Journal of Applied Pharmaceutical Science Vol. 3 (11), pp. 089-093, November, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.31116
ISSN 2231-3354

Anti-diabetic effects of ethyl acetate and n-butanol fractions of *Acacia nilotica* leaves methanolic extract on alloxan-induced diabetic wistar rats

[1]Tanko Y., [1]Muhammad A., [1]Elaigwu, J [1]Mohammed K.A., [1]Jimoh, A., [1]Sada N.M., [2]M.K Dallatu, [3]Yerima M and [1]Mohammed A

[1]Department of Human Physiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. [2]Department of Chemical Pathology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto State, Nigeria. [3]Department of Pharmacology and Therapeutic, Ahmadu Bello University, Zaria,Kaduna State, Nigeria.

ARTICLE INFO

Article history:
Received on: 28/07/2013
Revised on: 16/09/2013
Accepted on: 11/11/2013
Available online: 29/11/2013

Key words:
Anti-diabetic, Alloxan, Ethylacetate, n-butanol *Acacia nilotica*.

ABSTRACT

Evaluation of hypoglycaemic activity of ethyl acetate and n-butanol fractions of *Acacia nilotica* on alloxan induced diabetic Wistar rats has been investigated. Two doses of the ethylacetate fraction 50 and 100 mg/kg was administered. As regard to 50mg/kg caused a significant ($P<0.05$) reduction in the blood glucose levels when compared with control at 3,5,7,9 and 12 days of treatment with percentage glycaemia change of 49.1,54.8,60.5, 58.8 and 69.7 respectively. However, the dose of 100mg/kg ,there was a significant decrease ($p<0.05$) at 3 5 7, 9 and 12 days treatment when compared to control untreated with percentage glycaemia change of 50.1,56.8, 52.8, 69.9 and 59.6 . Also two doses of n-butanol, 100 and 200 mg/kg fraction was administered to the diabetic rats. The dose of 100 mg/kg, there was a significant decrease ($p<0.05$) after 7 and 12 days of treatment when compared to untreated control. As regard the dose of 200 mg/kg, there was a significant decrease ($p<0.05$) at 3, 5 ,7,9 and 12 days of treatment when compared to control untreated with percentage glycaemic change of 20.7,35.3,52.3, 44.2 and 40.9 respectively. The preliminary phytochemical screening revealed the presences of saponin, flavonoid, tannin and alkaloid. The median lethal dose (LD50) in mice was calculated to be 471.2 mg/kg bodyweight. This result suggests that the Ethylacetate and n-butanol fractions of leaves methanolic extract of *Acacia nilotica* possess antidiabetic effects on alloxan - induced diabetic Wistar rats.

INTRODUCTION

Diabetes mellitus is recognized by chronic hyperglycaemia and is associated with long term damage, dysfunction and failure of various body organs by involvement of micro and macro-vasculature (Hink *et al.,* 2001). The micro-vascular involvement mostly effects retina, renal glomeruli and peripheral nerves, while macro-vascular involvement results in dyslipidemia, formation of reactive oxygen species (ROS), advance glycation end product (AGEs), platelet hyper-reactivity and endothelial dysfunction (Cosentino *et al.,* 2003). Disturbance in endothelial function and coagulation pathway may lead to platelet activation, adhesion and aggregation. A large number of anti-diabetic medicines are available in the pharmaceutical market for diabetes and its related complications; however, currently no effective therapy is available to cure the disease. WHO Expert Committee on Diabetes has recommended investigating traditional herbal medicines (Sundaram and Mitra, 2007), and in this regard more than 400 medicinal plant species have been compiled. These herbal products are gaining popularity in developing and developed countries due to their lesser side effects and low cost (Sundaram and Mitra, 2007).

This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger) (Shoback *et al.,* 2011). When hyperglycemia is present, its severity may change in time, depending on the underlying process. Diagnosing and appropriate management approach to any disorder of glucose intolerance necessitates strong understanding of the mechanism involved in the disease process (Rebarber *et al.,* 2007). Hyperglycemia occurs when the blood glucose level becomes

* *Corresponding Author*
Tanko Y. Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria. Email- yusuftanko@yahoo.com

higher than 11.1 mmol/l (200 mg/dl), but symptoms may not start to become noticeable until even higher values such as 250–300 mg/dl or 15–20 mmol/l. In a normal state, the body can carefully balance the amount of insulin and glucose and thus regulate blood glucose levels. Normal blood glucose levels are: fasting (before eating) 70-99mg/dl and 140mg/dl or less two hours after a meal (Sharon, 2013).

