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Effects of Ethanolic Extract of Vinegar Leaf (*Andrographis paniculata*) on Blood Sugar Level of Alloxan-Induced Diabetic Albino Rat (*Rattus norvegicus*)

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ABSTRACT

This study aimed at examining antidiabetic efficacy of ethanolic extract of vinegar leaf on blood sugar level of alloxan-induced diabetic albino rats. 25 male rats of 137-224g body weight were grouped into five groups of five animals per group. Group 1 was used as non-diabetic control, group 2, 3, 4 and 5 were induced with diabetes by administering 150mg/kg of alloxan intraperitoneally. Group 2 served as diabetic control. After 2 days of developing diabetes by group 3, 4, and 5, they were treated with vinegar ethanolic extract at concentrations of 200mg/kg, 400mg/kg, and 800mg/kg respectively. Blood glucose levels were tested at three days intervals. Result indicated that the alloxan-induced diabetic rats expressed hyper-glycemia in group 3, 4 and 5 as against the control of non-diabetic sugar level. When the extract was administered to group 3, 4, and 5 there was a significant reduction in the glucose levels ($p<0.05$) compared with control group 2 after 12 days. The result also showed that there was a corresponding increase in weight as the sugar level increases ($p<0.05$) and a decrease in weight as the sugar level decreases. Vinegar leaf has positive hypoglycemic effect and should be recommended for diabetic patients.

Keywords: diabetic, vinegar leaf, alloxan, anti-diabetic, glucose

INTRODUCTION

Diabetes sets in when there is a problem with the hormone insulin which often results in abnormally high level of glucose in the blood as is generally known. Insulin resistance can also induce an imbalance in glucose metabolism that generates chronic hyperglycemia, which in turn triggers oxidative stress and cause an inflammatory response that leads to cell damage [1]. Hyperglycemia up to fourfold increase in neuronal glucose, with intracellular glucose metabolism leads to neuronal damage [2]. One of the most commonly used chemicals to induce diabetes especially for experimental purpose is alloxan [3]. This drug induces diabetes by intracellular production of reactive oxygen species (ROS) formed in a cyclic reaction involving alloxan and its reduced product called di- uric acid [4]. Alloxan has been widely used to induce experimental DM in animals such as rabbits, mice and dogs with different grades of disease severity by varying the dose of Alloxan used [5], causing "Alloxan induced Diabetes", a form of insulin-dependent diabetes mellitus similar to type 1 diabetes. In Africa and the world all over, different plants have been used to treat different health issues which include nervous [6], reproductive [7], [8], [9], anti-nutritional [10], [11] complications. *Andrographis paniculata* is a herbaceous plant, otherwise known as "King of Bitters", in the family of Acanthaceae [12]. A. *paniculata* grows vertically to a height of 30 – 110 cm with glabrous leaves and white flowers with rose-purple spots on the petals. It grows abundantly in southeastern Asia – India, Pakistan and Indonesia but is cultivated extensively in China, Thailand, the East and West Indies, and Mauritius [13]. Since *Andrographis paniculata* has medicinal properties, it is grown quite easily. It has been traditionally used as a remedy against hypertension, inflammation [14], cobra bite [15] and Antidiabetics [16]. Vinegar or kalmegh (*Andrographis paniculata*) is a native herb of India and China and has been used for years in herbal medicine [17]. *A. paniculata* contains therapeutically active principles like andrographalide, neoandrographalide, deoxyandrographalide and didehydroandrographalide [4]. *A. paniculata* has a broad range of pharmacological effects including anticancer, antihepatitis, anti-inflammatory, antioxidant, antimicrobial, antimalarial and immunomodulatory effects [18]. The phytochemical components of vinegar leaf include organic acids (formic, lactic, malic, citric, succinic, and tartaric), amino acids, peptides, vitamins, mineral salts, and polyphenolic compounds (e.g., catechin, caffeic, ferulic acid) [19]. This work tends to specifically examine the antibiabetic

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activity of *Andrographis paniculata* in alloxan – induce diabetes in male rats as to further contribute to the antidiabetic potential of *A paniculata* in male rats literatures.

