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Effect of Seven Keys Herbal Formulation on Plasma Concentrations of Liver Transaminases of Alloxan-Induced Diabetic Rats

Eguavoen Collins[1], Ekpo Daniel Emmanuel[2*] and Ebeire Ekene Nwoke[3]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.
[2]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.
[3]Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, Delta State University, PMB 1, Abraka, Delta State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author EEN designed the study, wrote the protocol and supervised the work. Author EDE wrote the first draft of the manuscript. Authors EC and EDE managed the literature searches; analyses of the study performed the spectroscopic analysis and managed the experimental process. Author EC identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was designed to investigate the effect of seven keys herbal formulation (*Allium sativum, Xylopia aromatica, Tetrapleura tetraptera, Ficus carica, Nauclear latifolia, Starculia aurens* and *Combretum micranthum*) on plasma concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of alloxan-induced diabetic rats.
Study Design: Twenty five (25) male albino rats of wistar strain weighing 120-160 g were used for the study. They were acclimatized to the laboratory environment for seven (7) days and were subsequently divided into five (5) groups of five rats each prior to experimentation. Group 1 and 2 served as the normal control (NC) and diabetic control (DC) respectively, and received placebo

**Corresponding author: E-mail: ekpodaniele@gmail.com;*
Co-author: E-mail: eguavoencollins@yahoo.com;

treatment with distilled water. All other groups were diabetic and treated with seven keys herbal formulation via oro-gastric intubation orally for 14 days. Group 3 served as the standard control treated with 5 mg/kg body weight of Glibenclamide. Groups 4 and 5 rats were treated with 200 mg/kg and 400 mg/kg body weight of seven keys to power respectively.

Place and Duration of Study: The study took place at the Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, Delta State University Abraka, Nigeria, between January and February 2014.

Methodology: Diabetes was induced in rats via single intra-peritoneal injection of alloxan monohydrate 100 mg/kg body weight, after which they were treated with standard drug and seven keys herbal formulation daily for 14 days. At the end of the fourteenth (14^{th}) day period of treatment, the animals were subjected to overnight fast after which they were anesthetised in chloroform vapour, and blood sample were collected via cardiac puncture. The animals were subsequently euthanized in chloroform vapour, and plasma aspartate aminotransferase and alanine aminotransferase activity was assayed.

Results: The result showed that the administration of seven keys to power significantly ($P<0.05$) lowered AST and ALT concentrations in Group 5 (19.7±5.50 and 21.20±3.04 IU/L) when compared to Group 2 (33.5±1.91 and 44.46±2.55 IU/L) to near normal as seen in Group 1 (17.46±3.94 and 17.14±2.19 IU/L) respectively.

Conclusion: This shows that the liver functions of the rats were better preserved in a dose dependent manner as indicated in the group that received a high dose of the herbal formulation. Hence the drug formulation can be recommended for use in diabetic complications especially in cases of hepatotoxicity.

Keywords: Seven keys; herbal medicine; aspartate aminotransferase; alanine aminotransferase; diabetes mellitus.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple aetiology characterised by chronic hyperglycaemia with disturbances of carbohydrates, fats and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. It can also be referred to as a metabolic disorder which is characterized by high blood sugar (glucose) levels established over a prolonged period of time and results from defects in insulin secretion, or its action, or both [2]. This high blood sugar produces the classical symptoms of polyuria, polydipsia and polyphagia. Normally, blood glucose levels are tightly controlled by insulin, a hormone produced by the beta cells of the pancreas. Insulin is water soluble hormone whose receptor is a tyrosine kinase [3]. It functions primarily to lower blood glucose level by providing a mechanism for the uptake, utilization and storage of glucose. When the blood glucose elevates (after a carbohydrate rich meal), insulin is secreted from the pancreas to normalize the blood glucose level. The inability of the pancreas to produce sufficient insulin or the cells of the body to respond to the insulin produced results in a state of hyperglycaemia and subsequently causes diabetes [4]. Diabetes mellitus is also known as a multifactorial disease which is characterized by hyperglycaemia, lipoprotein abnormalities [5], raised basal metabolic rate [6,7,8], defect in reactive oxygen species scavenging enzymes [9] and altered intermediary metabolism of major food substances [10]. It is a major degenerative disease in the world today. The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and 4.4% by 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 [11], with complications such as hypertension, atherosclerosis and microcirculatory disorders amongst others [12].

