

Antihyperglycemic Activity of Ethanolic Extracts of Azadirachta Indica Root Bark and Zanthoxylum Chalybeum Stem Bark in Alloxan-Induced Diabetic Mice

Okiror Simon Peter

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Abstract - Zanthoxylum chalybeum stem bark and Azadirachta indica root bark are used by communities in Africa and Asia to manage diabetes mellitus. This study determined the anti-hyperglycemic effect of Zanthoxylum chalybeum ethanolic stem bark extract and Azadirachta indica ethanolic root bark extract in Alloxan monohydrate-induced diabetic mice. The plants were obtained from Usuk sub-county, Eastern Uganda and extracts prepared and study done at Natural Chemotherapeutics Research Institute, Kampala, Uganda. Mice were divided into Zanthoxylum chalybeum (n=8), Azadirachta indica (n=8), combination of Zanthoxylum chalybeum and Azadirachta indica (n=8), normal control (n=8), positive control groups (n=8) and untreated diabetic (n=8). Diabetes was induced in each mouse in experimental groups by a single dose intraperitoneal injection of Alloxan monohydrate at 150mg/kg body weight. The plant extracts were administered orally to the experimental mice at dosages of 200mg/kg body weight for 28 days. The negative control group was left untreated while the positive control group was treated orally with Metformin (10mg/kg body weight). The effect of the extracts on blood glucose of the individual extracts and when combined were determined in all mice in the experimental and positive control groups. The combined extracts of Zanthoxylum chalybeum and Azadirachta indica (1:1) exhibited significant antidiabetic activity compared to when the extracts were used individually ($P < 0.05$). These results suggest that the ethanolic stem bark extract of Zanthoxylum chalybeum combined with extracts of Azadirachta indica root bark possesses synergistic antihyperglycemic activity. This study thus corroborates the traditional use of the plants for management of diabetes. However, further research is required to explore different dosages and the possible mechanism of action of Zanthoxylum chalybeum and Azadirachta indica in the treatment of Diabetes mellitus.

Keywords - Zanthoxylum chalybeum; Azadirachta indica; Diabetes mellitus; Antihyperglycemic activity; Alloxan-induced diabetes; Ethanolic plant extracts; Synergistic effect; Traditional medicine; Mice model; Metformin.

I. INTRODUCTION

Background

Diabetes mellitus characterized by hyperglycemia is one of the most severe non-communicable diseases in the world (Agwaya et al., 2016). Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with

disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Emily, 2009; Diamond, 2010; Vineeta & Janeshwer, 2014). The prevalence of Diabetes mellitus is growing rapidly (Anggit et al., 2014; Agwaya et al., 2015). In the last 20 years, there has been a great increase and rise in diabetes and its complications globally. The WHO estimated in its reports that in 2000, 171 million

people in the world had Diabetes mellitus and this is projected to increase to 366 million by 2030 (WHO, 2006) and in Africa, it is estimated that as years go by, the prevalence of Diabetes mellitus in the continent will increase highly due to people's way of living (Aubert, 1998). The population that is at a higher risk of getting Diabetes mellitus is between 35 to 65 years due to their nature of work mostly the white collar jobs and the retiring age that spends most of their time doing nothing which lead to decrease of their metabolic activity (Agwaya, 2016). In Uganda, the prevalence of Diabetes mellitus has increased and is a killer disease among the elderly population. The age-adjusted death rate due to Diabetes mellitus was found to be 39.88 people per 100,000 of the Ugandan population, which was the 50th highest in the world, according to the World Health Rankings, 2014. Diabetes is a major risk factor for the development of cardiovascular diseases (Nandutu et al., 2015).

