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Oral Glucose Tolerance and Histopathological Studies of Organs in Alloxan-Induced Diabetic Rats Treated with Tumeric Extracts

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***Abstract*— Diabetes mellitus (DM) is a growing health concern worldwide. The use of plants for treatment of DM is widely practiced in Africa. Tumeric is traditionally used as antidiabetic medications. The present study evaluated the effects of ethanol extraction of these plant on the blood glucose tolerance and the pathology of pancreatic β-cell mass, liver and kidneys in diabetic rats. DM was induced in adult male Albino rats, using intraperitoneal injection of 120 mg/kg BW alloxan. The diabetic rats were assigned into three groups, two of which were treated with extract of tumeric (50 mg/kg) and the rats of the third group, as the untreated group received ordinary diet. Glucose Tolerance Test (GTT) which is the determination of body ability to utilize glucose. This test can be used to diagnose pre-diabetes and diabetes. Administration of these extracts tended to aids the utilization of glucose and also decrease the blood glucose concentration, while the blood glucose of the untreated rats remained significantly high and uncontrolled. Histopathologically, tissue sections of the pancreas in the treated rats did not show a significant difference compare to the untreated diabetic rats which has a disrupted pancreas islets cell. The liver of the treated diabetic rats with tumeric extract revealed slight improvement in the hepatic tissue compared to those of the untreated diabetic rats. This study indicated a significant anti-hyperglycemic effect of tumeric and supported its traditional usage in treatment of diabetes mellitus.**

***Index Terms*— Diabetes mellitus, tumeric, pancreatic islets, intraperitoneal, glucose tolerance.**

I. INTRODUCTION

Diabetes mellitus is a multifactorial illness with imperfection in reactive oxygen species (ROS), scavenging enzymes, lipoprotein abnormalities, hyperglycemia, high basal metabolic rate and high oxidative stress induced damage [1]. It is a disease of the pancreas resulting in disorder of glucose metabolism. Symptoms of diabetes include hyperglycaemia, frequent urination, increased thirst, and increased hunger. Hyperglycaemia in diabetic patients is associated with alterations in glucose and lipid metabolism and modification in liver enzyme levels [2]. It has been recognized as a major risk factor for Cardiovascular Diseases (*CVD*), such as atherosclerosis, heart attacks and stroke. Blood glucose levels are controlled by a complex interaction of multiple chemical and hormones in the body including the hormones insulin made in beta cells of the pancreas.

Diabetes mellitus develops due to diminished production of insulin (in type I) or resistances to its effects (in type II and gestational), both leads to hyperglycemia, which largely causes the acute signs of diabetes and changes in energy metabolism [3]As a result of the deficiency of insulin or inadequate insulin function there is an inadequate transfer of glucose into the cells; the utilization of glucose for energy and cellular products and its conversion to glycogen or fat and storage as such are depressed, thereby leading to accumulation of glucose in the blood, causing hyperglycemia. Fat may be mobilized from adipose tissue and broken down to provide a source of energy, which is eventually withdrawn from the body by the liver and broken down to glycerol and fatty acids leading to oxidation by the hepatic cells to ketone bodies and metabolizes by cells to produced energy, carbon dioxide and water. Only a limited amount of ketone acids can be utilized by cells as such if ketogenesis proceeds rapidly, exceeding the rate at which they can be metabolized, the ketone acids accumulate in the blood causing ketosis or ketone acidosis [4].Tissue protein may also be broken down to amino acids which are used in gluconeogenesis contributing to the hyperglycemia. Both the uptake of amino acids by the cells and body protein synthesis are decreased. Insulin-dependent diabetes mellitus (IDDM) usually has a sudden onset in a severe, acute form. In non-insulin-dependent diabetes mellitus (NIDDM) the onset is most often insidious going undetected and untreated for a considerable period of time.

