	34.51
Negative Control	97.63 ± 0.89	26.79	9.47
Chemotherapeutic control	78.00 ± 0.79	14.73	8.50
Chemo-preventive Control	75.50 ± 0.69	34.32	4.00

Values are Means ± SE of triplicate.
Means in a column are not significantly (P< 0.05) different.

The means of oral glucose tolerance test in Type 2 diabetic rats are expressed in **Table 4.** The Table categorized the data information on glucose tolerance level at baseline, 30min, 1hr, 2hrs and 3hrs after induction of Type 2 diabetes in rats by using Fructose and Streptocotozin solution for Type 2 diabetes induction. Under Negative control group the means of oral glucose tolerance level ranged from the baseline of (106.0) mg/dl to a peak of (155.0) mg/dl after 30 min. of glucose administration. However after 3hr the glucose level declined from (155.0 to 70.50)mg/dl.

Under the Nettle plant extract treatment the means of oral glucose tolerance level ranged from the baseline of (102)mg/dl to a peak of (374.50)mg/dl of glucose level in 30 min. of glucose administration. After 3hr. the glucose level declined from (374.50 to 85.00)mg/dl. Also under Metformin drug treatment the means of oral glucose tolerance level ranged from the baseline of (140.0) mg/dl to a peak of (237.0) mg/dl after 30min. of glucose administration. However after 3hr, glucose level declined from (237.0 to 141.80)mg/dl.

Results of the oral glucose tolerance test indicated that after 30min of Type 2 diabetes induction using Fructose and Streptocotozin solution in the rats, there were glucose spikes in the means of blood glucose levels reaching maximum peaks of (155.0, 374.50, and 237.60)mg/dl for Negative control group, Nettle plant treated group and Metformin drug treated group respectively. These spikes in blood glucose suggested that the glycemic index of glucose were high in blood glucose resulting in excess blood glucose level.

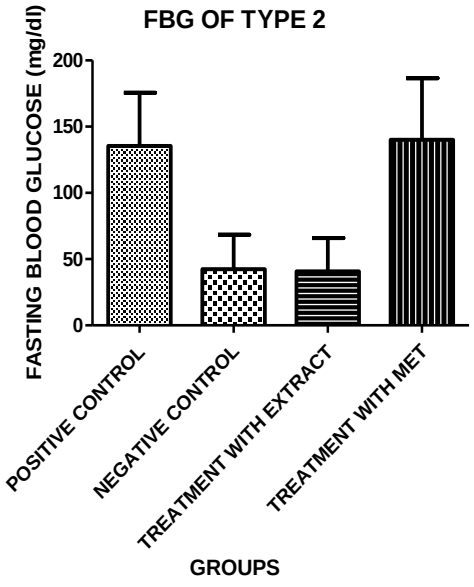

Figure 1. Means of Blood Glucose Tolerance level in Type 2 diabetic rats.

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After 3hr of Type 2 diabetes induction in the experimental rats, the means of oral glucose tolerance declined to (70.50; 85.00; and 141.80)mg/dl under Negative control group, Nettle plant treated group and Metformin drug treated group respectively, suggesting that after 3hrs of Type 2 diabetes induction, the liver was able to metabolize excess glucose into glycogen which resulted in reduction of the blood glucose levels.

The reduction in blood glucose level could also be attributed to the effectiveness of Nettle plant extract to lower blood glucose level to the minimum when compared to Metformin drug treated group.

Table 4. Means of Oral glucose tolerance of Type 2 in diabetic rats.

Groups	Negative control group. (mg/dl)	Treatment using Nettle plant extract (mg/dl)	Treatment using Metformin drug. (mg/dl)
Baseline	106.00	102.0	140.0
30min.	155.0 0	374.50	237.60
1hr	136.00	159.50	201.20
2hr	104.50	115.00	161.80
3hr	70.50	85.00	141.80

Values are Means ± SE of triplicate.
Means in a column are not significantly (P< 0.05) different.
(mg/dl) = milligram per deciliter of blood

Antioxidant enzymes involved in the inhibition of free radicals effects and depressing the oxidative stress effect in experimental rats were: Superoxide Dismutase (SOD), Catalase and Glutathione detoxifying enzymes among others. The mean of antioxidant enzyme activity levels in counteracting free radical effects are presented in **Table 5.** The Table classified the Antioxidant enzyme activity against the free radicals under: Positive control group, Negative control group, and Epinephrine, Chemopreventive and Chemotherapeutic groups.

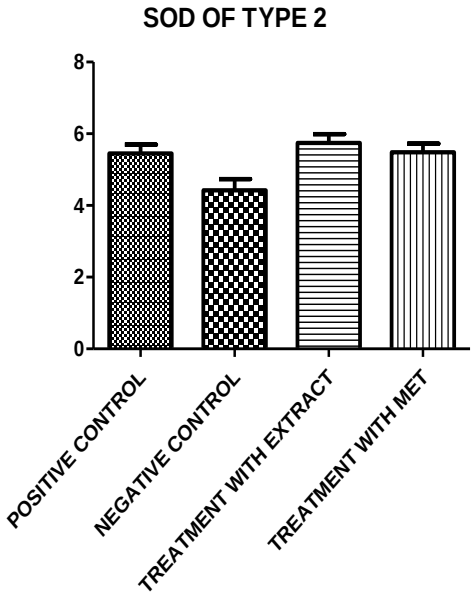

Figure 2. Means of Superoxide Dismutase enzyme activity in diabetic rats

Under the Positive Control group the mean of Superoxide Dismutase (SOD) enzyme reactions against free radical was (5.453) mg/ml at the initial level. Under the Negative group the means of (SOD) enzyme activity declined from (5.453 to 4.420) mg/ml during allergy induction using the Alloxan, suggesting that SOD enzyme activity was suppressed by free radical. However, under Chemopreventive group, the means of SOD enzyme activities increased from (4.420 to 5.745) mg/ml. This increase in antioxidant enzyme activity indicated that SOD together with Nettle leaf extract could were effective to control and suppress the activity of the free radicals, as well controlling the oxidative stress effects.

