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Effect of Crude Extracts of *Ocimum Gratissimum* and *Vernonia Amygdalina* on Urea and Creatinine in Non Diabetic and Diabetic Rats

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ABSTRACT: Diabetes mellitus is a progressive disease. Most investigators have focused on electrolytes derangement while the effect of OG and VA on Urea and Creatinine in diabetic state has been less attended. The present study has been conducted to find out urea and creatine changes in diabetic renal tissue and the ameliorative effects of herb extracts. Diabetes was induced by injection of streptozotocin 65mg/kg. The diabetic control group and normal control received only distilled water 10mg/kg. Both non diabetic groups and diabetic treated groups received 208mg/kg of *Ocimum gratissimum* and 52mg/kg of *Vernonia amygdalina*. The treatment lasted for 28 days. At the end of the experiment, result analysis for diabetes showed that serum creatinine levels were significantly reduced at ($p < 0.05$) in the diabetic control group while the non diabetic group treated with OG AND OG+VA was decreased at ($p < 0.05$) compared to normal control. Urea results showed a statistical increase at ($p < 0.05$) in diabetic control group compared to the non diabetic control while treatment with VA decreased at ($p < 0.05$) the creatinine level compared to diabetic control and non diabetic control group. Post treatment with OG, VA, OG+VA and Insulin was able to restore the Urea and Creatinine level. Daily administration of OG and VA to the different groups shows that leaf extracts of OG and VA can positively restore kidney functions in a diabetic state.

I.INTRODUCTION

Diabetes is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not responds to the insulin that is produced (1). This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst), and polyphagia (increased hunger). (2) Postulated that diabetes and its treatments can cause many complications such as hypoglycemia, ketoacidosis, or non-ketotic hyper osmolar coma. These may occur if the disease is not controlled adequately. Serious long-term complication includes; cardiovascular disease, chronic renal failure, retinal damage (which can lead to blindness) nerve damage (of several kinds), and micro vascular damages, which may cause erectile dysfunction and poor wound healing.

According to (3), many studies showed that hyperglycemia is among the contributing factors involved in most diabetes complications through excessive production of reactive oxygen species (ROS). Many investigators considered also that glucose-induced oxidative nitrosative stress is critical pathogenic mechanism that initiates a cascade of downstream metabolic and neurovascular perturbations (4). The development of diabetes-associated complications in the renal system was also found to be directly attributed to the increased polyol pathway activity in the kidneys of diabetic subjects. The accumulated sorbitol, in the renal tissue of diabetic animals, can increase the cellular osmolarity, resulting in water retention; cell edema and increases in cytosolic Na^+ concentration. All may contribute to the etiology of diabetic nephropathy (5).

Diabetic nephropathy is the largest single cause of end-stage renal failure worldwide. Nephropathy is defined as partial loss of functions of kidney associated with nephrotic syndrome, glomerulosclerosis, persistent albumin-uria, declining glomerular filtration rate (GFR), elevated arterial blood pressure and fluid retention. Again, the accumulation of glycogen in the kidney tubules as a result of the ensuring hyperglycemia is thought to be responsible for the progression of diseases in diabetic nephropathy. (6) The results of kidney biochemistry showed that STZ diabetic rats had a significant elevation in urea and creatinine levels indicative of renal cell injury.

(7) Also, demonstrated that a combination of the aqueous leaf extract of *Vernonia amygdalina* and *Ocimum gratissimum* induces significant reduction in the blood glucose of STZ induced diabetes. In other separate study, the author further observed that *V. amygdalina* extract alone reduced the glycemic level significantly which further lend credence that both extracts has peripheral action similar to that of insulin or glucose metabolism which can be attributed to the bioactive molecules present in the indigenous vegetables. Similar observations have been reported by other authors (8; 9). According to these authors, the nutrient composition also revealed that *Vernonia amygdalina* contained moisture and fiber and so, contributes lesser glucose to the blood glucose pool.