Diabetes mellitus is mainly of two types, type I and type II diabetes. Type I diabetes often referred to as juvenile diabetes that is insulin dependent is known to affect only 5% of the diabetic population (Shamim, 2014). Type II is also called non-insulin dependent in adults over the age of 40 years. Another type of diabetes is called Gestational diabetes which is a type of diabetes that tends to occur in pregnant women due to the high sugar levels as the pancreas does not produce sufficient amount of insulin. Taking no treatment can lead to complications during childbirth (Aiswarya et al., 2015). Chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and eventually damage of the body organs. The best solution for people with Diabetes mellitus mostly type I is induction of insulin and type II is insulin and other anti-hyperglycemic drugs which have serious adverse effects (Grant, 2003) like sulphonylureas and rapid-acting secretagogues/insulinotropics like glibenclamide, glipizide, rapaglinide, reduce hepatic glucose production, biguanides drugs like metformin, delay digestion and absorption of intestinal carbohydrate, α -glucosidase inhibitors like acarbose or improve insulin action, thiazolidinediones like pioglitazone, rosiglitazone (Luna & Feinglos, 2001; Bailey, 2009).

Over the last few decades, the role of medicinal plants as a primary tool in the preservation of health and management of diseases is realized with great concern (Shamim, 2014). The World Health Organization reported that more than 80% of the population in sub-Saharan Africa relies on herbal medicine for treatment of diseases including Diabetes mellitus (WHO, 2002; Keller et al., 2012), which is because of hyperglycemia. *Azadirachta indica* and *Zanthoxylum chalybeum* are commonly used in management of diseases like malaria, fevers, skin infections, wounds and diabetes in Uganda. Root and stem barks are commonly used to treat diseases, root extracts are considered stronger than stem extracts (Tabuti et al., 2011). Medicinal plants are embraced by many due to the use of synthetic drug molecules that have harmful side effects (Agarwal et al., 2014). The management of Diabetes has also reduced the cases of cardiovascular diseases that are associated with diabetes. Although *Zanthoxylum chalybeum* and *Azadirachta indica* are reported to be used in the treatment and management of Diabetes, no scientific study has been done to evaluate the synergetic effect of ethanolic extracts of *Azadirachta indica* root bark and *Zanthoxylum chalybeum* stem bark.

Thus, the study investigated the effect of ethanolic *Zanthoxylum chalybeum* stem bark extract and *Azadirachta indica* root bark on Alloxan monohydrate-induced diabetes mellitus in mice. In the study, oral administration of *Zanthoxylum chalybeum* and *Azadirachta indica* at doses of 200mg/dL for four weeks was evaluated in diabetic mice. *Zanthoxylum chalybeum* belongs to the Rutaceae family, *Zanthoxylum chalybeum* is a delicious shrub or tree with a round but open crown, it can grow from 1.5-10 meters tall, and the bole can be 15-40cm in diameter with large wood spines to 2cm long (Orwa et al., 2009). *Azadirachta indica* is also known as Neem tree in English, is a tree in the mahogany family Meliaceae that is native to India and the Indian subcontinent (Biswas et al., 2002).

Problem statement

Diabetes mellitus in Uganda is now at a high increase among the population, resulting into hyperglycemia. Most of the local population in the country cannot afford the synthetic antidiabetic analogues available on the market in the country. However, the limitation to the use of the synthetic drugs is their harmful side effects and expense. Several studies have been done on *Azadirachta indica* and *Zanthoxylum chalybeum* about their antihyperglycemic activity individually but not their combined activity. Research has not yet been done to determine the synergetic effect or activity of the two medicinal plants that could probably have a stronger antihyperglycemic activity. This is because the local populations in different areas are using *Azadirachta indica* and *Zanthoxylum chalybeum* individually yet Diabetes mellitus especially type II continues to be a problem.

General objective

To determine the individual and synergetic antihyperglycemic activity of ethanolic extracts of *Azadirachta indica* root bark and *Zanthoxylum chalybeum* stem bark in diabetic induced mice.

Specific objectives

To determine the phytochemical profile of the crude extracts To determine the antihyperglycemic activity of ethanolic extracts of *Azadirachta indica* root bark and *Zanthoxylum chalybeum* stem bark in diabetic mice.

To determine the synergetic antihyperglycemic activity of a mixture of ethanolic extracts of *Azadirachta indica* root bark and *Zanthoxylum chalybeum* stem bark in diabetic mice.