A subject with a consistent range between 100 and 126 (American Diabetes Association guidelines) is considered hyperglycemic, while above 126mg/dl or 7mmol/l is generally held to have diabetes. Chronic levels exceeding 7 mmol/l (125 mg/dl) can produce organ damage (Sharon, 2013).

Acacia, also known as a thorn tree, whistling thorn or wattle, is a genus of shrubs and trees belonging to the subfamily Mimosoideae of the family Fabaceae, first described in Africa by the Swedish botanist Carl Linnaeus in 1773. Many non-Australian species tend to be thorny, whereas the majority of Australian *Acacias* are not. The generic name was derived from *akakia*, this name was given by early Greek botanist-physician Pedanius Dioscorides to the medicinal tree *A. nilotica* in his book *Materia Medica*. This name derives from the Greek word for its characteristic thorns (*akis*, thorn) (Quattrocchi, 2000).

The bark of *Acacia nilotica* has a history of use as an anti-diarrhea folk medicine and dysentery treatments in Africa and India. A decoction of the bark taken orally acts as an astringent, easing the symptoms of diarrhea and accompanying intestinal pains (Bargar, 2013). The bark of *Acacia nilotica* (booni) tree is useful in the treatment of eczema. In India, about 25 grammes of the bark of booni tree and the mango bark are usually boiled in about 1 liter of water and the fapours allowed to forment the part of the body affected by eczema. After the formentation, powder and ointment made from the plant infusion will then be applied to the affected part (Rahaman, 2010).

The aim of this study is to determine the effects of N-butanol and Ethylacetate fractions of *Acacia nilotica* methanol leaves extract on blood glucose level in alloxan-induced diabetic wistar rats.

MATERIALS AND METHODS

Plant material

The leaves of *Acacia nilotica* was collected from Ahmadu Bello University, Zaria, Nigeria. The plant material was identified and authenticated by a taxonomist, in the herbarium section in the Department of Biological Science Ahmadu Bello University, Zaria, Nigeria, where a voucher specimen (No. 698) has been deposited for future reference.

Extraction of Plant Material

The leaves extract of *Acacia nilotica* were air dried under the shade and grinded into free powder using morter and pestle. 200 grams of the powdered material was macerated in 40% distilled water and 60% methanol at room temperature for 24 hours. It was then filtered using a filter paper (whatman size 1). The filtrate was then partitioned with ethylacetate to get ethylacetate fraction which was evaporated to dryness in an oven at 37 °C. A greenish-brown residue weighing 8.5 grams (1.7%w/w) was obtained and kept in a sealed container at 4°C in a refrigerator until use.

Another 200 grams of the powdered material was macerated in 40% distilled water and 60% methanol at room temperature for 24 hours. It was then filtered using filter paper (Whatman size 1). The filtrate was then partitioned with n-butanol to get the n-butanol fraction which was evaporated to dryness in an oven at 37°C. A brownish residue weighing 6.5 gram (1.3 % w/w) was obtained and kept in a sealed container at 4°C in a refrigerator until use.

Chemical used

Alloxan monohydrate was purchased from Sigma chemicals (St Louis U.S.A). The Biphasic Isophane Insulin AS Mixtard 30 HM Pen fill (Novo Nordisk AIS 2880 Bagsvaerd, Denmark. NAFDAC Reg no 04-1601). Accu-chek glucometer (Lifescan, Inc 2010 Milpitas, CA 95035, U.S.A) was use for the determination of blood glucose levels.

Preliminary phytochemical screening

The fractions were subjected to preliminary phytochemical screening test for the presences of secondary metabolites according to the method described by Trease and Evan (1983).

Acute toxicity studies (LD_{50})

The LD_{50} determination for each of the fractions was conducted separately using modified method of Lorke (1983). For each of the fractions, the evaluation was done in two phases. In phase one, three groups of three rats each, were treated with 10, 100 and 1000 mg fraction /kg body weight intraperitoneally (ip) respectively.