The disease is mainly classified as insulin dependent diabetes mellitus (IDDM) also known as Type 1 diabetes mellitus caused by immunological destruction or loss in the function the insulin-producing beta cells of the islets of Langerhans in the pancreas resulting in insulin deficiency. Non-insulin dependent diabetes mellitus (NIDDM), also known as Type 2 diabetes mellitus which is characterized by impaired insulin secretion or peripheral resistance to the action of insulin, as well as gestational diabetes amongst others. The majority of type 1 diabetes is of the immune-mediated nature, in which beta cell loss is a T-cell-mediated autoimmune attack [13]. The defective responsiveness of body tissues to

insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus remains a chronic metabolic disease of the human race. Management of the disease has been daunting, coupled with the absence of appropriate treatment. Drugs such as insulin and oral hypoglycaemic agents have been used for treatment of the disease. However, no cure exists to date [14].

Herbal medicines have been used since ages for the treatment of diabetic patients and they are currently accepted as a complementary or alternative treatment for diabetes mellitus [curcuma longa]. The field of herbal medicine has expanded significantly over the last few years, and these herbal drugs are gaining popularity in developing countries as well as developed countries due to their natural origin. Side effects associated with the use of orthodox drugs such as insulin and oral hypoglycaemic agents may have prompted the use of herbal therapy, and this has been authorized by the World Health Organization [14]. Africa alone is endowed with a rich biodiversity; a significant number of plants from this continent have been used by traditional healers for the treatment of various illnesses including diabetes. Myriads of plants and some potentially active compounds such as saponins, tannins, alkaloids, flavonoids and glycosides isolated from some of these plants have been reported to play important role in diabetic therapy [14].

According to an estimate, there are around 25,000 effective plant-based formulations used as folk medicine in curing many ailments and diseases [15]. In 2002, Chopra and Doiphode reported that Ayurveda is the most ancient health care system [16]. Ayurvedic practitioners have identified a number of medicinal preparations and surgical procedures for curing various ailments and diseases. Even in this era of modern medicines, many drugs have come to the drug market from plant sources used by the indigenous communities [17].

Medicinal plants are the most important source of life saving drugs for the majority of the world's population [18]. Plants have been an important source of medicine for thousands of years. Even today, the world health organization estimates that up to 80% of people still rely on traditional remedies such as herbs for their primary healthcare [19,20]. Many of the currently available drugs have been derived directly or indirectly from herbal sources. Herbal medicines have proved to be highly effective, economical and safe alternative tools for treatment of various human diseases. The medicinal plants are known to contain several phytochemicals such as carotenoids, terpenoids, alkaloids, flavonoids, polyphenols, tannins, saponins, enzymes, proteins, minerals and vitamins amongst others. These phytochemicals possess anti-diabetic, antioxidant, antimicrobial, anti-inflammatory, anticancer activities etc. Their traditional applications provide valuable clues for selection of plant products for the development of drugs based on their active chemical ingredients [15].

The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed [21]. Plants are also, the source of many modern medicines [22]. These plants are known to contain certain chemical ingredients which are used for the treatment of number of diseases. Recently, the interest in medicinal plants has tremendously increased due to failure of modern medicines to provide effective treatment without any toxicity and side effects [23]. Besides that, herbal drugs are cost effective too. With the onset of scientific research in natural products it is becoming clearer that medicinal plants have a potential in today's synthetic era. With the progress of new technologies, new avenues have been opened in purifying active components from the plants and establishing their chemical structures or even to synthesize and modify them chemically. So the ancient knowledge coupled with the modern scientific principles can come into the forefront and provide us with powerful remedies to several diseases [23]. Medicinal plants have one or more parts with medicinal properties [24]. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value, which are yet to be discovered. Large numbers of plants are constantly been screened to determine their toxicity level. The traditional use of any plant for medicinal purposes warrants the safety of such plant, particularly with regards to mutagenicity, nephrotoxicity, carcinogenicity and hepatotoxicity [25].