A similar finding (10) also showed evidence that a high intake of dietary fiber is associated with enhanced insulin sensitivity and therefore, may have a role in the prevention and control of diabetes.

Also, on the effect of *O. gratissimum* on the blood creatinin and urea levels, (6) discovered that; even though there was a decrease in the blood creatinin and urea levels, that the decrease/increase was dose dependent i.e. the higher the dose of *O. gratissimum*, the more increase the levels of both urea and creatinin will be. These by implication then mean that at a higher dose, *O. gratissimum* could induce more nephrotoxicity. Further studies by (11) showed that the hypoglycemic activities of some plants (*V. amygdalina* and *O. gratissimum*) have been attributed to their fiber and antioxidant contents. This is also consistent with the work of (12). A combination of these mechanisms could have resulted in the significant hypoglycemic activities observed in the above study, which is likely to be sustained and better than that of a single extract.

The kidneys are especially designed to filter large quantities of plasma, reabsorbs substances that the body must conserve and secrete substances that must be eliminated. The kidneys contribute in maintaining glucose homeostasis. When blood sugar is high, it can put too much stress on the kidneys causing serious damage to the blood vessels leading to kidney complication (13).

Urea and creatinine are one of the parameters used in diagnosing the functioning of the kidney. Urea formation is influenced by a number of factors such as liver function, protein intake and rate of protein catabolism (14).

II.MATERIALS AND METHODS

A.COLLECTION AND IDENTIFICATION OF PLANT MATERIALS. The plant materials were collected from the medicinal farm of the department of Pharmacology and natural medicine, Faculty of Pharmacy, University of Uyo. The plants were identified by the Botanist in the Department of Botany, University of Uyo. The plant was also identified by the Herbarium Officer of the faculty.

B. PREPARATION OF CRUDE EXTRACTS OF THE PLANTS. The fresh leaves of *Ocimum gratissimum* and *Vernonia amygdalina* were separately rinsed with water to remove sands and debris and then allowed to air dry. The leaves were cut into small pieces and sun dried for two days. The dried leaves were pulverized into fine powder to give a gram weight of 425 g of O G and 527 g of V A. These 425 g of OG and 527 g of VA were macerated in 300 ml of distilled water respectively for about 12 hours and stirred at regular intervals. The mixtures were filtered and the filtrate was concentrated to dryness in a water bath at 45˚C. The weight of the dried extracts was 57 g and 76 g for O G and V A respectively. The extracts were refrigerated at 4˚C until when required for use.

C.ACUTE TOXICITY TEST **(LD_{50}):** The LD_{50} was estimated using Lorke's method (15). Mice weighing between 20 g to 25 g were used for the experiment. A total of 30 mice of both gender were randomly selected for the research. The animals were weighed and grouped into 10 groups of 3 rats per group. The dosage ranges between 500 mg/kg to 5000 mg/kg b.w for both O G and VA. Rout of administration was intraperitoneal. This was to ensure quick absorption and action. After single administration, the animals were observed for 24 hours. Physical signs of toxicity and mortality were recorded. The medium lethal dose (LD_{50}) was *calculated to the 208mg/kg for OG and 52mg/kg for VA. The physical sings of toxicity included excitation, paw licking, increased respiratory rate, writhing, convulsion and death.*

D.ANIMAL PREPARATION:

Fifty six (56) female albino wistar rats weighing 150g to 200 g were obtained from the animal House unit Department of Physiology, University of Calabar and were housed in a cross ventilation room in the animal house unit of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo. The animals were kept in conventionally and environmentally adapted wooden cages with wire netting under uniform condition of daylight, night darkness and normal room temperature, with wood shavings as their beddings and were allowed to acclimatize for two weeks before the commencement of the research. The animals were kept in dry and hygienic condition with access to feed and water *ad libitum.* The animals were carefully checked and monitored every day for any changes. The experiments complied with the guidelines of our animal ethics committee which was established in accordance with the internationally accepted principles for laboratory animal use and care.