MATERIALS AND METHODS

Experimental Animals

All procedure adopted in the experiment in regard to animal handling complied with the International Guideline for the care and use of Laboratory Animals (IGCULA) as stated by the Ethical Committee, Faculty of Science University of Port Harcourt, River State. Twenty (25) Wistar albino rats (all male) weighing between 124-288g body weights, were obtained from the Animal House of the Faculty of Pharmacy, University of Port Harcourt, Nigeria. The rats were divided into five groups (5) of five (5) animals based on closely related weights in each cage and were allowed access to water and standard pellet food ad libitum. Prior to the administration of the extract, the rats were fasted for 12 hours and glucose levels were checked using the glucose test kit. The rats were housed in a facility with 12-12 h light-dark cycle that is maintained at 25°C.

Collection of plant material Fresh leaves of A. paniculata were obtained from University Botanical Garden University of Port Harcourt. The plant was identified at Botany Unit, of the same Institution. A voucher specimen was also deposited in the Herbarium of the same Institution for reference purpose.

Preparation of Plant Extract.

The leaves of *Andrographis paniculata* were collected and washed completely with distilled water to remove sand and contaminants. The leaves were air-dried for three weeks at room temperature and ground into powder using Christy Milling Machine (Christy and Norris Ltd., Process Engineers, Chelmsford, England blender. Powdered sample of the plant (252g) was obtained and soaked in 2 liters of ethanol for 48 hours with occasional shaking. The resulting mixture was filtered using Whatman No.1 filter paper. The filtrate was evaporated to dryness using a water bath system. From the extract, 200mg/kg, 400mg/kg, and 800mg/kg concentrations were prepared using distilled water and then stored in well stoppered containers and kept in a refrigerator at 4°C until used.

Phytochemicals

Phytochemical checking of *Andrographis paniculata* extracts was done according to established principle of [20] for the presence of alkaloids, flavonoids, saponins, tannins, cardiac glycosides, cyanogenic glycosides and anthracene glycosides.

Procurement of the alloxan. The alloxan was procured from a local chemical store in Choba, Rivers State

Diabetic inducement using alloxan. The glucose levels of 4 rats which act as the positive control were measured using a glucometer. The remaining 16 rats were intraperitoneally administered 150mg/kg dose of alloxan to induced diabetes according to the method of [21]. A diabetes Development period of 2 days (48 hours) was allowed after which the blood glucose levels were analyzed to determine the presence of diabetes according to the method of glucose-enzyme kit analysis [22]. During blood sample analysis, animals were held by their heads and the tails were rubbed with alcohol to prevent infection and blood samples were collected from the tail of the rats. Group 2 was taken as the negative control while group 3, 4 and 5 with the alloxan-induced diabetic rats were administered *Andrographis paniculata* ethanolic extract.

Extract Administration The rats were divided into five (5) groups and each group contains five (5) rats. Group 1 was the non-diabetic control (positive control) and was given normal saline of 1.0 ml/kg body weigh while Group 2 was the diabetic control group (negative control). Groups 3, 4 and 5 were treated with 200mg/kg, 400mg/kg, and 800mg/kg of the extract respectively. The animals were given the extracts once every morning orally using a cannula before meals. After 3 days of *A. paniculata* ethanolic aqueous administration, blood samples were collected from the tail vein (non-surgical) and tested using the glucose-enzyme test kit method by [22]. The animals were given the extract for 12 days and blood sugar levels were checked every 3 days.

Determination of weight The weights of the rats were obtained using a laboratory digital scale before alloxan- inducement and after alloxan inducement 2 days interval and after A. *paniculata* treatment. This was to ascertain weight difference that would be due to increase in glucose (hyperglycemia) before treatment and decrease in glucose (hypoglycemia) after treatment.

Statistical analysis The results were statistically checked using ANOVA on the computer based excel package. The results were expressed as Mean ± S.E.M.

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RESULTS

The results of glucose levels before and after the diabetic alloxan-inducement and the administration of aqueous ethanolic extract of *A. paniculata* are presented below in table 1. Table 2 gives the results of the weight of the rats before and after diabetic alloxan-inducement and during administration of aqueous ethanolic extract of *A. paniculata*. Graphical representation of the effects of are expressed in fig 1.0. where groups 2,3,4 and 5 had their glucose levels spiked up at at two days after Alloxan administration compared to the group 1 which did not have any appreciable increase in glucose level over the period

Glucose level on different days, before and after treatment.

