Vol 6 | Issue 1| 2016 | 1-10.

e-ISSN 2248 - 9169
Print ISSN 2248 - 9150

International Journal of
Experimental Pharmacology

www.ijepjournal.com

PRECLINICAL COMPARATIVE STUDY TO EVALUATE HYPOGLYCEMIC, BIOCHEMICAL, ANTIOXIDANT EFFECT AND HISTOLOGICAL CHANGES IN ALLOXAN - INDUCED DIABETIC RATS TREATED WITH PROCESSED AND UNPROCESSED *CURCUMA LOGNA* L.

S. Nikam[*1], V. M. Parashare[2], V. S. Nade[2], Pande Shishir[3], A. Tajanpure[2]

[1]Dept. of Biochemistry, KTHM College, Gangapur Road, Nashik, Maharashtra, India.
[2]Dept. of Pharmacognosy, MVPS's College of Pharmacy, Nashik, Maharashtra, India.
[3]Department of Rasshastra & B.K., ASS Ayurved Mahavidyalaya, Nashik, Maharashtra, India.

ABSTRACT

Since the time of the Charak and Sushruta many herbal medicines in different oral formulations have been recommended for Madhumeha and confident claims of cure are on record. The present study was carried out to investigate the effects of processed and unprocessed *Curcumin longa* L. on biochemical parameters, and lipid peroxidation in alloxan-induced diabetic rats. Acute Toxicity study was carried as per the OECD guidelines. Wistar rats (250 - 300 kg) were selected and grouped into hyperglycemic, control, standard drug Gilpizide, unprocessed turmeric and processed turmeric. Besides control group Alloxan was administered in all animals as a single dose (50 mg/kg, i.v.) to induce diabetes. A dose of 300 mg of processed and unprocessed turmeric/kg of body weight was orally administered daily for four weeks after induction of diabetic. Glipizide dose of 5 mg/kg of body weight was used as the standard which produced a significant reduction in blood glucose level. The biochemical parameters such as glucose, Cholesterol, Triglycerides, High density lipoprotein (HDL), Low density lipoprotein(LDL), Very low density lipoprotein (VLDL) and glycosylated Haemoglobin were checked using standard kits and methods before and after administration of the unprocessed and processes turmeric. Increase in reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) clearly showed the antioxidant property of the unprocessed turmeric is more than processed turmeric. Histological changes in the pancreas were also examined. There was significantly reduction in the glucose, Cholesterol, Triglyceride, LDL, VLDL and glycosylated Haemoglobin ($p \leq 0.05$), while HDL increases significantly. The present results showed that unprocessed turmeric exerted antioxidant and anti-hyperglycemic and anti-lipidemic effects more than processed turmeric and consequently may alleviate pancreas damage caused by alloxan-induced diabetes.

Keywords: Alloxan, Antioxidant, Unprocessed Turmeric.

INTRODUCTION

Diabetes mellitus (Madhumeham) was known to ancient Indian Physicians and an elaborate description of its clinical features have been described in siddha system of medicine [1]. Diabetes was discovered as early as 700-200 B.C. until the time insulin was invented, it was managed principally by using medicinal plants and traditional practices [2].Worldwide the major health problem is Diabetes mellitus (DM) which includes insulin dependent Diabetes mellitus and insulin independent Diabetes mellitus [3].

Corresponding Author

S. Nikam
Email id: parashare@gmail.com

There are 2 major types of diabetes mellitus namely, Type-1 and Type-2 diabetes mellitus. In Type-1 diabetes mellitus, the body completely stops producing insulin, a hormone that enables the body to use glucose found in foods for energy. People with Type I diabetes must take daily insulin injections to survive. This type of diabetes usually develops in children or young adults but can occur at any age. In Type-2 diabetes mellitus results when the body does not produce enough insulin or is unable to use insulin properly (insulin resistance). This form of diabetes usually occurs in people who are over 40 years of age, over weight and have a family history of diabetes.

It is characterized by chronic hyperglycemia and disturbed the metabolism of carbohydrate, fat, and protein so also called metabolic disorder [4]. In this disorder due to deficiency of insulin secretion in the body or due to unable or improper use and store of glucose or due to diminished effectiveness of insulin body is unable to use glucose coming from the diet. As a result glucose backs up in the blood stream causing one's blood glucose to rise. In Type-2 diabetes mellitus results when the body does not produce enough insulin or is enable to use insulin properly (insulin resistance). This form of diabetes usually occurs in people who are over 40 years of age, over weight and have a family history of diabetes [5].