A fourth group served as control. The rats were observed for clinical signs and symptoms of toxicity within 24 hours. Based on the results of phase one for the Ethylacetate fraction, fifteen fresh rats with three per group were each treated with 140, 225,370 and 600 mg fraction/kg (ip*)* respectively. A fifth group served as control.

Clinical signs and symptoms of toxic effects and mortality were then observed for seven days. Also based on the results of phase one for the n-butanol extract, fifteen fresh rats with three per group were each treated with 200, 400,600 and 800 mg fraction/kg (ip*)* respectively. A fifth group served as control. Clinical signs and symptoms of toxic effects and mortality were then observed for 72 hours.

The LD_{50} were then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose i.e. the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second phase.

Experimental Animals

A total of 30 Wistar rats of both sexes between the ages of 10 to 12 weeks old and weighed between 120-150grams were used for antidiabetic . The animals were housed in the Animal House, Department of Human Physiology, ABU, Zaria, Nigeria The animals were randomized into experimental and control groups and were kept in polypropylene cages. The animals were fed on standard feeds (Vital feeds, Jos Nigeria) and allowed access to water *ad libitum*. The "Principle of laboratory animal care " (NIH publication No 85- 23)'' guideline and procedures were followed in this study (NIH publication reserved 1985).

Induction of experimental diabetes mellitus

The animals were fasted for 16–18 hours with free access to water prior to the induction of diabetes. Induction of diabetes was carried out by single intraperitoneal injection of Alloxan monohydrate (Sigma St Louis, M.O., USA) dissolved in 0.9%$^{v/v}$ cold normal saline solution at a dose of 150 mg/kg body weight (Katsumat *et al.*, 1999).

Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20 % glucose solution intraperitoneally after 6h. The rats were then kept for the next 24h on 5 % glucose solution bottles in their cages to prevent hypoglycemia (Dhandapani *et al.,* 2002). The diabetes was assessed in alloxan-induced rats by determining the blood glucose concentration 72 hours after injection of alloxan. The rats with blood glucose level above 200mg/dl were then selected for the study.

Experimental design

After the induction of diabetes, the alloxan induced diabetic wistar rats were randomly assigned into the following groupings;

- Group 1 (n = 5) ---------- were treated with distilled water
- Group 2 (n = 5) ----------were treated with insulin (6 i.u/kg body weight i.p)
- Group 3 (n = 5) ---------- were treated with 50mg/kg of ethyl acetate fraction of *Acacia nilotica* i.p
- Group 4 (n = 5) ---------- were treated with 100mg/kg of ethyl acetate fraction of *Acacia niloticca* i.p
- Group 5 (n = 5) ---------- were treated with 100mg/kg of n-butanol fraction of *Acacia nilotica* i.p
- Group 6 (n = 5) ---------- were treated with 200mg/kg of n-butanol fraction of *Acacia nilotica* i.p

Determination of blood glucose levels

Fasting blood glucose levels were determined by using the glucose oxidase method (Trinder, 1969) with Accu-chek glucometer and The blood samples were collected at an interval of 0, 1, 3, 5, 7, 9 and 12 days. The blood results were reported as mg/dl (Rheney and Kirk, 2000).

Statistical Analysis

Blood glucose levels were expressed in mg/dl as mean ± SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group by Dunnett's method. Values of $p< 0.05$ or less were taken as significant (Duncan *et al.,* 1977).

RESULTS

Phytochemical screening

Preliminary phytochemical screening of the two fractions of *Acacia nilotica* fractions revealed the presence of saponin, flavonoid, tannin and alkaloid.

Acute Toxicity Studies.

The signs of toxicity were first noticed after 4-5 hours of extracts administration. There were decreased locomotor activity and sensitivity to touch and pain. Also there was decreased feed intake, tachypnoea and prostration after 12-18 hours of fraction administration. Early deaths were recorded after 48 hours after fractions administration. The LD_{50} were then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose i.e. the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second phase.

For the n-butanol fraction, there was 0% mortality at 1000mg/Kg and 33.3% mortality was the next highest lethal dose at 1600mg/Kg. The LD_{50} of the n-butanol fraction was thus; $\sqrt{600 \times 800} = 774.5$ mg/Kg.