In group 2 there was a significant increase ($p<0.05$) in glucose level after inducement with alloxan extract compared with group 1 positive control. In group 3 there was a significant increase ($p<0.05$) in glucose level after inducement with alloxan extract and significant decrease ($p<0.05$) in glucose during and after 200mg/kg A.*paniculata* administration compared with negative control group 2. In group 4 there was a significant increase ($p<0.05$) in glucose level after inducement with alloxan extract and significant decrease ($p<0.05$) in glucose during and after 400mg/kg A*. paniculata* administration compared with negative control group 2. In group 5 there was a significant increase ($p<0.05$) in glucose level after inducement alloxan extract and significant decrease ($p<0.05$) in glucose during and after 800mg/kg A*. paniculata* administration compared with negative control group 2. It was observed that groups 3, 4 and 5 had 96.18% 99.33%, 82.08%, of the glucose reduction by *Andrographis paniculata* ethanoic extract in within the first six days of the treatment (Fig 2). At the 12th day, groups 3, 4, and 5 induced with 200mg/kg, 400mg/kg and 800mg/kg of *Andrographis paniculata* have had a reduction of glucose level to 86.13%, 84.60% and 84.81 % of the originally hyper glycemic value after inducing with alloxan (Fig 2).

Table 1: showing glucose level on different days, before and after treatment (mmol/l)

Groups	**A.*paniculata*** doses (mg/kg)	Initial glucose level	Glucose level after inducing (%)	Glucose levels in an interval of 3 days during treatment				
				Day3 (%) []	**Day 6**(%) []	**Day 9**(%) []	**Day 12**(%) []	
Group1		3.65±0.11	4.01±0.24 (9.59)	4.525±0.25 (23.83)	4.55±0.39 (24.66)	4.1±0.23 (12.33)	4.625±0.17 (26.71)	
Group2		4.175±0.26	31.775±1.25 (661.07)	31.675±1.98 (658.68)	30.5±0.64 (630.54)	31.25±0.47(648.50)	30±1.22 (618.56)	
Group3	**200mg/kg**	4.4±0.16	29.725±1.18(575.56)	10.325±3.29(134.65)	5.1±0.44 (15.91)	5.075±2.22(15.34)	4.125±0.29 (-6.30)	
Group4	**400mg/kg**	3.8±0.13	31.325±2.32 (724.34)	14.725±6.40 (287.5)	5±0.40 (31.58)	5±2.22 (31.58)	4.825±0.38 (26.97)	
Group 5	**800mg/kg**	4.325±0.28	25.35±3.27 (486.81)	15.0±4.45 (247.22)	7.7±2.99 (78.24)	6.2±1.46 (43.51)	3.85±0.50 (-10.88)	

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Data are given as mean ± SEM of significance difference $p<0.05$