Curcuma longa, or turmeric is a perennial herb and member of the Zingiberaceae (ginger) family and is cultivated extensively in Asia mostly in India and China. Dried *Curcuma longa* is the source of turmeric, the ingredient that gives curry powder its characteristic yellow color. It has many names such as Curcum in the Arab region, Indian saffron, Haridra (Sanskrit, Ayurvedic), Jianghuang (yellow ginger in Chinese), Kyoo or Ukon (Japanese) [5].Turmeric has been used in Asian cuisines for both its flavor and color and in the Chinese and Ayurvedic medicine particularly as an anti-inflammatory and for the treatment of jaundice, menstrual difficulties, hematuria, hemorrhage, and colic. It is official in the Pharmacopoeia of China as well as in others. It

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is one of the plants that have been shown to demonstrate the hypoglycemic activity in normal diabetic rat. The active component in turmeric is curcumin. Curcumin has been discovered to have anti-oxidant, anti-inflammatory and anti-diabetic properties which is of great importance to health. Despite a good number of standard drugs on the treatment of diabetes, these drugs are fast becoming less effective and some patients becoming resistant and/or non responsive to them. There is a growing public interest in dietary supplements and botanicals that has hypoglycemic property. There is a continuous search for herbal based anti diabetic agents. World Health Organization recommendations on diabetes mellitus, investigations of hypoglycemic agents of plant origin used in traditional medicine are important [6]. This study therefore investigates the ameliorative effect of Turmeric extract and its ability to restore tissue loss in alloxan induced diabetic rats.

In view of the above, the present study was planned to investigate the ameliorative effect of turmeric extract and its ability to restore tissue loss in alloxan induced diabetic rats.

II. MATERIALS AND METHODS

PLANT MATERIAL

The turmeric plant was purchased from Oja oba market in Ado- Ekiti, Ekiti State, Nigeria.

PLANT EXTRACTION

Turmeric plant was air dried and ground into fine powder. It was then stored in a air tight container for further analysis. A quantity of 250g of turmeric powder was subjected to maceration with 750ml of ethanol in a beaker for 2 days. The extract was filtered to clear liquid by using Whatman No. 1filter paper. Solvent was evaporated on a rotary evaporator at 40◦C under reduced pressure.

III. EXPERIMENTAL ANIMALS

The study was performed on twenty (20) male wistar albino rats weighing between (170g -234g). The animals were housed in ventilated cages in the Animal House of College of Medicine, Ekiti State University, Ado Ekiti, under standard environmental conditions and maintained with free access to water and standard laboratory diet. Prior to the study, the animals were acclimated for two weeks before the administration of the different dosage of the drugs. The rats were divided into 3 groups each comprising four rats (n=4).The following treatment was given to animals of different groups:

Group A Normal control (diet only)

Group B 50mg/kg body weight of turmeric extract + 120mg/kg b.wt alloxan

Group C Diabetic control(120mg/kg body weight alloxan

IV. INDUCTION OF DIABETES

Male Wister Rats (170g -234g) obtained from the animal house of the College of medicine, Ekiti State University (Ado- Ekiti, Nigeria) were acclimatized for a period of 2 weeks before the start of the experiments. Diabetes was induced by the administration of single intraperitoneal injection of 120mg/ kg body weight of alloxan monohydrate (dissolved in 0.9% normal saline) after an overnight fast(8-12hrs) and after the induction, all the rats were given free access to food and water [7]. Hyperglycaemia (blood glucose level 250mg/dl and above) was confirmed 72h after alloxan induction.

V. DETERMINATION OF BLOOD GLUCOSE

Glucose measurement was done with Randox kit utilizing the glucose oxidase method. All reagents are ready-to-use liquids and suitable for use on a wide range of chemistry analysers. The animals were anaesthetized with chloroform and thereafter sacrificed by cervical dislocation.

Principle of the Glucose Oxidase Method

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazzone to form a red – violent quinoneimine dye as indicator.

Reaction

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{quinoneimine} + 4H_2O$$

VI. DETERMINATION OF ORAL GLUCOSE TOLERANCE TEST

Blood glucose concentration was evaluated at 0, 30, 60, 90 and 120 minutes interval after treatment in both cases.

VII. HISTOPATHOLOGICAL EVALUATION

Fifteen days after diabetes induction, appropriate tissue samples were collected from pancreas, liver, kidneys, heart, and were then fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm thicknesses, and stained with hematoxylin-eosin for light microscopic examination and histopathological examinations (Luna, 1968). The slides were examined microscopically for morphological changes.

VIII. STATISTICAL ANALYSIS

Glucose profiles between different groups and timings were analyzed by GraphPad Prism at probability level of 0.05%.