E.*EXPERIMENTAL GROUPING*:

Group Division	Group description	Administered Dosage	Duration of Study

Group 1	Non Diabetic Control (Positive control)	10 ml of normal saline	28 days
Group 2	Non Diabetic treated group with OG.	OG 208 mg/kg	28 days
Group 3	Non Diabetic treated group with VA	VA 52mg/kg	28 days
Group 4	Diabetic control (Negative control)	10 ml of normal saline	28 days
Group 5	Diabetic treated group with OG	OG 208 mg/kg	28 days
Group 6	Diabetic treated group with VA	VA 52mg/kg	28 days
Group 7	Diabetic combined treatment group with OG and VA	OG 208 mg/kg and VA 52 mg/kg	28 days
Group 8	Diabetic treated group with Insulin	0.16iu	28 days

There were a total of seven (7) rats per group and the extracts and drugs were administered according to the animals' body weight daily for 28 days.

F.INDUCTION OF TYPE 1 DIABETES: Type 2 diabetes was induced on the rats by the injection of single dose of 65 mg/kg intraperitoneally of streptozotocin which was dissolved in 0.5 M of Citrate buffer at a pH of 4.5. The state of diabetes was also observed in these groups of rats after 48 hrs of induction by the symptoms of polyphagia, polyuria and polydipsia.

***G.EXTRACT ADMINISTRATION AND OBSERVATIONS*:** A day after diabetes induction has been confirm, the administration of extracts began. The extracts as well as the standard drugs were administered on daily basis via oral rout to the animals at a dose of 208 mg/kg of O G and 52 mg/kg of VA for 28 days. Administration was facilitated by the use of a syringe and esophageal cannula. Blood glucose level and body weight were monitored on a weekly basis for 28 days duration of the work.

***H.COLLECTION OF SAMPLES*:** At the end of the treatment protocol (i.e., after the 28thday of administration), rats were anaesthetized with chloroform while those samples that are needed for immune studies e.g. kidney and pancreas were anaesthetized using Ketamin. Blood samples were collected from the heart via cardiac puncture and serum was separated by centrifugation at 3000 g for 15 min, and then stored at -20°C until used. Also, the kidney and the pancreas were also collected from the rats for analysis.

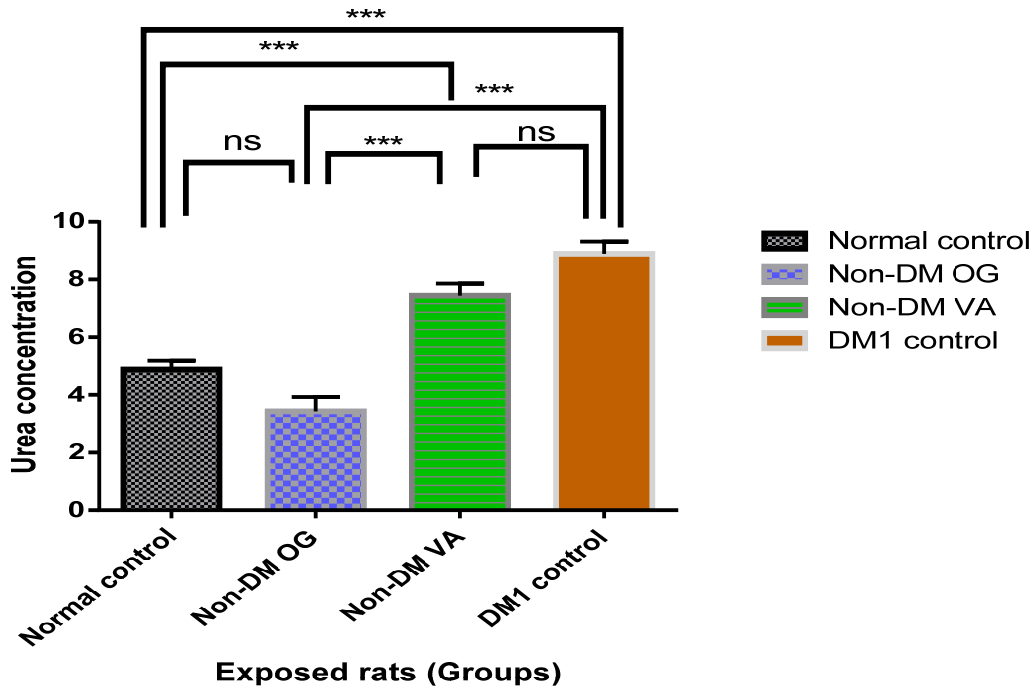
***I.STATISTICAL ANALYSIS*:** Statistical analysis was carried out using windows (SPSS version 15.0). Data were analyzed using one way ANOVA followed by post hoc test-least significant difference (LSD), while charts were