Diabetic hyperglycaemia causes a variety of pathological changes in small vessels, arteries and peripheral nerves.The biochemical pathways involved in the pathogenesis are such as glucose-induced activation of protein kinase C isoforms which increased formation of glucose-derived advanced glycated end-products; such as carboxy-methyllysine, a crystalline lens protein. Secondly it increasesthe glucose flux through the aldose reductase pathway that is increased flux of glucose into the polyol pathway [6]. Vascular endothelial cells are an important target of hyperglycaemic damage. The endothelium is the biological active inner layer of the blood vessels, which serves as an important locus of control of vascular and thus organ functions .Diabetes initiate atherosclerosis through endothelial activation and therefore endothelial dysfunction Endothelial dysfunction could contribute to the pathogenesis of albuminuria both directly, by causing increased glomerular pressure and the synthesis of a leaky glomerular basement membrane, and indirectly, by influencing glomerular and epithelial cell function in a paracrine fashion. The endothelial dysfunction causes micro-albuminuria. The close linkage between micro-albuminuria and endothelial dysfunction in diabetes is a risk marker for atherothrombosis [7].

The relevance of each of these pathways is supported by animal studies. Hyperglycaemia increases the production of reactive oxygen species inside cultured bovine aortic endothelial cells.[8]Hyperglycaemia can increase oxidative stress through several pathways. A major mechanism appears to be the hyperglycaemia-induced intracellular reactive oxygen species (ROS), produced by the proton electromechanical gradient generated by the mitochondrial electron transport chain and resulting in increased production of superoxide [9]

Accumulation of glucose in red cells is said to enhance the irreversible production of glycosylated haemoglobin which is a marker of glycosylation in red cells in alloxanized rats.

There is also evidence that glycation itself may induce the formation of oxygen derived free radicals in the erythrocytes of diabetic subjects. Glycosylated haemoglobin has been found to increase by about 16% in diabetic patients [10]. Greater concentration of free radicals found in diabetes mellitus is due to increased glucose auto-oxidation and also due to increased oxidation rate of major antioxidants [11]. Increased in blood glucose level continuously generating reactive oxygen species (ROS) and superoxide anions, which further aggravate the diabetic complication by damaging the protein, deoxy ribose nucleic acid and carbohydrate, which leading to increasing the oxidative stress.

The HALLMARK of diabetes mellitus is polyuria-excessive urine production, polydipsia-excessive thirst and polyphagia-excessive eating. It is also character-rized by chronic hyperglycaemia and glycosuria, caused by an absolute or relative deficiency of insulin. This derangement may results into the development of further metabolic and anatomic disturbances, among which the lipemia, hypercholesterolemia, loss of weight, ketosis, arteriosclerosis, gangrene, pathologic changes in the eye, neuropathy, renal disease and coma are more common [12]. Evidence indicates the development of diabetic late complications (cataracts, retinopathy, nephropathy and neuropathy and others) are associated with increased presence of free radicals. Free radicals are highly reactive species (O_2, OH, and H_2O_2) with one or more unpaired electrons.

Free radicals are formed in almost every cell of the body at an astonishing rate during normal oxidative metabolism. These free radicals cause oxidative stress. Environmental factors such as uv light, tobacco smoke, different xenobiotic, herbicides, pesticides, ionizing radiation etc. cause their formation in greater extent. Free radicals are inherently unstable since they contain extra energy hence to reduce their energy load; free radicals react voraciously with almost every cellular component and in the process interfere with the cells ability to function normally. Free radicals are involved in the etiology of aging and the pathogenesis of variety of diseases. The pathology associated with free radicals is derived from their ability to modify cellular and extra cellular macromolecules such as proteins, lipids and DNA and to disrupt cellular function but the mammalian cells have developed an elaborate defence system which neutralize and prevent oxidative damage and allow the cell to survive in an aerobic environment. Antioxidants or autoxidation agents reduce the effect of dangerous free radicals by binding together

with these harmful molecules, decreasing their destructive power. Antioxidants interact and stabilize the free radicals and prevent the damage caused by free radicals.

Enzymatic Antioxidants including certain antioxidants enzymes are produced within the body the most commonly recognized of these naturally occurring antioxidants are superoxide dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GSHPx) and as Non – Enzymatic Antioxidants including Vitamin E , B- carotene.

Along with hyperglycaemia and abnormalities in the diabetes is appreciated with micro and macro vascular complications, which are the major causes of morbidity and death in diabetic subjects. Management of diabetes without any side effects is still a challenge to the medical system. This leads to increasing demand for natural products with antidiabetic activity and less side effects **[**13].

According to the WHO (World Health Organization) an estimated 300 million during World, 57.2 million adults in India affected in the year 2025 [14]. Prevalence of diabetes in adults worldwide was estimated to be 4.0% in 1995 and to rise to 5.4% by the year 2025. The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in the year 2025. The major part of this numerical increase will occur in developing countries. There will be a 42% increase, from 51 to 72 million, in the developed countries and a 170% increase, from 84 to 228 million, in the developing countries. Thus, by the year 2025, >75% of people with diabetes will reside in developing countries, as compared with 62% in 1995 [15].

Apart from currently available therapeutic options like insulin, Sulphonyl urea, Biguanides, Thiazolidinediones etc. many herbal medicines have been recommended for the treatment of diabetes mellitus due to their less side effects and increased acceptability [16]. To date there are different groups of oral hypoglycaemic agents for chemical use, having characteristic profiles of side effects. Management of diabetes without any side effects is still a challenge to the medical system. This leads to increasing demand for natural products with antidiabetic activity and less side effects.