The seven keys herbal mixture is an herbal drug formulation which is made up of a combination of seven (7) plants extracts some of which have been shown to have anti-diabetic activity. It is commonly used in the treatment of measles, chicken pox and rashes. The effect of this herbal formulation on the liver function of diabetic

patients is yet to be reported. This makes the study important since this formulation can be used by intended patients who may also be diabetic. The drug primarily consists of plant extracts namely: *Allium sativum* 3 w/w, *Xylopia aromatica* 5 w/w, *Tetrapleura tetraptera* 7 w/w, *Ficus carica* 10 w/w, *Nauclea latifolia* 25 w/w, *Sterculi aurens* 15 w/w and *Combretum micranthum* 35 w/w in a water base.

Liver function tests (LFT's) are a group of clinical biochemistry laboratory blood assays designed to provide information on the state of a patient's liver. Liver transaminase enzymes (AST and ALT) are useful biomarkers of liver injury in patients with some degree of intact liver function [26]. Aspartate aminotransferase (AST) also known as serum glutamic-oxaloacetic transaminase (SGOT) or aspartate transaminase [27], is a Pyridoxal phosphate (PLP)-dependent transaminase enzyme which catalyses the reversible transfer of α-amino group between aspartate and glutamate [28]. It is an important enzyme in amino acid metabolism and is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it is commonly measured clinically as a marker for liver health [29,30]. Aspartate transaminase, as with all transaminases, operates via dual substrate recognition; that is, it is able to recognize and selectively bind two amino acids (Aspartate and Glutamate) with different side-chains [31]. Alanine aminotransferase (ALT) also known as serum glutamic-pyruvic transaminase (SGPT) or Alanine transaminase is an enzyme found in plasma and in various body tissues, but is most commonly associated with the liver. It is commonly measured clinically as a part of a diagnostic evaluation of hepatocellular injury in other to determine liver health [32]. It catalyses the transfer of an amino group from L-alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and L-glutamate [33]. ALT as well as all transaminases requires the coenzyme Pyridoxal phosphate (PLP), which is converted into pyridoxamine in the first phase of the reaction, when an amino acid is converted into a keto acid [34]. Both enzymes are similar, in that they are associated with liver parenchymal cells. While ALT is found predominantly in the liver, with clinically negligible quantities found in the kidneys, heart, and skeletal muscle, AST is found in the liver, heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells. As a result, ALT is a more specific indicator of liver inflammation than AST. Hirotsu et al. (2005) reported that AST may be elevated also in disease conditions affecting other organs, such as myocardial infarction, acute pancreatitis, acute haemolytic anaemia, severe burns, acute renal disease, musculoskeletal diseases, and trauma.

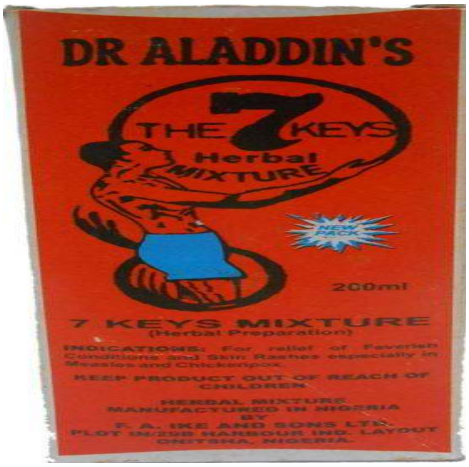

Fig. 1. The seven keys herbal formulation

2. MATERIALS AND METHODS

2.1 Materials

All drugs, chemicals, reagents used for this research were of analytical grade.

2.1.1 Drugs

Each tablet of Glibenclamide (5 mg) was reconstituted to suspension (mg/ml) with distilled water prior to daily oral administration. Seven keys herbal mixture (Abraka, Nigeria) was administered at a dose of 200 mg/kg and 400 mg/kg body weight.

2.1.2 Chemicals and reagents

Alloxan was purchased from (Sigma Aldriech St. Louis USA). A digital glucometer (Accu-Chek Active, Roche Diagnostic, Germany) was used for the determination of the blood glucose levels of the animals.