done using Microsoft excel. The data was expressed as mean ± SEM and values of $P < 0.05$ were considered significant.

III.RESULTS

EFFECT OF CRUDE EXTRACT OF OG AND VA IN NON DIABETIC RATS

The administration of nephrotoxic doses of STZ to rats resulted in development of oxidative stress damage in renal tissue. In this study, STZ induced nephrotoxicity showed a significant ($p<0.05$) increase in the serum urea concentration in the Group Labeled DM1 control (diabetic untreated group) rat when compared to the normal control group (Group I). Also, the administration of the extracts on the different non diabetic groups of rat (labeled non DM OG and non DM VA) showed that VA at $p<0.05$ significantly increased the level of urea when compared to the normal control while the administration of OG has no significant effect on urea level in a non diabetic state.



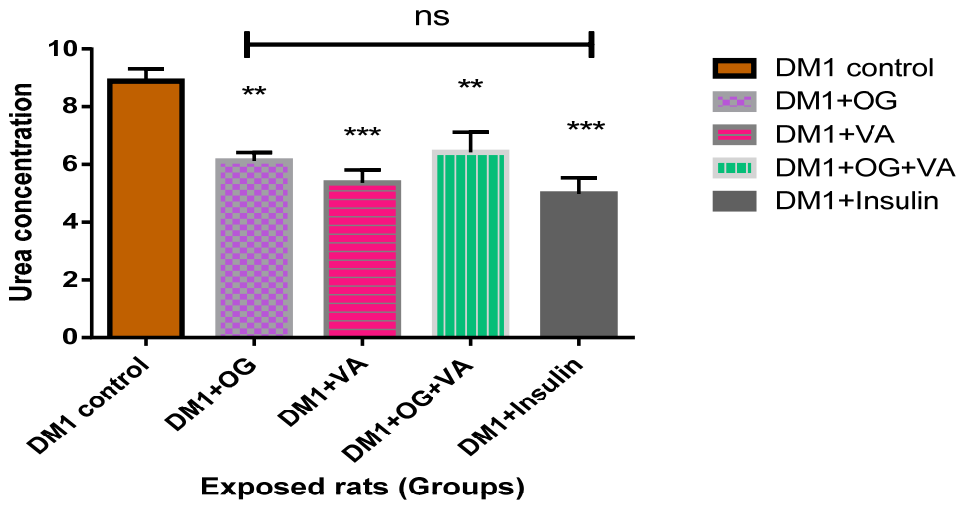
*** signifies P<0.05; ** signifies P<0.01; *** signifies P<0.001 while 'ns' signifies not significant.**

Fig 1: Effect of Crude Extract of OG and VA on Creatinine Level of Non Diabetic Rats.

EFFECT OF CRUDE EXTRACT OF OG AND VA IN THE UREA LEVEL OF DIABETIC RATS

The administration of nephrotoxic doses of STZ to rats resulted in development of oxidative stress damage in renal tissue (As can be seen in the DM1 control group). In this study, STZ induced nephrotoxicity showed a significant ($p<0.05$) increase in the serum urea concentration in the DM1 control Group (diabetic untreated group) rat when compared to the other diabetic treatment groups. Moreover, oral administration of OG, VA, combination of OG&VA and insulin into different experimental groups significantly ($p<0.05$) decreased the levels of urea in

the treatment groups when compared to the Group for DM1 control (diabetic untreated group). In summary, the levels of urea were significantly increased ($p<0.05$) in the diabetic untreated group of rats when compared to other treatment groups.