IX. RESULTS AND DISCUSSION

This study investigated the ameliorative effects of turmeric extracts on alloxan induced diabetic rats. There have been increasing demand for use of plant products with antidiabetic activity due to low cost, easy availability and lesser side effects [9]. Therefore plants materials are continuously scrutinized and explored for their effect as antidiabetic agents. Although many investigators reported that turmeric possesses a variety of medicinal properties such as hypoglycemic and hypolipidemic activities, to our knowledge, there are a few documents about medicinal

properties and side effects of turmeric in the treatment of diabetes mellitus. The results of the present study in figure 1 and 2 showed that oral administration of 50 mg/kg of turmeric extracts for 12 days decreased the blood glucose concentrations into normal range in the alloxan-induced diabetic rats. This result shows turmeric extract has a possible antihyperglyglycemic agent, confirming previous reports on this plant [10]. A previous study reported that turmeric extracts increase glycogen storage in liver and suppresses activities of gluconeogenic enzymes thereby reducing blood glucose in alloxan induced diabetic rats [11]. This could be a plausible explanation in human as well. Therefore, the results of the present investigation clearly indicate that the turmeric extracts have a glucose lowering effect on alloxan-induced diabetic rats.

The histopathologic sections of the liver of the untreated diabetic rats in figure 2 showed numerous pathological signs including degenerative changes in the hepatocytes which may be as a result of disorganization of the hepatic cords, congestion of the central veins with mild hepatocellular necrosis, cell vacuolization, fatty deposition and the sinusoids were infiltrated by mild nonspecific inflammatory cells caused by hyperglycemic effects. This was compared with previous study that reported that periportal vacuolization with central putrefaction was observed in the rat liver treated with single intraperitoneal infusions of alloxan at a dose of 120mg/kg body weight and the liver of the treated diabetic rats with turmeric extract revealed slight improvement in the structure of the hepatic tissue, except for a few mildly degenerated hepatocytes around the central vein of the treated rats which still had some cytoplasmic vacuoles, other hepatocytes and portal and sinusoidal areas were almost normal [12]. This was in agreement with a previous study that reported that there was an observation of swollen hepatocytes with vacuolar cytoplasm and hypertrophic nuclei, sinusoidal dilatation, and lymphocyte invasions in the periportal areas of alloxan-induced diabetic rats [9].

The histological studies of pancreas of the untreated diabetic rats in figure 4 showed shrinkage of islets of Langerhans, severe necrotic changes of the pancreatic islets, particularly the cells in the center of the islets, reduction in size and number of islets, disappearance of nucleus in some places that was might be caused by the administration of alloxan which resulted in the disruption of the islets of Langerhans and is in accordance with a previous study that reported that alloxan destroys the insulin secreting cells of the pancreas resulting in hypoinsulinemia and hyperglycemia while recovery group showed restoration of number and size of islets of Langerhans [13]. Islets cells of recovery group treated with turmeric extracts have regenerated considerably suggesting the presence of stable cells in the islets with the ability of regenerating which confirms the report on a previous study [14]. The kidneys of the normal control rats in figure 3 showed normal corpuscular histomorphology, undamaged bowman's capsule and habitually normal convoluted tubules. The histoarchitectural assortment of component part and staining intensity appear normal with no histopathological alterations compared to the kidneys of the untreated diabetic rats that showed that histoarchitectural assortment of component part and staining intensity appear with slight histopathological alterations which might be caused by alloxan toxicity [15]. The alterations may be related to the depletion of ATP, which finally leads to the death of the cells [16]. Kidneys of the turmeric treated rats showed little or no changes in the histoarchitectural structure which could be as a result of the effect of the turmeric extracts and this is in agreement with previous studies that stated that turmeric extracts protect the tissue against chemical damage [17, 18, 19] .

X. CONCLUSION

The results showed that ethanolic extract of Turmeric has antihyperglycemic effects in alloxan induced diabetic rats. Turmeric extracts in addition to possessing hypoglycemic properties, it could also be used to ameliorate the biochemical alterations induced by diabetes.