Plants have been the major source of drugs in Indian system of medicine and other ancient systems in the world. Earliest description of curative properties of medicinal plants was found in Rig Veda (2500- 1800 BC). Charaka Samhita and Sushruta Samhita give extensive description on various medicinal herbs. Information on medicinal plants in India has been systematically organized [17]. Medicinal plants have the advantage of having little or no side effects. There are many anti-diabetic plants, which might provide useful sources for the development of drugs, in the treatment of diabetes mellitus. The literature on medicinal plants with hypoglycaemic activity is vast. As many of these plants were used for many centuries and sometimes as regular constituents of the diet, it is assumed that they do not have many side effects. However chronic consumption of large amounts of traditional remedies must always be taken with caution as toxicity studies have not been conducted for most of these plants [18-21]. There is increasing demand by patients to use natural products with anti-hyperglycaemic activity due to side effects associated with the use of insulin and oral hypoglycaemic agents. The number of diabetic patients is increasing globally because of diverse changes in diets and all cultures.

Turmeric (*Curcuma loga*) is a rhizomatous herbaceous perennial plant of the ginger family, *Zingiberaceae.* It is native in southeast India, and needs temperatures between 20°C - 30°C.The fresh rhizomes are boiled in alkaline medium for about 45-60 minutes in and then dried in hot ovens after which they are ground into a deep orange-yellow powder [22]. Turmeric has been used in Asia for thousands of years and is a major part of Siddha medicine [23].

The most important chemical components of turmeric are a group of compounds called curcuminoids, which include curcumin (diferuloylmethane), desmethoxycurcumin, and bisdemethoxycurcumin. The best studied compound is curcumin, which constitutes 3.14% (on average) of powdered turmeric [24]. In addition there are other important volatile oils such as turmerone, atlantone, and zingiberene. Some general constituents are sugars, proteins, and resins [25-27]. The biological responses of turmeric have been largely attributed to Hepatoprotection, Antioxidant effect, antimicrobial effect, Stimulation of immune responses, pulmonary responses etc. Although *C. longa* has been investigated for its various medicinal properties, detailed studies on the unprocessed turmeric its hypoglycaemic effect, antioxidant potential, lipid peroxidation level in alloxan induced hyperglycaemic rats are still lacking.

Numerous animal models have been developed for the past few decades for studying diabetes mellitus and testing antidiabetic agents. One of the most potent method is to induce experimental hyperglycaemia is chemical induction by Alloxan. In addition, it has been widely used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of alloxan used [28].

The standard method of inducing diabetes in animal models is with alloxan, a toxic glucose analogue, which selectively destroys insulin-producing (beta) cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus called “alloxan-diabetes” in these animals, with characteristics similar type 1 diabetes in humans. The alloxan model of diabetes was first described in rabbits by Dunn, Sheehan and McLetchie in 1943 [28].

Most of the studies are performed using processed turmeric. Therefore, the purpose of the present study was to examine the influence of unprocessed turmeric by oral administration the levels biochemical parameters, and the

activities of some enzymes in plasma and different tissues of alloxan-induced hyperglycaemic rats.

Standardised turmeric has been used. Acute toxicity study was performed as per the guidelines of OECD. There was no mortality or morbidity observed in animals through the 21-day period following single oral administration of processed and unprocessed turmeric at all selected levels. The objective of the study was to compare and evaluate the Processed and unprocessed turmeric powder for antioxidant, hypoglycaemic properties and histopathological studies in normal and diabetic rats.

Experimental Design

Fresh Turmeric rhizomes (*Curcuma Longa L.*) were purchased from the Farmer of Rameshwar, District Sangali, Maharashtra, during the month of April. Sangali district is famous for the cultivation and production of Turmeric. The rhizomes were washed using water. The rhizomes were identified and authenticated by Botany research office (Botanist) from the office of the Botanical survey of India, Pune(MH).The rhizomes were cut into small and uniform pieces and air-dried for 3 days in sunlight which are further subjected to pulverization to get coarse powder. The fine powder was kept in air tight glass containers. The complete processed turmeric was also purchased from the same farmer, pulverised and store in air tight glass container.

Venues

The whole experimental work was conducted at the NDMVP's College of Pharmacy, the University of Pune.

Animals

Fourty Wistar rats (190-220 g) of either sex were employed in this study. They were housed in well ventilated cages and maintained under standard laboratory conditions at 25 ± 2°C,relative humidity 50 ±15% and normal photo period [12 h dark/12 h light] .They were used for the experiment. The rats were acclimatized for seven days in the laboratory and were fed with Commercial pellet diet [Hindustan liver, Kolkotta, India] and water was provided *ad libitum.*

All the animal experiments were conducted according to the ethical norms approved by CPCSEA, Ministry of Social Justice and Empowerment, Government of India, and ethical clearance was granted by Institutional Ethical Committee presentations held on (24\02 2014) at NDMVP samaj's College of Pharmacy, Nashik.