For the Ethylacetate fraction, there was 0% mortality at 370mg/Kg and 33.3% mortality was the next highest lethal dose at 600mg/Kg. The LD_{50} of the ethyl acetate fraction was thus; The LD_{50} was thus; $\sqrt{370 \times 600} = 471.2$ mg/Kg.

Effect of daily doses of ethyl acetate and n-butanol fractions of *Acacia nilotica* extract on alloxan induced blood glucose levels of diabetic wister rats.

There was a significant decrease ($p< 0.05$) in the blood glucose levels of the diabetic groups treated with ethylacetate fraction after the three days of treatment when compared to control untreated as shown in table 1. However, there was a significant decrease ($p< 0.05$) in the blood glucose levels of the diabetic groups treated with n-butanol fraction at the three days of treatment when compared to control untreated as shown in table 1. Also, there was a significant decrease ($p< 0.05$) in the blood glucose levels of the diabetic groups treated with standard biphasic isophane insulin after the three days of treatment when compared to control untreated as shown in table 1.

Table. 1: Effects of ethylacetate and n-butanol fractions of *Acacia nilotica* leaf extract on Blood glucose levels of alloxan induced diabetic wistar rats .

Groups	Blood glucose level(mg/dl)						
	0	1 day	3 days	5 days	7 days	9 days	12 days
Control (distilled water)	483.5 ± 10.7	445.5 ± 60.89	578.0 ± 13.61	478.7 ± 33.3	483.7 ± 40.0	425.2 ± 79.5	446.0 ± 20.6
Insulin treated (1mg/kg)	490.75 ±7.05	305.7 ± 89.9^{ns} (37.7%)	307.7 ± 82.24^{a} (37.3%)	119.2 ± 59.62^{a} (75.7%)	220.2 ± 31.2^{a} (55.1%)	157.6 ± 78.8^{a} (67.9%)	329.7 ± 99.4^{ns} (32.8%)
ethylacetate (50mg/kg)	486.2 ± 22.3	335.2 ± 27.1^{ns} (31.1%)	247.7 ± 48.1^{a} (49.1%)	219.7 ± 44.16^{a} (54.8%)	192.0 ± 21.7^{a} (60.5%)	200.2 ± 11.4^{a} (58.8%)	147.5 ± 27.8^{a} (69.7%)
ethylacetate (100mg/kg)	481.0 ± 68.4	318.2 ± 27.3^{ns} (33.9%)	240.2 ± 29.7^{a} (50.1%)	207.7 ± 28.37^{a} (56.8%)	227.0 ± 12.9^{a} (52.8%)	144.7 ± 26.4^{a} (69.9%)	194.0 ± 12.5^{a} (59.6%)
N-butanol (100mg/kg)	486.2 ± 22.3	405.7 ± 16.27^{ns} (16.6%)	315.75 ± 60.7^{ns} (35.0%)	349.2 ± 30.5^{ns} (28.2%)	308.2 ± 35.0^{a} (36.6%)	388.0 ± 23.3^{ns} (20.2%)	272.5 ± 49.6^{a} (43.9%)
N-butanol (200mg/kg)	481.0 ± 68.4	390.2 ± 9.35^{ns} (18.9%)	381.0 ± 45.43^{a} (20.7%)	311.0 ± 31.5^{a} (35.3%)	229.5 ± 32.2^{a} (52.3%)	261.0±41.32^{a} (44.15%)	284.5±51.5^{a} (40.9%)

Values are expressed as mean ± SEM; n = 5.
Value considered statistically when compared with control group: a = $p<0.05$ significant and ns = not significant.
% Glycaemic change = Glucose concentration (1,3,5,7,9 and 12)– fasting blood glucose x 100 / Fasting blood glucose
Parenthesis index ( ) means percentage glycaemic change.