2.2 Methods

2.2.1 Experimental animals

A total of 25 adult male albino rats of wistar strain weighing between 120-160 g were used for the study. They were obtained from the Animal House of the Delta State University Abraka, and

were kept in well ventilated laboratory cages. They were acclimatized to the laboratory environment for a period of seven (7) days under standard environmental conditions, with a 12 hour light/dark cycle prior to experimentation. They were maintained on standard animal feed and drinking water *ad libitum*, and were handled in accordance with National Institute of Health (NIH) Guide for care and use of laboratory animals.

2.2.2 Induction of experimental diabetes

Following seven (7) days of acclimatization to the laboratory environment, they were randomly distributed into five (5) groups of five (5) rats each. The baseline blood glucose levels were determined with the aid of a digital glucometer prior to induction of experimental diabetes. The animals were fasted overnight with free access to water before the induction of diabetes. Diabetes was induced by a single intra-peritoneal injection of Alloxan monohydrate at a dose of 150 mg/kg body weight dissolved in 0.9% cold normal saline (NaCl solution) [35]. Since Alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release, the rats were treated with 20% glucose solution orally after six (6) hours, and subsequently kept on 5% glucose solution bottles in their cages for the next 24 hours to prevent hypoglycaemia [36]. Following 48 hours of Alloxan treatment, blood samples were collected from tail vein of the rats and blood glucose concentrations of each rats was measured by the glucose oxidase method of Beach and Turner (1958) with the aid of a digital glucometer [37]. Rats having fasting blood glucose level greater than 200 mg/dl were considered diabetic and used for the study [38].

2.2.3 Experimental design

Soon after experimental diabetes mellitus was confirmed in the groups that were subjected to induction by Alloxan monohydrate, the treatment dosages administered for each of the groups are represented as follows:

Group 1: Non diabetic rats treated with distilled water (normal control)

Group 2: Diabetic rats treated with distilled water (diabetic control)

Group 3: Diabetic rats treated with 5 mg/kg body weight of Glibenclamide (standard control)

Group 4: Diabetic rats treated with 200 mg/kg body weight of seven keys to power

Group 5: Diabetic rats treated with 400 mg/kg body weight of seven keys to power

2.2.4 Collection of blood samples and preparation of sera for analysis

At the end of the 14th day period of treatment with the standard drug and seven keys herbal mixture, the animals were anaesthetized and decapitated in chloroform vapour and blood samples were drawn from the heart of the animals via cardiac puncture following overnight fast for 16-18 hours, and emptied directly into neatly labelled lithium heparin containers. The tubes were subsequently subjected to centrifugation at 3000 rpm for 10 minutes. The supernatant (plasma) was carefully pipetted into sterile plain tube and stored in the refrigerator for liver enzyme assay.

2.2.5 Assay of serum liver enzymes

Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using the assay kits of Randox Laboratories Ltd., United Kingdom according to the method of Reitman and Frankel [39].

2.2.5.1 Assay for serum liver aspartate aminotransferase (AST)

Aspartate aminotransferase was assayed by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl hydrazine. The intensity of the colour formed was then measured against the reagent blank at 540 nm. The procedure is as outlined in the Randox Diagnostic test kits.

2.2.5.2 Assay for serum liver alanine aminotransferase (ALT)

Alanine aminotransferase was assayed by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenyl hydrazine. The intensity of the colour formed was then measured against the reagent blank at 540 nm. The procedure is also as outlined in the Randox Diagnostic test kits.

2.3 Statistical Analysis

The data were analysed using IBM Statistical Product and Service Solutions (SPSS) version 18 and the results were expressed in U/L as mean ± standard deviation. Significant differences were established by the one-way analysis of variance (ANOVA). Mean values with $p<0.05$ were considered statistically significant.

3. RESULTS

3.1 Effect of Seven Keys on the Body Weight of Alloxan-induced Diabetic Rats

There was an increase in body weight of rats observed in the treatment groups (Groups 1, 3, 4 and 5) at the end of week 2 when compared to week 0. However, there was a reduction in the body weight of rats in the diabetic control Group 2 at the end of week 2 when compared to the body weight at week 0 and the normal control rats at week 2.