Research hypothesis

A significant number of members of Uganda and the African community are aware of medicinal plants especially *Azadirachta indica* (Neem) and *Zanthoxylum chalybeum* (Knob wood) that are used in the management of diabetics resulting into hyperglycemia and that the medicinal plants from contain essential elements.

Justification and significance of the study

There is literature examining the effects of *Zanthoxylum chalybeum* alone in diabetic rats and *Azadirachta indica* in diabetic mice. These were found to show effect of lowering blood sugar level to a lesser extent. The study demonstrated the potential usefulness of *Azadirachta indica* root bark and *Zanthoxylum chalybeum* stem bark when combined in treatment of diabetes on a pharmacological basis, hence adding to the available literature which will be a basis for further investigation in the efficacy, toxicity and mode of action of these plant use.

II. MATERIALS AND METHODS

Study design

The study was an experimental laboratory based study that was done between March and May 2017 to evaluate the antihyperglycemic activity of ethanolic extracts of *Zanthoxylum chalybeum* stem bark and *Azadirachta indica* root bark using diabetic induced laboratory mice. The mice were dosed with Alloxan monohydrate to induce diabetes, then followed by administration of the medicinal plants.

Study setting/study area

Plant materials of *Zanthoxylum chalybeum* stem bark and *Azadirachta indica* root bark were collected from Usuk Sub-County in Katakwi district in Eastern Uganda because of their availability. The area was mostly occupied by Itesoits. The area experiences high temperatures and heavy rainfall from March to May and from October to November (Mbonye et al., 2008). The experiment was carried out at Natural Chemotherapeutics Research Institute (NCRI) which was located adjacent the Ministry of Health headquarters along Lourdel road, plot 2A in Nakasero, Kampala, Uganda.

Sample collection

The healthy plant materials in form of stem barks of *Zanthoxylum chalybeum* and root barks of *Azadirachta indica* were collected using a panga with the help of a Botanist, identified by their local, common and scientific names and put in polythene bags, sealed and transported very fast to the laboratory for processing, a sample of the selected plants was identified and authenticated by a plant

taxonomist at the Makerere University herbarium found in Makerere University in Kampala, Uganda. The voucher specimens were deposited with reference Voucher number OSP/ZC03/001 for *Zanthoxylum chalybeum* and OSP/AI03/001 for *Azadirachta indica* at the Herbarium for future references.

Materials and reagents

Distilled water, Metformin (purchased from a pharmaceutical outlet in Kampala, USV Ltd), 2M Hydrochloric acid, amyl alcohol, methanol, ferric chloride solution, concentrated hydrochloric acid, magnesium turnings, Sodium hydroxide, concentrated sulphuric acid, chloroform, ammonia solution, glucometer (One Touch Ultra Glucose Monitor-Life scan), analytical weighing balance (Sartorius, GMBH GOTTINGEN, Type L2200P Germany), a rotary evaporator (A Büchi Rotavapor RE-2000B), timer then plastics and glassware. Most of the reagents were provided by the Institute at analytical grade.

Preparation of plant crude extracts

Plant material processing and crude extract preparation

The stem barks of *Zanthoxylum chalybeum* and the root barks of the *Azadirachta indica* were washed with running tap water. The cleaned plant material was then dried in the drier for 72 hours at 50°C, weighed using an analytical weighing balance and then crushed into powder using a grinding machine (Brook Crompton Series 2000) to increase their surface area for reaction; the plant material was weighed, and then macerated in 95% ethanol (300ml) for 72 hours using extracting solvent. After extraction, the ethanolic plant extracts of *Zanthoxylum chalybeum* and *Azadirachta indica* were poured in a flat bottomed flask filtered separately and each were concentrated in a rotary evaporator (A Büchi Rotavapor RE-2000B) at 50°C in separate flasks, each was dried using a hot air oven to remove last traces of the solvent, finally obtaining the crude extracts of the two medicinal plants. The extracts were put in cleaned and labeled conical flasks then stored in the refrigerator (Lec SPD 703G) at 4°C until used for the experiment.