DISCUSSION

A number of anti-diabetic medicines are available in the pharmaceutical market to reduce the ill effect of diabetes and its related complication, but no satisfactory effective therapy is available to cure the disease (Kim *et al.,* 2006). More than 400 medicinal plant species with anti-diabetic effect are compiled. These herbal products are gaining popularity in developing and developed countries due to their lesser side effect and low cost (Modak *et al.*, 2007). Alloxan monohydrate is one of the chemical agents used to induce diabetes mellitus. It induces diabetes by partial destruction of β-cells of islets of langerhan's. This results in decreased insulin levels and hyperglycemia leading to type I diabetes mellitus. Different doses of the two fractions of ethyl acetate and n-butanol were administered to the alloxan induced diabetic Wistar rats. As regard to the ethylacetate fraction 50 mg/kg and 100 mg/kg were administered. The dose of 50 mg/kg of the ethyl acetate fraction when compared to the control there was a significant decrease $p<0.05$ in the blood glucose levels with the highest percentage glycaemic decrease of 69.7% after 12 days of administration of the ethylacetate fraction. Also in relation to the dose of 100mg/kg of the ethylacetate fraction when compared to control there was a significant decrease in the blood glucose levels after 3,5,7, 9 and 12 days of administrations of the ethylacetate fraction with percentage glycaemic decrease of 50.1,56.9,52.8,69.9 and 59.6% respectively.

As regard to the n-butanol fraction, two doses 100 and 200 mg/kg were administered. In relation to the dose of 100 mg/kg there was a significant decrease $p<0.05$ in the blood glucose levels after 7 and 12 days of treatment with the highest percentage glycaemic decrease of 43.9% after 12 days when compared to control. However, the dose of 200 mg/kg there was a significant $p<0.05$ in the blood glucose levels when compared to control after 3,5,7,9 and 12 days with percentage glycemic decrease of 20.7, 35.5, 52.3, 44.2, and 40.9% respectively. In relation to the standard positive control biphasic insulin 6.i.u/kg when compared to control there was a significant decrease ($P<0.05$) after 3, 5, 7 and 9 days of administration with percentage glycaemic decrease of 37.3, 75.7, 55.1, 67.9% respectively. Medicinal plant extracts have been valuable anti-diabetic agents and may involve one or more active components responsible for blood glucose reduction (Marles and Farnsworth, 1995; Grover *et al.,* 2002). Flavonoids of different plant origin showed a promising anti-diabetic activity, as demonstrated in diabetic animal models (Zarzuelo *et al.,* 1996; Nojima *et al.,* 1998; Kim *et al.,* 2004).

The preliminary phytochemical screening of the fractions of *Acacia nilotica* revealed the presences of saponin, flavonoid, tannin and alkaloid. Flavonoid and terpenes isolated from the other antidiabetic medicinal plants has been found to stimulate secretion or possess an insulin like-effect (Marles and Farnsworth 1995). Effect of the flavonoids quercetin and ferulic acid on pancreatic β-cells leading to their proliferation and secretion of more insulin have been proposed by Mahesh and Menon (2004) and Sri-Balasubashini, *et al.,* (2004) as the mechanism by which they reduced hyperglycaemia caused by alloxan induced diabetic rats. The flavonoids present in *Acacia nilotica* may also be acting similarly thereby decreasing the high blood glucose levels of alloxan induced -diabetic rats. In conclusion, the experiment evidence obtained in the present laboratory animal study indicate that the ethylacetate and n-butanol fractions of *Acacia nilotica* possess anti-diabetic properties which suggest the presence of biologically active components which may be worth further investigation and elucidation.

REFERENCES

Barger R., Medicinal uses of *Acacia nilotica*. *WiseGeek,* 2003.

Cosentino, F., Eto, M., Paolis, P. D., Loo, B., Bachschmid, M and Ullrich, V. High glucose causes upregulation of cyclooxygenase 2 and alters prostanoid profile in human endothelial cells, Role of protein kinase C and reactive oxygen species. *Circulation*; 2003 107:1017–23.

Dhandapani, S., Ramasamy, S.V., Rajagopal, S. and Namasivayam, N. Hypolipidemic effect of *Cuminum cyminum* L. on alloxan-induced diabetic rats. *Pharmacol. Res,* 2002; 46 (3): 251-255.

Duncan, R.C., Knapp, R.G and Miller, M.C. . Test of hypothesis in population Means. In: *Introductory Biostatistics for the health sciences*. John Wiley and Sons Inc.

NY: 1997,71-96.