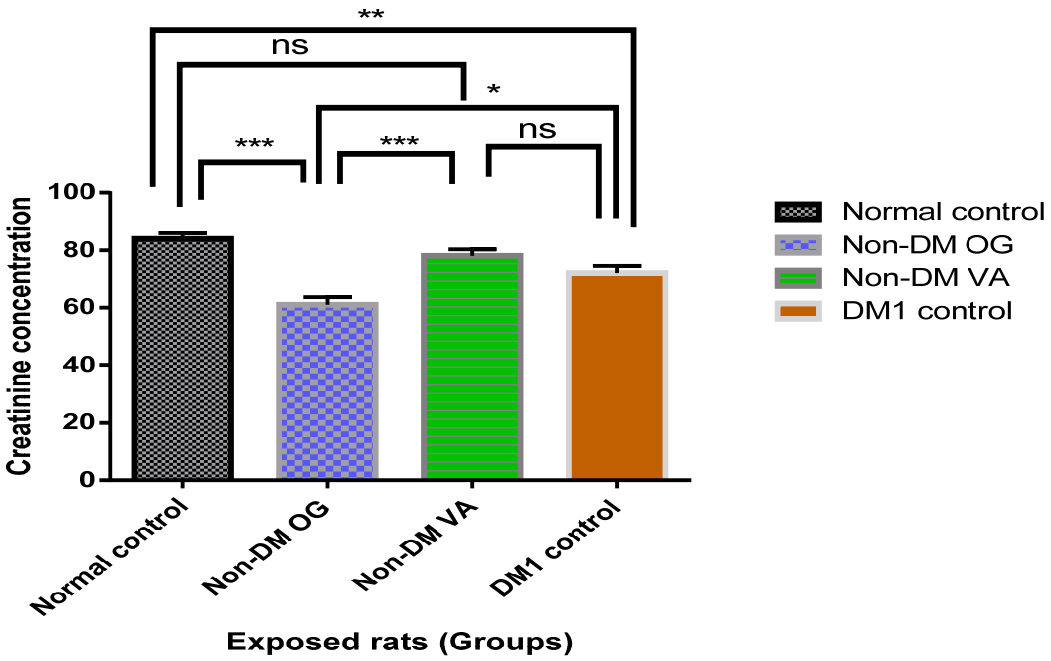


*** signifies P<0.05; ** signifies P<0.01; *** signifies P<0.001 while 'ns' signifies not significant.**

Fig 2: Effect of Crude Extract of OG and VA on Urea Level of Diabetic Rats

EFFECT OF CRUDE EXTRACT OF OG AND VA IN CREATININE LEVEL OF NON DIABETIC RATS

In this study, STZ induced nephrotoxicity showed a significant ($p<0.05$) decrease in the serum creatinine concentration in the group labeled DM1 control (diabetic untreated group) rat when compared to the normal control group. Also, the administration of the extracts on the different non diabetic groups of rat (labeled non DM OG and non DM VA) showed that the group for Non DM OG at $p<0.05$ significantly decreased the level of creatinine when compared to the group for normal control and diabetic untreated control group. The administration of VA into non diabetic rats has no significant effect on creatinine level.