3.2 Effect of Seven Keys on Plasma Concentration of Aspartate Aminotransferase

Intra-peritoneal induction of experimental diabetes with Alloxan monohydrate resulted in a significantly higher ($p<0.05$) plasma concentration of aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in Group 2 when compared to Group 1. Statistical analysis of results obtained from this study revealed that oral administration of seven keys herbal formulation (200 mg/kg and 400 mg/kg body weight) recorded a significantly lower ($p<0.05$) plasma concentration of aspartate amino transaminase (AST) in Groups 4 and 5 in a dose dependent manner when statistically compared to the diabetic control group as shown in Table 2. There was a significant decrease ($p<0.05$) in AST concentration in Group 5 (400 mg/kg) when compared to Group 4 (200 mg/kg) which is comparable to the normal control (Group 1) and the standard control (Group 3).

3.3 Effect of Seven Keys on Plasma Concentration of Alanine Aminotransferase

There was a significant reduction ($p<0.05$) in plasma concentration of Alanine aminotransferase (ALT) in the groups administered with 200 mg/kg and 400 mg/kg body weight of seven keys herbal formulation (Groups 4 and 5) when statistically compared to the diabetic control group as shown in Table 2. Also, there was a significant decrease ($p<0.05$) in AST concentration in Group 5 (400 mg/kg) when compared to Group 4 (200 mg/kg) which is also comparable to the normal control (Group 1) and the standard control (Group 3).

Table 1. Effect seven keys herbal formulation on the body weight of diabetic rats

Groups treatment		Weight (g) Week 0	Weight (g) Week 1	Weight (g) Week 2
1	Normal control	118±4.89*	134±4.00*	155±3.87*
2	Diabetic control	154±5.09	159±4.00	146±5.09
3	Diabetic + 5 mg/kg Glibenclamide	152±5.83	160±4.18	156±4.85*
4	Diabetic + 200 mg/kg 7 Keys	162±5.83	166±5.09	166±8.12*
5	Diabetic + 400 mg/kg 7 Keys	154±9.27	161±8.12	161±5.09*

Values are expressed as mean ± standard deviation of five replicates, n=5.
**p<0.05: Significantly different from diabetic control*

Table 2. Effect seven keys herbal formulation on AST and ALT concentrations of diabetic rats

Groups	Treatment	AST (U/L)	ALT (U/L)
1	Normal control	17.46±3.94*	17.14±2.19*
2	Diabetic control	33.50±1.91	44.46±2.55
3	Diabetic + 5 mg/kg Glibenclamide	23.74±4.31*	26.12±3.05*
4	Diabetic + 200 mg/kg 7 Keys	29.66±2.52*	36.68±3.75*
5	Diabetic + 400 mg/kg 7 Keys	19.70±5.50*	21.20±3.04*

Values are expressed as mean ± standard deviation of five replicates, n=5.
**p<0.05: Significantly different from diabetic control*

4. DISCUSSION

Diabetes mellitus, commonly referred to as diabetes is a metabolic disorder characterized by high blood sugar (glucose) levels over a prolonged period of time and results from defects in insulin secretion, or its action, or both [40]. The liver is the largest solid organ in the body. It is the Centre of all metabolic activities in the body [41]. Drugs and other foreign substances are metabolized and inactivated in the liver. Essential functions of the liver tend to be lost in the development of hepatic disease or disorder [42]. Disease conditions such as diabetes, drugs and toxins could cause hepatic cell damage. The damage to hepatocytes causes the release of intracellular constituents into circulation. Measurement of serum / plasma concentrations of liver enzymes therefore provides a valuable tool for clinical diagnosis of liver damage as well as toxicity studies.

Certain constituents of the seven keys herbal formulation such as *Allium sativum* [43,44], *Tetrapleura tetraptera* [45,46], *Ficus carica* [47], and *Nauclear latifolia* [48,49,50], have been shown to possess hepato-protective effect. The liver enzymes AST and ALT are biomarkers of liver function. Hence, they are usually monitored during disease conditions in other to ensure maintenance of the functional integrity of the liver.