Chemicals

Alloxan and Nitroblue tetrazolium was purchased from Sigma chemicals (St. Louis MO, USA). Oxidized glutathione, Glucose oxidase peroxidasekit (Auto -span), Cholesterol, triglycerides, High density lipoprotein (HDL)Low density lipoprotein (LDL) estimation kits (Randox kit.) were purchased from Local Distributor. Hydroxylamine hydrochloride and all other chemical used were of Analytical grade reagents. Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative which is present as alloxan hydrate in aqueous solution. The alloxan model of diabetes induction was first described in rabbits by Dunn, Sheehan and McLetchie in 1943

Acute toxicity test

The acute oral toxicity studies [29] of processed and unprocessed turmeric were carried out as per the OECD guidelines, draft guidelines 423 adopted on 17 December 2001 received from CPCSEA, Ministry of Social Justice and Empowerment, Government of India. The animals of either sex were segregated into six groups consisting of six rats each and were fasted for 18 hand use. Orally administration of the stepwise doses in which the starting dose of unprocessed turmeric was 100mg/kg of animal weight. The increasing doses were administered up-to 3000mg/kg of animal weight in a range of 100mg/kg. Additionally, 3 mice were kept as control. Same procedure was repeated for processed turmeric. Since no mortality was observed in both studies, the behavioural pattern was unaffected. Hence, the dose selected for all the extract were as per the OECD guidelines No 423, fixed dose methods for evaluation of anti-diabetic activity was 300mg/kg, body weight (1/10 of 3000 mg/kg body weight.)

Drug preparation and administration

The turmeric powder is insoluble in water. Processed and unprocessed turmeric powder mercerized in the carboxy Methyl Cellulose (CMC) Sodium Salt was used as a drug and administered orally by intragastric intubation.

Induction of diabetic

In this method, experimental rats were fasted in individual cage for 24 hours. Care was taken to avoid carophagy. Forty Wistar rats of either sex weighing 190 - 220 gm were divided into five groups of six animals each and grouped as follows [30].

Group I:
Served as normal control and did not receive any treatment
Group II:
Served as diabetic and received single dose of alloxan monohydrate (50mg/kg, intra peritoneal.
Group III
Alloxan monohydrate + unprocessed turmeric (300 mg/kg, p.o.)
Group IV:
Alloxan monohydrate + processed turmeric (300 mg/kg, p.o.)
Group V:
Alloxan monohydrate + Glipizide (5mg/kg, p.o.) and served as Standard.

Diabetes was induced in the rat by injecting alloxan monohydrate intra peritoneally in a single dose of 50mg/kg in 0.95% of weight/volume NaCI, to the overnight fasted rat. Diabetes was confirmed in the alloxan treated rats by measuring the fasting blood glucose concentration 8-10 days post injection [31].

The fasting blood glucose level was measured. The animal whose glucose level did not rise to more than 250mg/dl was rejected. Only animals that presented with glycaemic levels equal to or above 250 mg/dL were submitted to treatments, which consisted of a daily administration of the processed and unprocessed turmeric for 21days.It was considered as a first day and blood samples (Approximately 0.5ml) were collected from rats by puncturing the retro orbital sinus, under mild ether anaesthesia for biochemical estimations. The study was continued for 21 days. For the lipid profile study the animals whose serum cholesterol, triglycerides, HDL and LDL values are found to be increase were selected for the study. The highest value on an average obtained in all animals is as listed, triglyceride 142 mg/dl, cholesterol 137mg/dl, HDL 19mg/dl. LDL 66mg/dl. Induction of diabetic in all animals increases the glycosylated haemoglobin, an average value for that is 9.7%.

Collection of Blood and tissues samples

The blood samples (Approximately 1ml) were collected on 1st, 7^{th}, 14th, and 21^{st} day by puncturing the retro orbital sinus, under mild ether anaesthesia in a EDTA bulb for biochemical estimations. The Plain bulbs, for serum, are used in which, 0.5 ml blood sample was added. Both the blood samples were centrifuge at 5000 RPM for 20 min. At the end of the studies, on 21^{st} day the animals were sacrificed, pancreas was quickly removed. The samples were rinsed using isotonic saline for three times and were fixed at 40% natural buffered formalin.

Determination of Biochemical parameters

Glucose, Cholesterol, Triglycerides, HDL cholesterol were estimated on 1^{st}, 7^{th}, 14^{th} and 21^{st}.days of study. The insulin level was also determined using the samples collected on 1^{st}, 14^{th} and 21^{st} day. Estimation of lipid profile was performed using the Randox (Crumlin, Co. Antrim, Northern Ireland) diagnostic kit. In the assay of Cholesterol the cholesterol oxidase-phenol + aminophenazone (CHOD-PAP) colorimetric method was used. The Randox TG assay is based on the glycerol-3-phosphate oxidase (GPO)/PAP colorimetric method. In the estimations of HDL and LDL Randox direct clearance method was used. The Plasma has been used to determine glucose level by glucose oxidase enzymatic method using diagnostic kits (Span diagnostic Ltd.)All reagents are ready-to-use liquids and enzymes suitable for use on a wide range of chemistry analysers. The Techo kit with calibrator was used to estimate glycosylated haemoglobin. To assay the Serum insulin level by achemi-immunoassay method, kit supplied by (Bhabha Atomic Research centre, Mumbai MH) was used.