Phytochemical screening

The extracts were then analyzed for principle chemical groups using standard procedures and tests as described by Subrata et al., 2011 summarised on page 17.

Preparation of doses

The body weights of the animals were measured using a weighing balance (Sartorius, GMBH GOTTINGEN, Type L2200P Germany) for calculation of the volume of medication to be administered. The volume from the stock solution of *Zanthoxylum chalybeum* and *Azadirachta indica* crude extracts that was to be administered to the study animals was calculated from the formula below;

Volume to be administered = Dose (mg/kg) * weight (kg)

Concentration of stock solution (mg/ml)

Experimental animals

Twenty-four (24) female and Twenty-four (24) male mice (*Mus musculus*) approximately 8 weeks of age of about 20-30 grams were purchased from the small animal breeding house at the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala. The animals were allowed time of seven (7) days to acclimatize in the animal facility at the Natural Chemotherapeutic Research Institute, MoH in Wandegaya, Kampala, Uganda. The mice were maintained in a controlled environment under standard conditions of temperature ($28 \pm 2^\circ\text{C}$) and humidity (50-70%) with an alternating dark and light cycle. The mice were caged randomly in groups of four in cages made from wire mesh with wood shavings, shredded paper and cotton wool will be used as bedding material. The mice were fed with commercially available pelleted mice pencils (NUVITA) and water ad libitum for the six weeks of the experiment. The mice were treated according to the international guidelines on the use of laboratory animals in biomedical research (OECD, 2005).

Sample size calculation

Sample size calculated using Graph pad Prism 7 statistical program where a sample size of 8 mice per

group had a 90% power to detect the difference between means (Malu, 2014) at $\alpha=0.05$.

Inclusion and exclusion criteria

Only animals obtained from the same colony were included in order to minimize genetic variations in the results obtained. In addition, only healthy animals were included in this study and this was identified by their physical appearance and mental well-being. Sick animals were not included in this study as per the ethical guidelines on the use of laboratory animals (OECD, 2005).

Experimental procedure

Induction of Diabetes in mice

Diabetes mellitus was induced in the mice by a solution of Alloxan monohydrate dissolved in

distilled water at a dose of 150 mg kg⁻¹ after 8 hours of starving the mice according to Syiem et al., 2002, that was injected through intraperitoneal route. The Alloxan monohydrate solution caused Diabetes mellitus since it led to massive release of insulin from the pancreas. The mice were orally (gavage) further kept on a 5% glucose solution to prevent hypoglycemia. Mice which developed Diabetes mellitus observed by hyperglycemia at 72 hours after injection of Alloxan (i.e., blood glucose concentration >250 mgdl⁻¹) observed using a Fast plasma glucose test were selected for subsequent tests.

Experimental design

Table 2.1. The treatment regime among the groups

Group	Treatment	No of mice
1	Normal mice + Distilled water	8
2	Diabetic mice + 200mg/kg body weight <i>Azadirachta indica</i> crude extract	8
3	Diabetic mice + 200mg/kg body weight <i>Zanthoxylum chalybeum</i> crude extract	8
4	Diabetic mice + 200mg/kg body weight of <i>Azadirachta indica</i> and <i>Zanthoxylum chalybeum</i> crude (1:1)	8
5	Diabetic mice + 10mg/kg body weight metformin (Conventional antidiabetic medicine)	8
6	Untreated diabetic mice + distilled water	8

Glucose measurements following treatment of Alloxan- induced mice with extracts

Five (5) mice were randomly selected from each of the six (6) groups and blood samples collected by a prick on the lateral tail vein at day 0 i.e., before start of treatment, day 7, day 14 and day 28 after consecutive treatment to determine the plasma glucose using a glucometer (One Touch Ultra Glucose Monitor-Life scan). Blood glucose was

measured using a glucometer and results expressed in mg/dl.

Data Analysis and interpretation

The data from the study was analyzed using Graph pad prism 7 (Inc., USA). The glucose concentration results were expressed as mean value $\pm$ Standard Error of the Mean (SEM). The mean and SEM of the treatment groups was generated by use of the analysis of variance (ANOVA) test. The significant difference between and within the treatment groups

was considered significant at set $P < 0.05$. The results of phytochemical screening were presented in a table form.