Hink, U., Li, H., Mollnau, H., Oelze,C M., Matheis, E and Hartmann, M. Mechanisms underlying endothelial dysfunction in diabetes mellitus.*Circulation*; 2005;88:14–22.

Katsumata, K.Y., Katsumata., T.O., Katsumata, K.. Potentiating effects of combined usage of three sulfonylurea drugs on the occurrence of alloxan-induced diabetes in rats. *Horm Metab Res*,1999; 25: 125-126.

Kako, M., Miura, T., Nishiyama, Y., Ichimaru, M., Mariyasu, M and Kato, A. Hypoglycemic activity of some Triterpenoid Glycosides. *J.Nat Prod*, 1997;60:604-605.

Kim, H.Y., Moon, B.H., Lee, H.J and Choi, D.H. Flavonoids glycosides from the leaves of *Eucommia ulmoides* with gycation inhibitory activity.*J Ethnopharmacol*, 2004;93:227-230.

Kim J.S., Ju B.J., Choi C.W and Kim S.C. Hypoglycemic and hyperlipidemic effect of four Korean medical plants in alloxan induced diabetic rats. *American Journal of Biochemical and Biotechnology*, 2006; 2: 154-160

Lork D .A New Approach to Practical Acute Toxicity Testing Archieves of Toxicology: 1983; 275-287.

Marles, R., Farnsworth., N.. Antidiabetic plants and their active constituents. *Phytomedicine*, 1995; 2, 137–165.

Modak M., Dixit P., Londhe J., Ghaskadbi S., Paul A and Devasagayam J. Indian herbs and herbal drugs used for treatment of diabetes. *Journal of Clinical Biochemical Nutrition*, 2007; 40: 163-173.

Nojima, H., Kimura, I and Chen, F. Anti-hyperglycemic effects of N-containing sugars from *Xanthocercis zambesiaca*, *Morus bombycis*, *Aglaonema trubii* and *Castanosperrmin australe* in STZ-diabetic mice.*J Nat Prod*, 1998; 61:397-400.

Mahesh, T. and Menon, P.V. Quercetin alleviates oxidative stress in streptozotocin induced diabetic rats. *Phytotherapy Research*, 2004; 18: 123-127.

Quattrocehi U. 2000. CRC world dictionary plants name. *CRC press.* P.6 Rahaman O. 2010. A review of uses of *Acacia nilotica* (booni) in alternative medicine. *Searchwarp.com.* Rheney, C.C and Kirk, K.K. Perfomance of three blood glucose meters.*Ann Pharmacother*, 2000; 34(3):317-21. Reher, G., Slijepcevic, M and Krans, L. Hypoglycemic activity of triterpenes and tannins from *Sarcopoterium spinosum* and two *Sanguisorba* Species. *Planta Med*,1991; 57: A57-A58.

Sharon H. What is diabetes? *Diabetes Care,2013*

Shoback, edited by David G. Gardner. Dolores. 2011. Greenspan's basic & clinical endocrinology (9th ed.). New York: McGraw-Hill Medical.

Sri Balasubashini, M., Rukkumani, R., Viswanathan, P. and Menon, P.V. Ferulic acid alleviates lipid peroxidation in diabetic rats. *Phytotherapy Research*, 2004; 18: 310-314.

Trease, G.E and Evans, M.C. Textbook of pharmacognosy, 12th ed Balliere Tindall, London, 1983; pp.343-383.

Trinder, P. Determination of Glucose in blood using glucose-oxidase with an alternative oxygen acceptor. *Ann Clin Biochem*, 1969; 6: 24-27.

Zarzuelo, A., Jimenez, I and Gamez, M.J. Effects of luteolin 5-O-beta-rutenoside in STZ-induced diabetic rats. *Life Sci*, 1996; 58:2311-2316.

How to cite this article:

Tanko Y., Muhammad A., Elaigwu, J Mohammed K.A., Jimoh, A., Sada,N.M., M.K Dallatu, Yerima M and Mohammed A., Anti-diabetic Effects of Ethyl acetate and n-Butanol Fractions of *Acacia nilotica* methanol Extract on Alloxan - Induced Diabetic Wistar rats., J App Pharm Sci, 2013; 3 (11): 089-093.