Statistical analysis

The obtained data was subjected to statistical analysis. Data was expressed as mean ± standard deviation. The Comparative analysis between variables was done using student t-test (Unpaired) in Graph pad prism software. The statistical analysis was done by applying ONE WAY ANOVA followed by Dunnett's multiple comparison tests. Statistical significance was set at $P < 0.05$ and it is considered significant in relation to control and standard. All statistics were done using Graph-pad prism (version6).

Preparation of Tissue homogenate

All groups rats pancreas were successfully removed and labelled properly. Pancreas tissues from each animal were minced and 10% (W/V) tissue homogenate was prepared using 0.1 M Phosphate buffer (PH 7.4) in a Potter- Elvehjem type homogenizer. All the test tubes were labelled carefully and then subjected to centrifugation at 1000xg, 4^0C for 10minutes.A pellet from all the test tube was discarded. The supernatants were subjected for centrifugation at 12.000xg for 20 min. at 4^oC to obtained mitochondrial supernatant (PMS).The supernatants were sieved separately through two layers of muslin cloth and immediately used to estimate the activity of catalase and superoxide dismutase (SOD), GSH.

Estimation of antioxidant enzymes

Catalase activity was determined according to the method of luck (1974).3 ml H_2O_2- phosphate buffer of pH 7 (12.5mM H_2O_2 in 67 mM Phosphate buffer) was pipetted directly in the cuvette. 0.05 ml of tissue homogenate (10%) mitochondrial supernatant was added to the buffer solution. The content was mixed and the decrease in the absorbance was followed and noted at 240nm for 3 minutes. The results were expressed as µmole of H_2O_2 decomposed/ min/mg protein using Molar extinction coefficient of H_2O_2 (71 mM-1cm.l) at 240nm. The enzyme solution containing H_2O_2-free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05 units.

Superoxide dismutase activity was assayed by the method of Kono. An aqueous solution of hydroxylamine was prepared daily and its pH was adjusted to 6.0 with 2 M sodium hydroxide. Control consist of a mix of 2 ml of NBT (Nitroblue tetrazolium 1.5mM)), 0.5 ml hydroxylamine hydrochloride. It is non-enzymatic reaction. In a test, enzymatic reaction, 2 ml of NBT was mixed with 0.5 ml of hydroxylamine hydrochloride and appropriate amount of mitochondrial supernatant was added the development of blue colour was measured at 560nm for 2 minutes. Enzyme activity was expressed as Units /mg protein where one unit of enzyme is defined as the amount of enzyme inhibiting

NBT reduction by 50%.

Estimation of lipid peroxidation [32,33] in terms of malonaldehyde (MA) thiobarbituric acid reaction was performed. The reaction mixture contained 0·1 ml of tissue homogenate, 0·2 ml of 8·1% sodium dodecyl sulphate (SDS),1·5 ml of 20% acetic acid and 1·5 ml of 0·8% aqueous solution of thiobarbituric acid (TBA).The pH of 20% acetic acid was adjusted with 1 N NaOH to 3·5.The mixture was finally made up to 4·0 ml with distilled water and heated at 95°C for 60 min. After cooling under tap water,1·0 ml of distilled water and 5·0 ml of a mixture of n-butanol and pyridine (15:1 by vol) was added and the mixture was shaken vigorously on a vortex mixer. After centrifugation at 2200 g for 5 min the absorbance of the organic layer (upper layer) was measured immediately at 532 nm. Lipid peroxide formation in tissues clearly requires substrates, the unsaturated fatty acids of the tissue lipids, and a catalytic system. Either of these factors may limit or control the extent of peroxide formation.

Histological study

Collected pancreatic tissues were tissue fixed at 40% natural buffered formalin, dehydrated by passing through a graded series of alcohol, and embedded in paraffin blocks. Using hand rotatory microtone 5 mm sections were developed. Haematoxylin and eosin were used for staining.

RESULT AND DISCUSSION

Acute toxicity study

In the acute toxicity study the various observations showed the normal behaviour of all treated rats. None of the studied processed turmeric and unprocessed turmeric drug showed any significant toxicity sigh during the first 4 hrs. The daily observations for 14 days do not show any mortality. The drug was found to be safe at the all tested levels of concentration and at the higher tested dose. No toxic effects were observed at a higher tested dose of 3000m g/kg of body weight. There were no lethal effects in any of the group till the end of the study. One tenth of this dose level was taken as effective dose.