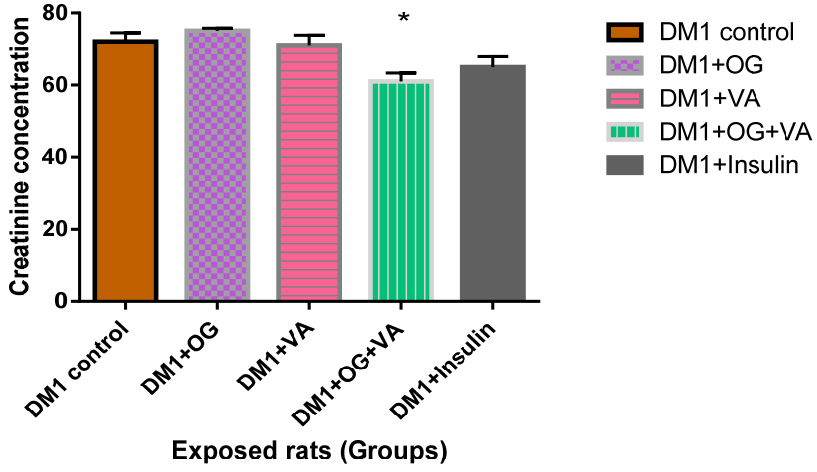


*** signifies P<0.05; ** signifies P<0.01; *** signifies P<0.001 while 'ns' signifies not significant.**

Fig 3: Effect of Crude Extract of OG and VA on Creatinine Level of non Diabetic Rats.

EFFECT OF CRUDE EXTRACT OF OG AND VA IN CREATININE LEVEL OF DIABETIC RATS

In this study, STZ induced nephrotoxicity showed a significant ($p<0.05$) decrease in the serum creatinine concentration in the group labeled DM1 control (diabetic untreated group) rat when compared to the normal control group. Also, the administration of the extracts on the different diabetic treatment groups of rat (labeled DM +OG, DM+VA, DM+ OG+VA and DM+ insulin) showed that the group for DM+ OG+VA at $p<0.05$ significantly decreased further the level of creatinine when compared to the group for diabetic control (DM1 control). The administration of VA, OG and Insulin into diabetic rats has no significant effect on creatinine level when compared with the DM1 control (diabetic control).



*** signifies P<0.05; ** signifies P<0.01; *** signifies P<0.001 while 'ns' signifies not significant.**

Fig 4: Effect of Crude Extract of OG and VA on Creatinine Level of Diabetic Rats

IV.DISCUSION

In response to World Health Organization, drawing attention to the use of herbal medicine as being of great importance to the health of the individual and communities, this study has confirm / justify the effectiveness, relative nontoxic nature of OG and VA in the management of urea and creatinine in diabetes.

There was no significant change in the serum urea level in the diabetic treated group compared to the normal control. But, there was a significant increase in the diabetic control. This is in consonant with reports by (1) in which combined treatment with *Ocimum gratissimum* and V*ernonia amygdalina* causes a significant decrease in serum urea in a diabetic state. In this study, the significant decrease in urea by treatment with *Vernonia amygdalina* alone and the group treated with combine herbs is consistent with an earlier work done (6). Urea level in diabetic control was significantly higher with respect to the normal control. This observation is expected. Gluconeogenesis is sustained by increased proteolysis which release free glycogenic amino acids circulated in plasma. These are deaminated in the liver with the consequences of increased urea in blood.

This study shows that the combined treatment of diabetes with leaf extracts of *Vernonia amygdalina* and *Ocimum gratissimum* shows a defense against damage due to the effect of STZ; supposedly decreasing the degree of proteolysis. This observation was also reported by (1). These studies further show that *Ocimum gratissimum* and *Vernonia amygdalina* has significant antidiabetic effect, as the administration of the leaf extract positively restores kidney function in a diabetic state. So, this study therefore suggests that leaf extract of *Vernonia amygdalina* and *Ocimum gratissimum* apart from their hypoglycemic action could protect the kidneys against impairments due to diabetes.

V.CONCLUSION

This study shows that the combination of the leaf extracts of *Vernonia amygdalina* and *Ocimum gratissimum* as well as the individual usage of the extracts, shows a defense against damage due to the effect of STZ; supposedly decreasing the degree of proteolysis. This observation was also reported by (1). It also has significant antidiabetic effect, as the administration of the leaf extract positively restores kidney function in a diabetic state by ameliorating the levels of Urea and Creatinine. So, this study therefore suggests that leaf extract of *Vernonia amygdalina* and *Ocimum gratissimum* apart from their hypoglycemic action could protect the kidneys against impairments due to diabetes.