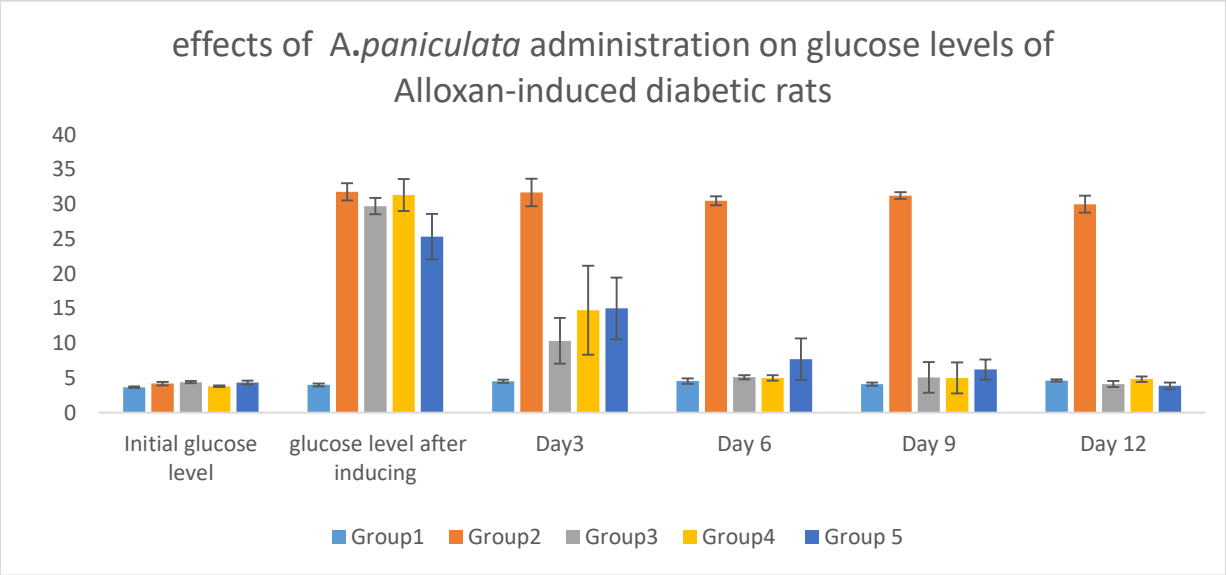

Fig 1 Effects of A. paniculata administration on glucose levels of alloxan induced diabetic rats

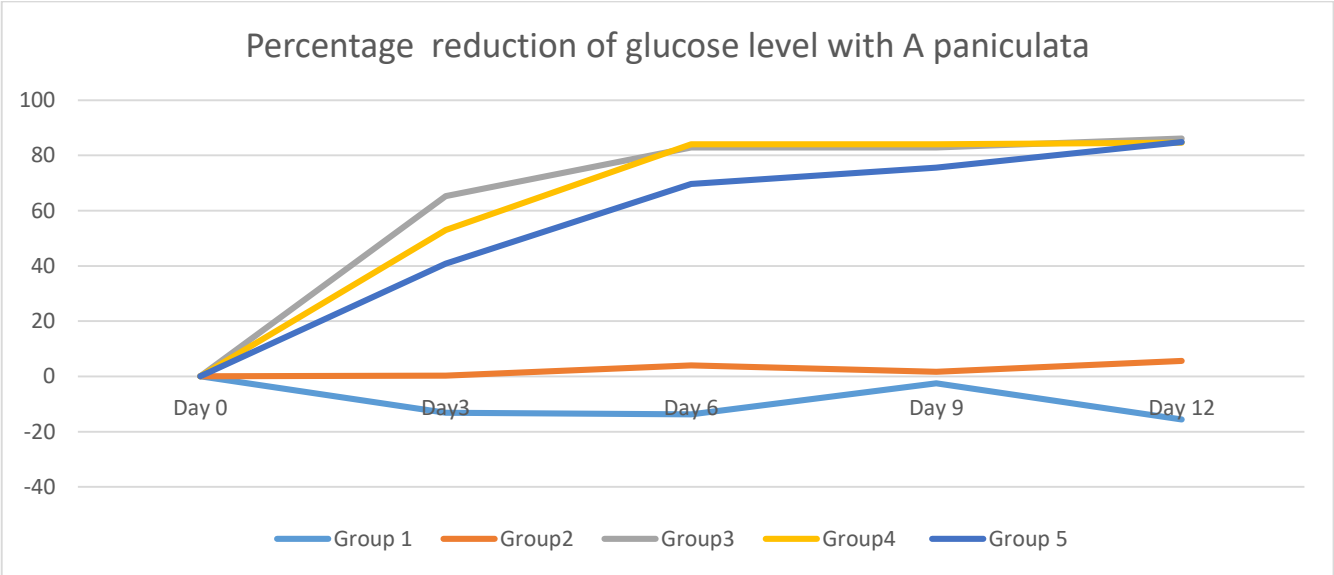

Fig 2: Percentage reduction of glucose level with A paniculata

Animal weight within the experiment period

In group 2 there was a significant decrease $p<0.05$ in weight during the experiment after the alloxan inducement compared with the positive weight control in group 1. There was a decrease in the initial weight in group 3 compared with positive control group one but a decrease after inducement with alloxan compared with negative control group 2. There was a decrease in the initial weight in group 4 compared with positive control group 1 but a decrease after inducement with alloxan compared with negative control group 2. There was a decrease in the initial weight in group 5 compared with positive control group 1 but a decrease after inducement with alloxan compared with negative control group 2.