Ethical considerations

Approval to carry out the study was sought from the Institutional Review Board at the College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB), Makerere University, Kampala. The Food and Drug Authority (FDA) and OECD guidelines for testing of chemicals in laboratory animals were strictly followed and adhered to when handling the animals. The mice were maintained in a controlled environment under standard conditions of temperature ($28 \pm 2^\circ\text{C}$) and humidity (50-70%) with an alternating dark and light cycle. After completion of the study, the mice were humanely put to sleep using an over dose of Chloroform and the carcass was properly disposed by digging a hole.

Quality control and assurance

Animals of the same parental origin and of about the same age were used to minimize inter- individual variations in response to treatment. Only freshly prepared reagents and extracts were used. Animals were starved for four hours before administration to enhance absorption of the treatment administered. Good laboratory practice and standard operating procedures were important throughout the entire experiment. Three control groups (Positive control that were given with Metformin, a normal control with Normal saline and untreated diabetic group) were also used during the study.

Study Limitations and mitigation

The sample size required for such a study was expected to be high, but due to the high costs required to maintain these animals for such a period. The sample size was reduced to minimal to obtain the expected results.

III. RESULTS

This study was done to determine the antihyperglycemic activity of ethanolic extracts of *Zanthoxylum chalybeum* stem bark and *Azadirachta indica* root bark. *Zanthoxylum chalybeum* is known as knob wood in English and *Azadirachta indica* known as Neem tree. These medicinal plants used in the study were obtained from Usuk, Katakwi district and the study was done at the Natural Chemotherapeutics Research Institute, Wandegaya, Ministry of Health.

Quantitative phytochemical analysis of the crude medicinal plants

The results of the analysis of ethanolic extracts of *Zanthoxylum chalybeum* stem bark showed a significant presence of Alkaloids (+++) and Steroids (+++) in abundance followed by moderate amounts of Saponins (++) and Flavonoids (++) , trace amounts of Glycosides (+) and Reducing sugars (-) were found absent. The results from the analysis of the *Azadirachta indica* root bark ethanolic extract showed a significant presence of Alkaloids (+++), in abundance followed by moderate amounts of Steroids (++) and Reducing sugars (++) and trace amounts of Glycosides (+) but also Saponins (-) were absent (Table 4.1).

Table 3.1: Phytochemical components of ethanolic extracts of *Zanthoxylum chalybeum* stem bark and *Azadirachta indica* root bark.

Chemical Class	Phytochemical Test	Relative abundance of <i>Zanthoxylum chalybeum</i>	Relative abundance of <i>Azadirachta indica</i>
Alkaloids	Mayer's Test	+++	+++
Glycosides	Kedde Test	+	+

Saponins	Frothing Test	++	-
Steroids	Burchourd Test	+++	++
Flavonoids	FeCl ₃ Test	++	+
Reducing Sugars	Fehling's Test	-	++

Key: - Absent + Trace amounts ++ Moderate +++ Abundant

Effect of Zanthoxylum chalybeum and Azadirachta indica on glucose levels in Alloxan induced diabetic mice

In the study, administration of the extract of Zanthoxylum chalybeum stem bark and Azadirachta indica root bark extract individually caused a significant decrease in fasting blood glucose in

diabetic mice at the dose level of 200mg/kg BW ($p < 0.05$) compared to untreated diabetic mice. Results show that Zanthoxylum chalybeum lowered blood glucose levels of the mice from 450.03 ± 34.08 mg/dL to 174.90 ± 2.44 mg/dL after 28 days of repeated treatment and Azadirachta indica root bark extract (200mg/kg BW) reduced blood glucose levels from 379.25 ± 28.45 mg/dL to 179.05 ± 1.54 mg/dL after 28 days of repeated treatment (Table 4.2).

Table 3.2: Effect of Zanthoxylum chalybeum and Azadirachta indica extracts on blood glucose levels compared with the controls.