In the present study, diabetes was induced in adult male wistsar rats via single intra-peritoneal injection of 100mg/kg body weight of Alloxan monohydrate. Results from our study showed that there was an increase in body weight of the animals treated with the herbal formulation after 14 days. Also, induction of diabetes with Alloxan led to a significant increase in the activities of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the diabetic animals as compared to the normal control group. Assaying the activities of these liver function enzymes in plasma or serum can be used as an indirect marker to assess the integrity of liver as well as the extent of liver damage after being exposed to any pharmacological agent such as Alloxan [51]. These enzymes are biomarkers of liver function whose plasma concentration rising above homeostatic limit could be associated with various forms of disorders which affect the functional integrity of the liver [52,53]. Since the liver tissues are grossly damaged during diabetic condition, elevated plasma concentrations of liver enzymes in the bloodstream following Alloxan induced diabetes was observed in our study, and this is consistent with the report and findings of others researchers [54].

In addition, the increase in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations could also be as a result of the metabolic changes in the liver following administration of toxin, cirrhosis of the liver, hepatitis as well as liver cancer [55]. Similarly in our study, it was also observed that there was a significant increase in the plasma AST and ALT concentrations in Alloxan-induced diabetic rats. The Increase in the levels of these enzymes during diabetes is as a result of leakage of cellular enzymes from the liver tissue into the plasma [56]. The increased levels of plasma enzymes (AST and ALT) observed in diabetic rats resulted in liver damage, increased permeability and necrosis of hepatocytes [57]. The significant increase observed in the level of plasma aminotransferases in the diabetic control group when compared to the normal rats in this study could be due to hepatocellular damage because these enzymes which are normally located in the cytoplasm are released into the circulation after cellular damage [58].

There was a significant decrease ($P<0.05$) in the AST and ALT level in Group 4 and Group 5 when compared to the diabetic control Group 2. This observed effect may be as a result of medicinal properties of some constituent mixture of the herbal formulation which possess several antioxidant properties with hepato-protective effect. The mechanism by which seven keys herbal formulation exerts this effect could possibly be by the prevention of the intracellular enzyme release and membrane stabilizing effects. This is because the herbal formulation contains herbal ingredients such as *Nauclea latifolia*, *Tetrapleura tetraptera* and *Alium sativum* which are rich in strong antioxidants] [59,60]. The presence of phytochemicals with antioxidant and medicinal properties such as tannins, alkaloids, flavonoids, polyphenols and saponins in *Nauclea latifolia* and *Alium sativum* leaf extracts suggest why it has protective effects on the liver.

The decrease in AST and ALT level observed for the drug formulation (seven keys to power) was dose dependent, being most prominent in the group treated with 400mg/kg body weight of the herbal mixture. This shows that the liver function of the rats was better preserved in the group that

was treated with high dose of the herbal mixture when compared to those treated with a lower dose as well as the standard drug. The activity of AST and ALT was decreased in the group that received a higher dosage of the herbal formulation when compared to the standard drug (Glibenclamide). However, for the group treated with the standard drug (Group 3), the plasma AST and ALT activities was lower when compared to Group 4 which received a lower dose of the dose of the herbal formulation. This suggests that more of the drug (seven keys to power) was required to reach the target sites (site of action) in sufficient amounts so as to give an efficacious response.

5. CONCLUSION

Our study confirms the fact that induction of diabetes with Alloxan monohydrate results in an elevated concentration of plasma liver transaminases (AST and ALT) which could be as a result of peroxidation reactions, arising from biotransformation of the Alloxan drug during diabetes mellitus, and these reactions can inflict oxidative injury to cellular components resulting in leakage of these enzymes into the blood stream. From our results, we tend to conclude that the herbal mixture of seven keys to power plays vital role in the prevention of hepato-cellular injury caused by alloxan in diabetic rats. This effect was more prominent at a higher dose of the drug when compared to the standard drug Glibenclamide.

CONSENT

It is not applicable, as no patients or human subjects were used for the study.

ETHICAL APPROVAL

All procedures involving animals in this study conformed to the guiding principles in the NIH Guide for care and use of laboratory animals [61], and the Faculty of Basic Medical Sciences, Delta State University code of ethics for the use of laboratory animals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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