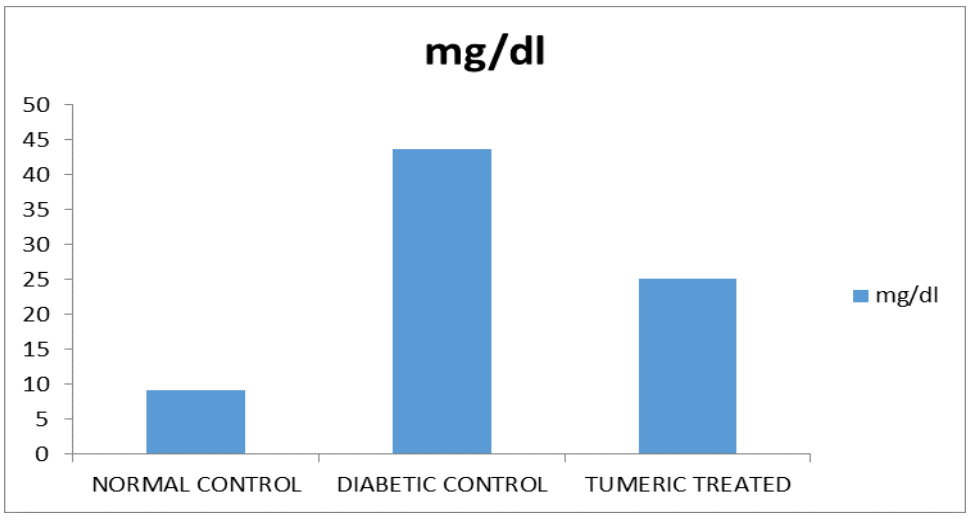

Figure 1: Effect of turmeric extract on blood glucose concentration in control and diabetic rats

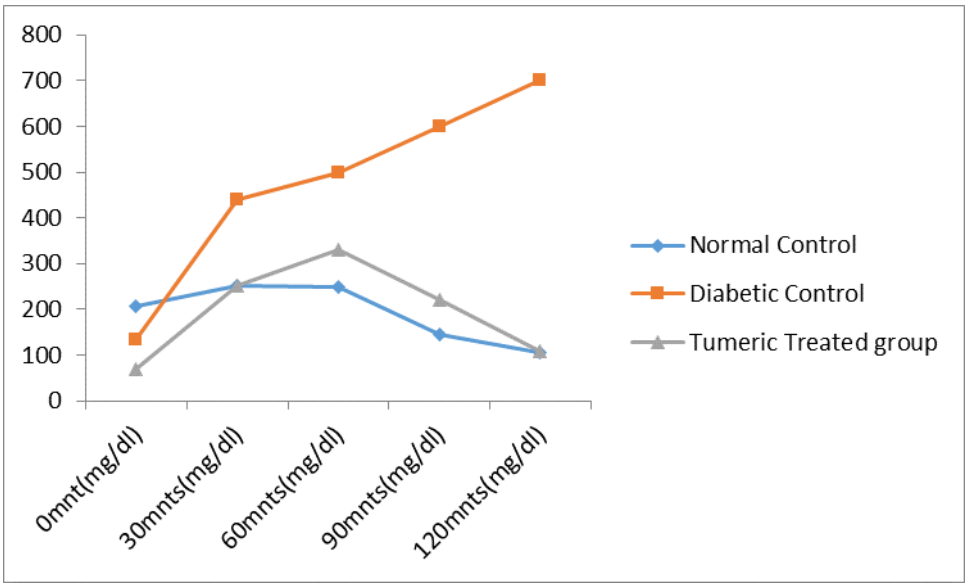

Figure 2: The effect of turmeric extract on blood glucose tolerance concentration in control and diabetic rats

HISTOLOGICAL STUDIES

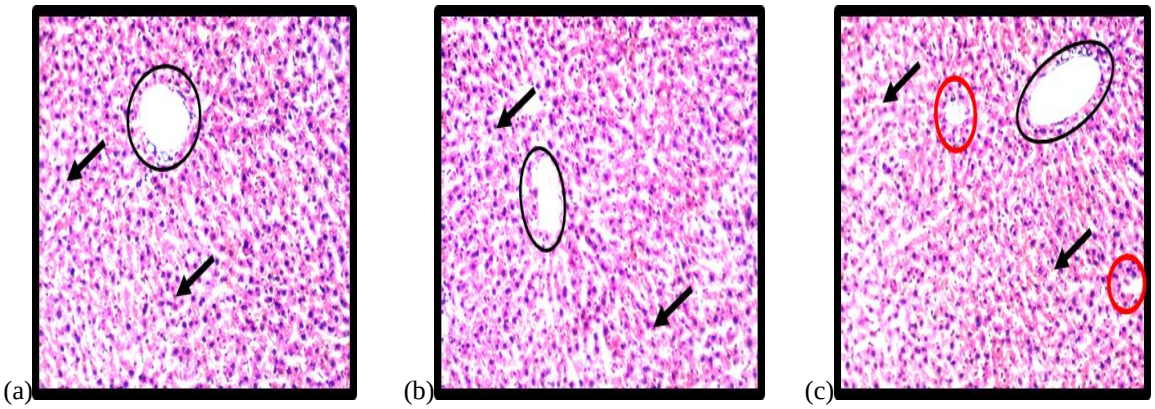

FIGURE 3 (a-c): Histopathology of the section of the liver of rats showing the effects of turmeric extract treatment on the liver of normal and alloxan- induced diabetic rats. (a) Normal control, (b) Diabetic treated with turmeric extract and (c) Diabetic control