Parameters	Normal control	<i>Z. Chalybeum</i> treated group (200mg/kg BW)	<i>indica</i> treated group (200mg/kg BW)	Untreated diabetic group	Metformin treated group (10mg/kg BW)
Glucose (mg/dL)					
Day 0	98.55 $\pm$ 5.94	450.03 $\pm$ 34.08	379.25 $\pm$ 28.45	306.90 $\pm$ 42.57	428.23 $\pm$ 29.31
Day 7	95.40 $\pm$ 5.04	260.85 $\pm$ 4.12	251.95 $\pm$ 6.64	411.90 $\pm$ 53.57	253.23 $\pm$ 15.63
Day 14	115.87 $\pm$ 5.76	177.80 $\pm$ 8.19	198.90 $\pm$ 6.52	496.90 $\pm$ 53.57	141.95 $\pm$ 20.73
Day 28	87.75 $\pm$ 6.58	174.90 $\pm$ 2.44	179.05 $\pm$ 1.54	<i>Mice died</i>	121.36 $\pm$ 10.06

Synergetic activity of Zanthoxylum chalybeum and Azadirachta indica on glucose level in Alloxan induced diabetic mice

In the study, interestingly upon administration of the combination of Zanthoxylum chalybeum and Azadirachta indica in the ratio of 1:1 lowered the blood glucose levels of the mice much greater than

when the two medicinal plants were used individually from 356.90 ± 26.79 mg/dL before treatment to 133.10 ± 4.62 mg/dL after twenty-eight (28) days of treatment. Results show that ethanol extracts possessed almost the similar effect as that of reference antidiabetic drug Metformin (10mg/kgBW) ($P > 0.05$). The blood glucose levels of normal mice remained in the normal range of 80 to

120 mg/dL. Blood glucose levels of untreated diabetic mice were significantly ($P < 0.05$) and continuously elevated throughout the experimental period. There were no results for blood glucose level of the mice that was recorded on day 28 since the five mice had died (Table 4.3).

Table 3.3: The effect of a combination of *Zanthoxylum chalybeum* and *Azadirachta indica* extracts on blood glucose in Alloxan induced diabetic mice compared with the controls

Parameters	Normal control		<i>Zanthoxylum chalybeum</i> and <i>Azadirachta indica</i> treated group (200mg/kg BW)	Untreated diabetic group	Metformin treated group (10mg/kg BW)
Glucose (mg/dL)					
Day 0	98.55 ± 5.94		356.90 ± 26.79	306.90 ± 42.57	428.23 ± 29.31
Day 7	95.40 ± 5.04		236.58 ± 8.41	411.90 ± 53.57	253.23 ± 15.63
Day 14	115.87 ± 5.76		151.23 ± 2.58	496.90 ± 53.57	141.95 ± 20.73
Day 28	87.75 ± 6.58		133.10 ± 4.62	<i>Mice died</i>	121.36 ± 10.06

Discussion

Azadirachta indica and *Zanthoxylum chalybeum* are common herbs in Africa and Asia reported to be used to treat malaria, wounds, sickle cell and also used to treat skin infections (Biswas et al., 2002; Tabuti et al., 2016). The medicinal plants used in this study were obtained from Usuk Sub-County in Katakwi district in Eastern Uganda. The area was mostly occupied by a Itesoits. The area experiences high temperatures and heavy rainfall from March to May and from October to November. In Uganda, *Azadirachta indica* and *Zanthoxylum chalybeum* are currently being used by traditional medicine

practitioners and claimed to be effective in the management of diabetes. This study determined the antihyperglycemic activity of the ethanolic extracts of *Azadirachta indica* root bark and *Zanthoxylum chalybeum* stem bark in Alloxan-induced diabetic mice was carried out at Natural Chemotherapeutics Research Institute.