Biochemical parameter

It was observed that due to hyperglycemia there was an increase in the glucose, triglyceride and total cholesterol levels. Plasma glucose, serum Triglycerides (TG), Total Cholesterol (TC), Low Density Lipoproteins(LDL), and glycosylated Hb (HbA1C)levels were significantly elevated and High Density Lipoprotein (HDL) level was decreased in group II(diabetic rats) when compared with Group I(control rats). In the Group I, blood glucose levels have almost similar level till the end of the study (80.1205± 1.0358). At first day the average plasma glucose level recorded in group II was 277.8 mg% which was increased 283mg%., 338.7mg% 358.7mg% at 7^{th}, 14^{th}, 21^{st}day of studies respectively.

Diabetic rats fed on unprocessed turmeric drug (300mg/BW) that is Group III showed a significant decrease in plasma glucose level presented in table 1.

At the first day the average plasma glucose level of group II rats was 373.5mg% before the feeding of both drugs. This level was found to be reduced after treatment with processed turmeric and recorded as 228.467mg% and using unprocessed turmeric drug it was recorded as 157.75mg%. Plasma glucose in unprocessed treated animal was found to be reduced ($^{***}p\leq0.001$) when compare with diabetic animals. The serum cholesterol and triglyceride values recorded in all animals, the average cholesterol level of cholesterol was 137mg/dl at the first day of experiment and of triglycerides it was 141mg/dl%. The condition is called as hypercholesterolemia. The blood cholesterol levels shows significant reduction in group IV at the end of 21^{st} day,($p\leq0.001$).Similarly the reduction of Triglyceride level was recorded and it was 116mg% ($p\leq0.001$) when compared with group II. The high density lipoprotein value of group II compare with the group III and group IV and V and in all cases there was significant difference ($p \leq0.001$).At the first day of experiment in diabetic group the average value of HbA1C was recorded as 9.555%. At the end of the study values are significantly decreased in group IV 6.4%. A significant reduction of LDL, levels ($p< 0.001$) and increase in HDL level were observed after the treatment with unprocessed turmeric drug.($p< 0.001$).The cholesterol values of group V compared with group II and a significant difference noted was ($p \leq0.01$) whereas the statistical significant change in the cholesterol values of group IV with group II was found to be more significant. ($p< 0.001$) [Table no. 1], [Table no.2],[Graph no.1] and [Graph no.2].

BIOCHEMICAL PARAMETERS

Body weight analysis

The alloxan produced significant loss in the body weight as compared to the normal rat within 21 days of the study ($p<0.001$). At the end of 21^{st} day of the treatment the statistical analysis of body weight data was shows significant changes. The administration of unprocessed turmeric drug (300mg/body weight) restore the changes in the body weight to near normal level as seen in table($p<0.001$).There was no significant difference between the group III and group V. The changes in the body weight of Group IV are less significant. ($p< 0.01$)

Antioxidant enzymes

The activity of the antioxidant enzyme SOD, CAT, and lP in the normal and diabetic at is studied. The level of Superoxide dismutase (SOD), Catalase (CAT) and Lipid peroxidase (LP) decreased in the alloxan induced diabetic (group II) rats (Table 4).

Upon administration of unprocessed turmeric drug (Group III) with dose 300 mg/kg in rats indicate significant change in the level of SOD,CAT, LP as compared to the

diabetic control, glipizide treated (group V) rats graph1. Oral administration of unprocessed turmeric drug dose (300 mg/kg) brought to the value to near the normal, similar to the glipizide treated (group V) group rat but the oral administration of processed turmeric drug dose(300mg/Kg) does not show similar effects. It indicates the antioxidant potential of unprocessed turmeric.

The low level of SOD, CAT and LP in diabetic (group II) rats indicates the high levels of free radical hyperglycemia. There was an observed decrease in the concentration by increased oxidative stress by alloxan induced Hyperglycemia. The decrease in an antioxidant level in Group II rats might also be due to DNA damage by the alkylating agent alloxan which in turn increases free radical production.

Changes of histopathology of the pancreas

The histopathological examination of the pancreas of the non diabetic rats showed round and elongated islets which were evenly distributed throughout the cytoplasm. In diabetic control animals, the cells were irregular, not well defined and necrosis of the cells could be clearly appreciated. The standards group showed a mild protection from alloxan induced changes in the pancreatic islets. The unprocessed turmeric at 300mg/kg showed slight regeneration of beta cells when compared with the diabetic control.(A) Further study require electron microscopy study.