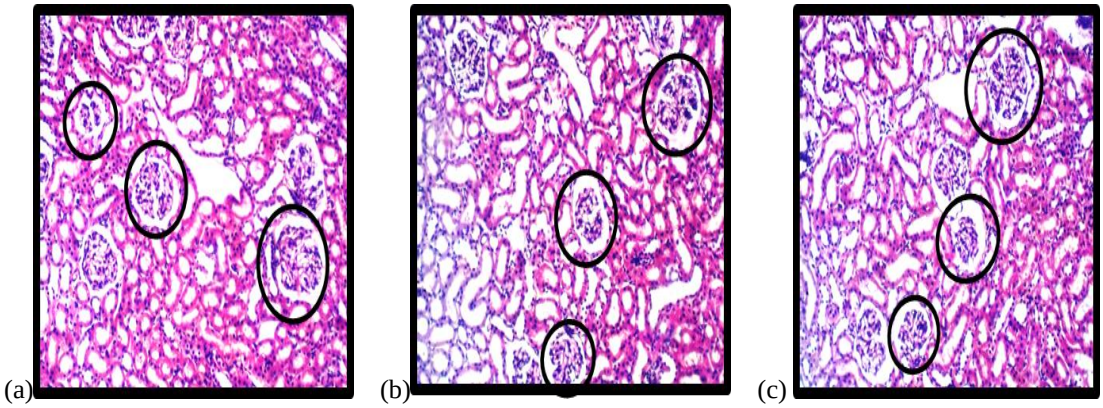

FIGURE 4 (a-c): Histopathology of the section of the kidney of rats showing the effects of turmeric extract treatment on the kidneys of normal and alloxan- induced diabetic rats. (a) Normal control, (b) Diabetic treated with turmeric extract and (c) Diabetic control

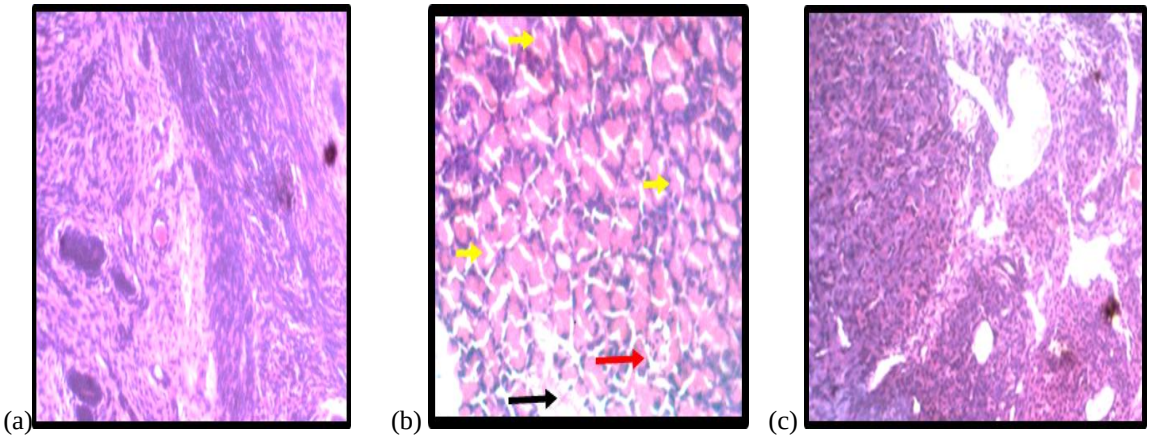

FIGURE 5 (a-c): Histopathology of the section of the pancreas of rats showing the effects of turmeric extracts treatment on the pancreas of normal and alloxan- induced diabetic rats. (a) Normal control, (b) Diabetic treated with turmeric extract and (c) Diabetic control

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