In this study of the medicinal plants, phytochemicals in both plants were reported. *Azadirachta indica* root bark was reported to have alkaloids, steroids, glycosides, flavonoids and reducing sugars. These results were in agreement with Rasheda et al., 2013,

a study done in Bangladesh since Rasheda also reported presence of flavonoids, steroids and glycosides. This may be because the same ethanolic extract was used. These finding were also similar to Biswas et al., 2002 who found *Azadirachta indica* to belong to a group of tetra triterpenoids and a small number of non-terpenoidal ingredients. The study also showed that *Zanthoxylum chalybeum* contains alkaloids, glycosides, saponins, steroids and flavonoids. These results were similar to those reported in Kenya by Kimani et al., 2015 that showed actually the same phytochemicals. This was because the extracts were from the same plant part-stem bark of the plant. The significant decrease in blood glucose levels in the extract treated mice in this study suggests that the different constituents of antidiabetic plants could have different sites of action in the body of the animals (Jarald et al., 2008)

The present study indicated *Zanthoxylum chalybeum* extract alone and *Azadirachta indica* extract alone to have significant antihyperglycemic effects in Alloxan monohydrate- induced diabetic mice. These results of *Azadirachta indica* extract alone were in accordance with studies of antihyperglycemic activity of *Azadirachta indica* by Shradha, 2010; Rasheda et al., 2013 and Anggit et al., 2014 in the Alloxan and streptozotocin-induced diabetic rats that also showed results of reduction of blood glucose levels in diabetic animals on administration of the *Azadirachta indica* extract. The results were in agreement probably because same extract was used (ethanolic extract). This study was also in agreement with that carried out in India by Kar et al that reported 95% alcoholic extract of *Azadirachta indica* extract in the dose of 250 mg/kg twice daily orally for one week that reduced blood sugar level by 55% and urine sugar by 100% ($p < 0.05$) in Alloxan induced diabetes in rats. Furthermore, effect of *Zanthoxylum chalybeum* extract on blood glucose level is similar to studies by Kimani et al., 2015 and Agwaya et al., 2016 of Uganda that reported significant reduction of blood glucose in animals treated with *Zanthoxylum chalybeum* stem extract. This may be due to the use of the same part of the plants where the extract was obtained from-the stem bark and secondary metabolites and suggested that it can increase glucose utilization to

lower plasma glucose in diabetic animals lacking insulin (Anggit et al., 2014). The antihyperglycemic activity of *Zanthoxylum chalybeum* stem bark and *Azadirachta indica* root reported in this study may be attributed to the secondary metabolites identified through phytochemical screening.

Study finding also show that significantly lower blood glucose was observed in mice treated with a combination of *Zanthoxylum chalybeum* and *Azadirachta Indica* in comparison to the groups treated with *Zanthoxylum chalybeum* extract alone and *Azadirachta Indica* extract alone. These results were similar to results of Anggit et al., 2014 a study carried out in Indonesia that showed a much lower fasting blood glucose level when two antidiabetic plants are combined during administration. This was because the various active compounds in the extracts of both plants may be able to synergistically increase the effect of lowering the blood glucose levels (Agwaya et al., 2016). In general, there is very little biological knowledge on the specific modes of action in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids and flavonoids that are frequently implicated as having antidiabetic effects (Loew and Kaszkin, 2002).

Furthermore, the study indicated that untreated mice had elevated blood glucose levels that were raised throughout the study. There were no results of fasting plasma glucose of the untreated diabetic mice that were recorded on day 28 since five (5) of the mice in that group had died. These results were different from a study carried out in Bangladesh by Rasheda et al., 2013 where no death was recorded. This could probably be due to the difference in the feed given to the mice. The death of the untreated diabetic mice was probably due to hyperglycemia and inability of the body to control blood glucose levels (Donkumar et al., 2011).

IV. CONCLUSIONS AND RECOMMENDATIONS

Conclusion

This study showed that *Zanthoxylum chalybeum* ethanolic stem bark extract and *Azadirachta indica* ethanolic root bark extract possesses antihyperglycemic activity. The extracts, however when used in combination possessed a more significant synergetic antihyperglycemic activity in reduction of blood glucose level. Both medicinal plants were seen to contained antioxidant compounds.

Recommendations

The toxicity of the combination of the two herbs should be researched.

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