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Table 2: showing weights in gram of rats before and after treatment on different days

Group	*A.paniculata* doses (mg/kg)	Initial weights	Weight after inducement in 2 days	Weight of rats during treatment			
				Day 3	Day 6	Day 9	Day 12
Group 1 Control	**-**	224.75±17.18	223.6±12.23	222.5±8.09	222.5±4.90	228.5±6.87	230.25±4.56
Group 2	**-**	161±14.21	151.5±10.02	160.7.96	155±5.46	154±5.67	149±3.56
Group 3	**200mg/kg**	137.25±3.23	138.25±4.34	140.53.76	137±4,87	139±7.36	139±7.89
Group 4	**400mg/kg**	167±7.32	155.25±8.65	141±5.94	139±8.73	137±6.45	140±54
Group 5	**800mg/kg**	205.75±13.32	138.25±6.45	202.665.62	186±4.76	176±4.78	173±5.78

Data are given as mean ± SEM of significance difference p<0.05

DISCUSSION

Diabetes Mellitus (DM) is the variable metabolic problem featured by hyperglycemia [23]. In the developing stage in children the common diabetes is type 1 diabetes (DM1) which leads to the alteration or destruction of the proper functioning pancreatic cells, resulting to insulin deficiency [24]. The inducement of diabetic condition in albino rats and administering a remediation through herbal therapy is a way of trying to find various ways of treatment for it, which this piece of work centered on. Alloxan administration in groups 2.3.4 and 5 induced a diabetes condition in the rats known as hyperglycemia in this research is in accordance with the report of [25]. On their Study of antihyperglycaemic activity of medicinal plant extracts in alloxan induced diabetic rats. Alloxan drug as used in this research to induce diabetes problem served as a model which was in line with the study of [26]. The manifestation of diabetes condition was seen to be after two (2) days which was in line with the 36 hours recorded by [26] in their study. The discovery of various herbal medicines the world all over is a thing of importance to medical and allied discipline like physiology, biochemistry, pharmacology and others. This is because according to WHO report in her projection in 2016 that diabetes will be the 7th leading cause of death in 2030 [27]. To this end, this work further deems it fit to examine the curative activity of *A.paniculata* ethanol aqueous extract on male albino rats induced in a diabetes condition by using alloxan drug. After 2 days of the inducement of the rats with a dose of 150mg/kg of alloxan drug the diabetes levels of all the groups (2,3,4 and 5) significant increased range from 31.775±1.25 - 25.35±3.27 as against 4.4±0.16 - 3.65±0.11 range of initial glucose levels in all groups. The implication was that the pancreatic beta cells that are responsible for the secretion of insulin that will regulate the level of glucose in the blood had been affected negatively as reported by [28] and this resulted to a disease condition known as hyperglycemia. In group 3 that was administered 200mg/kg of *A. paniculata* there was significant decrease in glucose level in day 3 down to 12th day of administration with 96.18% of the reduction experience within the first six days . This trend of significant decrease in glucose levels occurred in all the other rats exposed to 400mg/kg with 99.33% reduction in six days and 800mg/kg with 82.08% reduction in six days, when ethanolic aqueous extract of **A.** *paniculata* was administered to alloxan-diabetes rats as indicated in Table 1 and graphically expressed in Fig 2. This findings corresponded with the findings of [16] when they induce hyperglycemia on rats using streptozotocin and [29] that synergized herbal extract of *A. paniculata* (Burm. f) Ness and *Azadirachta indica* Juss that brought about a reduction in the level of glucose in the blood (hypoglycemia). Among the 3 groups that received treatment, group 5 rats which received 800mg/kg of the ethanolic extract of *A.paniculata* showed to be most effective than groups 3 and 4 as there was a steady decrease in glucose level. This proofs the effectiveness of *A.paniculata* as an antidiabetic herbal plant.The initial weights of the rats ranged from 224.75±17.18 of the control group 1 to137.25±3.23 of group 3. There was a quite significant decrease in weight after 2 days inducement with alloxan drug compared with the control of the initial weight. The decrease in weight was as a result of the body weight loss from metabolic disorders due to high blood glucose levels in diabetic animals that cannot enter the cells to produce energy. This weight loss of diabogenetic animals was in line with the findings of [30]. The weight loss in the animals during treatment with *A.paniculata* from day 3 to day 12 in an interval of 3 days was well pronounced in doses of 400mg/kg and 800mg/kg respectively. The decrease in weight of the animals was as a result of the evacuation of the excess of glucose through urine as also examined by [31].

CONTRIBUTION

The contribution of the work is to specifically examine the antidiabetic ability of *Andrographis paniculata* without synergy with another medicinal plant in the treatment of diabetes. Therefore, *Andrographis paniculata* as a single herb can also be used to treat a diabetic condition.

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