Table 1. The biochemical parameters studied are Gluc.(Glucose), Chole.(Cholesterol), Tg(Triglycerides) HDL(High density lipoprotein) LDL (Low density lipoprotein) and Gly-Hb (Glycosylated Hb that is HbA1C) in blood sample of Control and Experimental groups

Test blood	Group -I Normal i.e. Control	Group- II Diabetes Induced	Group-III Diabetes + Standard Gilpizide	Group-IV Diabetes + unprocessed Turmeric drug 300mg/kg of B.W.	Group- V Diabetes + processed Turmeric drug 300 mg/kg of BW
Gluc.	80.1205± 1.0358	359.8± 6.342^{a***}	144.46± 1.492^{b***e**}	157.75±2.529^{c***}	228.467±3.65^{d***}
Chole.	93.020±1.020	137.366± 1.714^{a***}	118.2± 0.642$^{b***\ ens}$	116.95±1.089^{c***}	122.5±1.075^{d**}
Tg	91.29±1.593	141.81± 2.228^{a***}	108.183± 0.828$^{b***\ e**}$	119.223±2.288^{c***}	114.68±1.932^{d***}
HDL	36.478±0.724	19.773± 1.773^{a***}	38.788±0.2482$^{b***\ e**}$	36.55±0.938^{c***}	37.66±1.188^{d***}
LDL	21.433±0.469	64.235± 0.829^{a***}	22.401±0.2104$^{b***\ e**}$	23.61±0.427^{c***}	45.47±0.800^{d**}
Gly- Hb	6.3667±0.102	9.655± 0.060^{a***}	8.0117±0.3722^{b***e**}	6.4333±0.105^{c***}	8.595±0.0802^{d**}

Values are expressed as mean±SD. Values are taken as a mean of six experimental animals and are statistically significant at p≤0.05.Satistical significance was compared within the group as follows,

a= Group II compared with Group I b= Group III compared with Group II

c= Group IV compared with Group II d= Group V compared with Group II

e=Group V compared with Group III *** P ≤0.001, **P≤0.01, *P≤0.05

ns= Non significant

Table 2. Body weight (Grams) changes recorded in Control and Experimental animal at the end of 21st day. It express the mean body weight of all groups of rats at the end of the study.

Group	Initial body weight	Final body weight
Normal Group I	198.40±2.721	211.46±2.376
Diabetic control Group V	207.317±2.956	197.683±2.156
Diabetic Induced Group II	211.0±3.024	171.7±4.367***
Diabetic+ unprocessed turmeric Group III	198.56± 4.639	197.41±4.151$^{a\ ns}$ restore the changes in body weight.
	212.000±5.445	177.90±5.648**

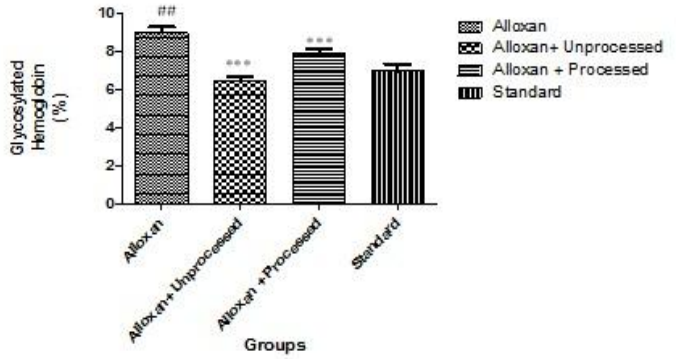
a= Group V compared with Group III Restore the change in the body weight.

Table 3. The antioxidant enzymes Superoxide dismutase (SOD), Lipid peroxidase (LP), Catalase studied in the pancrease tissue of all group animals at end of 21st day.

Group	Catalase	SOD	Lipid peroxidase
Normal Group I	17.57±0.7043	37.50±1.209	66.48±0.992
Diabetic control,group V	18.30±0.284[#]	36.67±0.9977	64.12±0.2884
Diabetic Induced , group II	9.133 ±0.466[c]	11.69±0.2252[f]	29.56±2.303[k]
Diabetic+ unprocessed turmeric drug,group III	19.43 ±0.3774[a]	35.86±1.535[d]	62.46±0.4748[j]
Diabetic+ Processed turmeric drug, group IV	10.87 ±0.4240[b]	21.28±0.670[e]	47.07±1.436[h]

Values are expressed as mean ± SD. Values are taken as a mean of six experimental animals and are statistically significant at p≤0.05.Satistical significance was compared within the group as follows,
a= catalase: Group V with Group III=ns(p=0.0946),
b= catalase: Group V with Group IV =***, c=catalase: Group V with Group II =***
d= SOD= Group V with Group III= ns(p=0.341),
e= SOD =Group V with Group IV=***, f= SOD= Group V with Group II =***
j= LP= Group V with Group III =*(p=0.0102), h = LP= Group V with Group IV =***
k= LP= Group V with Group II =***P≤0.001, **P≤0.01, *P≤0.05 ns= Non significant

Graph 1. Effect of unprocessed and processed Turmeric on Glycosylated Haemoglobin



Graph 2. Effect of unprocessed and processed Turmeric on Serum Glucose.