REFERENCES

1 E. O. Agbai., U. C. Ofoego, F. N. Nwodo., and C. O. Nwanegwo: Synergistic Effect of Methanolic Extract of Vernonia amygdalina del and Ocimum gratissimum on Kidney Functions in Streptozotocin-Induced Diabetic Wistar Rats in Comparison with Insulin. *Journal of Medical and Applied Biosciences* 5(1):116 – 131. (2013)

2 E. K. Jang, J. H. Seo, S. P. Lee; Physiological activity and antioxidative effects of aged lack garlic extract. *Korean Society of Food Science and Technology*. 40:443-448 (2007).

3 J. V. Hunt, R. T. Dean, and S. P. Wolff; Hydroxyl radical production and autoxidative glycosylation.Glucose autoxidation as the cause of protein damage in the experimental glycation model of diabetes mellitus and ageing.*Biochemistry Journal.* 256:205-209. (1988).

4 L. Hounsom, R. Corder, J. Patel, and D. R. Tomlinson; Oxidative stress participate in the breakdown of neuronal phenotype in experimental diabetic neuropathy. *Diabetologia*. 44: 424-428 (2001).

5 M. Nattrass; In "Recent advances in diabetes". 2nd edition, Nattrass, M. (ed), Edinburgh, London, Melbourne New York. P.1. (1986).

6 A. Y. Onaolapo, O. J. Onaolapo, and O. S; Adewole, Ethanolic extract of *ocimum gratissimum* leaves rapidly lowers blood glucose levels in diabetic wistar rats. *Macedonian Journal of Medical Science* 4(4):351-357. (2011).

7 S. E. Nielsen, S. Takeshi, L. Tarnow, M. Lajer, K. J. Schljoedt, A. S. Strup, T. Baba, P. Hans-Henrik and P. Rossing; Tubular and glomerular injury in diabetes and the impact of ACE inhibition.*Diabetes care*. 32(9):1684-1688. (2009).

8 O. Fuentes., A. Arancibis., and H. Alarcon, Hypoglycemic activities *of Bauhinia Candican* in diabetic induced rabbits: *Fitoterapis* 6:527-532 (2004).

9 Sepici, I. Gurbuz, C. Cevik, and E. Yesilade, Hypoglycemic effects of myrtle oil in normal and alloxan diabetic rabbits.*Journal of Ethnopharmacol*ogy 93:311-318. (2004).

10 K. Yionen, C. Saloranta, C. kronberg, C. Leif, A. Antti, M. Suvi and M. Virtanean, Association of dietary fiber with glucose metabolism in non-diabetic relatives of subjects with type 2 diabetes. *Diabetes Care* 26:1979-1985. (2003).

11 V. F. Socorro, Madeira, J. A. Francisco, Matos, Joseph, Leal-Cardoso, and N. David, Relaxant effects of essential oil of O.G on isolated ileum of the guinea pig. *Journal of Ethnopharmacology*.81:(1)1-4(2002).

12 A. Lemhadri, M. Eddouks; T. Sulpice, and R. Burcelin; Anti glycemic and anti –obesity effects of *Capparisspinosa* and *Chamaemelumnobile* aqueous extracts in HFD mice. *Americn Journal of Pharmacology and Toxicol*ogy 2(3):106-110. (2007).

13 Anupriya, Sharma, N. Hirulkar and Payal, Ranka Effectrs of hyperglycemia on electrolytes imbalances. *International Journal of Pharmaceutical and biological Archives*. 2(1):526-533. (2011).

14 K. A. Griffin, H. Kramer, A. K. Bidani; Adverse renal consequence of obesity. American Journal of Physiology-Renal Physiology, 294:685-696 (2008):

15 D. Lorke;. A new approach to practical acute toxicity testing.*Archives of Toxicol*ogy 54:275-287. (1983)

AUTHOR(S) CONTRIBUTION

All authors have contributed one way or the other to the success of this paper and there is no conflict of interest.