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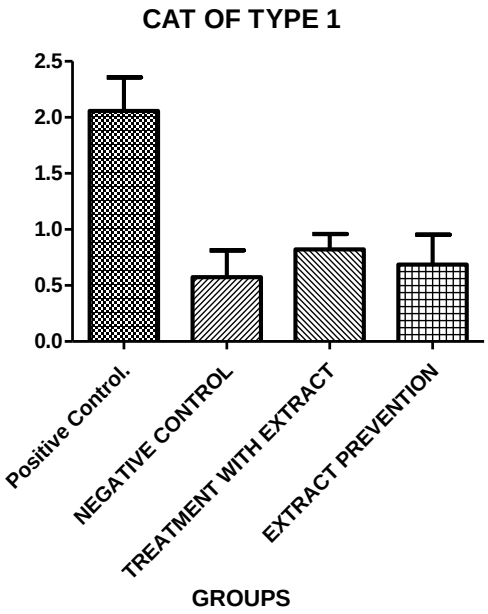

Figure 3. Means of Catalase enzyme activity in diabetic rats

Under the Positive Control group the mean of hepatic Catalase enzyme activity against free radical was (2.058) mg/ml, at initial level of enzyme activity. Under the Negative group the means of Catalase enzyme activity declined from (2.058 to 0.680) mg/ml, suggesting that allergy induction enabled free radicals to effectively suppressed the catalase enzyme activity and consequently increased the oxidative stress in the tissue of the experimental rats. However, under Chemopreventive group the means of Catalase enzyme activities increased from (0.680 to 1.090) mg/ml. This increase in enzymes activity of Catalase and Stinging Nettle plant extract were effective in controlling and the suppression of free radicals that would promote oxidative stress in the body of the rats.

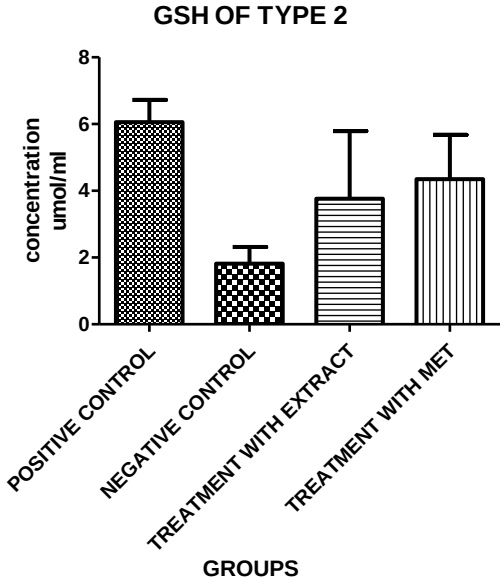

Figure 4. Means of Glutathione enzyme activity in diabetic rats.

Under the Positive Control group the mean of Glutathione activity against free radical was (6.060) mg/ml at initial enzyme activity. Under the Negative group the means of Glutathione enzyme activity declined from (6.060 to 1.820) mg/ml, indicating that allergy induction process using Alloxan solution enabled the free radical to suppress Glutathione

enzyme activity and consequently promoted oxidative stress in the body of the rat animals. However, under Chemopreventive control group the means of the antioxidant Glutathione enzyme activity increased from (1.820 to 3.765) mg/ml. This increase in Glutathione enzyme activity and Nettle plant extract suggested that both would in synergy were effective to inhibit free radical and consequently prevented oxidative stress in the experimental rats

Table 5. Means of Antioxidant enzymes activity against Free radical effects

Enzymes	Positive Control Group (Normal control group)	Negative Control Group (diabetes induction)	Chemo-preventive Group (using **Nettle plant extract**)	Chemotherapeutic Group treatment (using **Metformin drug**)
SOD	5.453 ±0.241	4.42 0 ± 0.320	5.745 ± 0.245	5.86 ± 0.235
Catalase	2.058 ± o.299	0.680 ± 0.020	1.090 ± 0.900	0.842 ± 0.084
Glutathione	6.060 ± 0.662	1.820 ± 0.500	3.765 ± 2.025	4.352 ± 1.324

SOD = Superoxide Dismutase
Values are Means ± SE of triplicate.
Means in a column are not significantly (P< 0.05) different.

5. CONCLUSION

The results of this study indicated that the reduction in blood glucose level by Stinging Nettle plant extract was effective in lowering the blood glucose level and consequently prevented diabetes mellitus disorder in the experimental rats. Also after 3hr of Type 2 diabetes induction in rats, the means of oral glucose tolerance level declined considerably under the treatment using Nettle plant extract and the treatment under Metformin drug. The liver function was able to recover from excess blood glucose level and liver rapidly converted the excess glucose into glycogen. Therefore the reduction in glucose tolerance level after 3hr of Type 2 diabetes induction could be attributed to Nettle plant extract effectiveness to depress the blood glucose level to a level of tolerance when compared to Metformin drug that is regularly prescribed for diabetes disorder. Also the result of this study revealed that application of Nettle plant extract improved the antioxidant effects of Superoxide Dismutase, Catalase and Glutathione enzyme activities against free radicals. They effectively inhibited the free radical reactions that would cause oxidative stress in the tissue of the experimental rats

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