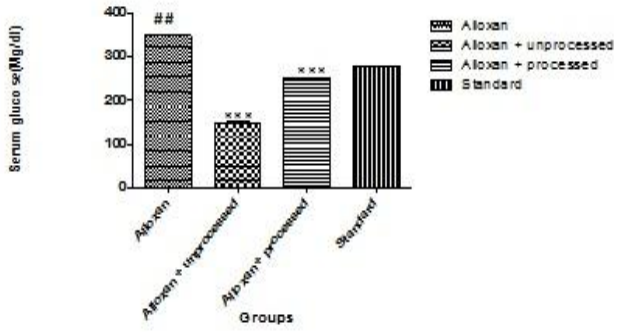


Fig 3. A unprocessed group

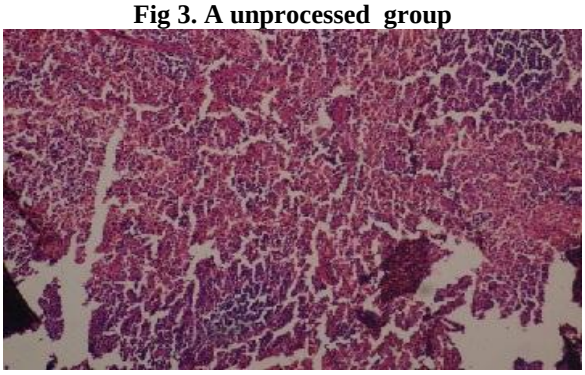
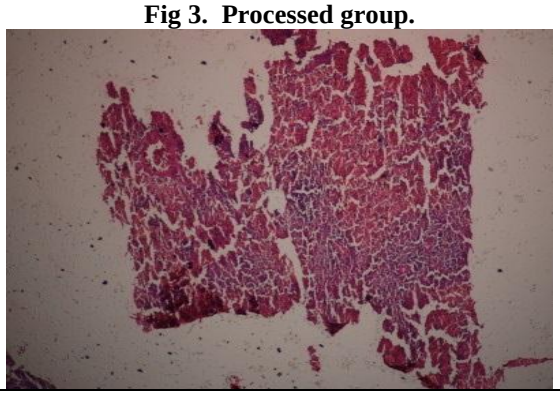


Fig 3. Processed group.



CONCLUSIONS

The unprocessed turmeric drug effectively reversed the alloxan-induced changes in the blood sugar level and the beta-cell population in the pancreas. The antioxidant activity of it also showed a protective effect when it was given prior to alloxan administration. The action of unprocessed turmeric powder plant extracts on the pancreatic beta-cells and absence of acute toxicity may offer a new hope to the diabetics in future in concern with the cheap and easily availability of the drug.

From the above discussion and results itis concluded that unprocessed turmeric drug at aldose (300 mg/kg) shows significant anti-hyperglycaemic activity than the processed turmeric drug at same dose in alloxan-induced diabetic rats. There is also an improvement noted in the unprocessed group animals, in the other parameters which include body weight, Lipid profile glycosylated haemoglobin as well as regeneration of β cells of pancreas. It might be of value in the treatment of hyperglycaemia .It is cost effective, cheaper source without side effect. Further

investigation is in necessary to determine the exact phytoconstituents (s) responsible for hypoglycaemic effect.

ACKNOWLEDGEMENT: None

CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

REFERENCES

1. Parthiban R, Anbu J. (2012). Antidiabetic activity of kovai kizhangu chooranam in Alloxan induced diabetic rats. *International journal of life science and pharma research*, 2(4), 201.
2. Kumar NK, Loganathan K. (2010). Hypoglycemic effects of *S. Oleracea*in Alloxan induced Diabetic Rats. *J Biotech &biochem*, 5 (2), 87-91.
3. Gandhi HR. (2001). Diabetes and coronary artery disease: importance of risk factors. *Cardiol Today*, 1, 31–4.
4. Baquer N Z, Gupta D, Raju J. (1998). Regulation of metabolic pathways in liver and kidney during experimental diabetes: effects of antidiabetic compounds. *Indian J ClinBiochem*, 13, 63–80.
5. Mohandas Rai, Paramesha S, Suchetha Kumari N, Damodara Gowda KM, Vidhya GG. (2011). Effect of garlic on total antioxidants in alloxan Induced diabetes mellitus rats. *International Journal of Biomedical and Advance Research*, 02, 2011, 09.
6. Cameron NE, Cotter MA. (1997). Metabolic and vascular factors in the pathogenesis of diabetic nephropathy. *Diabetes*, 46(2), S31–S37.
7. Casper G, *et al*. (2004). Vascular complications in diabetes mellitus: the role of endothelial dysfunction. *Clinical science*, 109, 143–159.
8. Nishikawa T, Edelstein D. (2000). Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage**.** *Nature*, 404, 787-790.
9. Al-Shamoun LA, Al-Khazraji SM, Twaiji HAA. (1998). Hypoglycemic effect of Artemisia herba alba. II. Effect of a valuable extract on some blood parameters in diabetic animals. *J Ethno Pharmacol*, 43, 167–171.
10. Inouye M, Hashimoto H, Mio T, Sumino K. (1998). Levels of lipid peroxidation product and glycated hemoglobin A1C in the erythrocytes of diabetic patients. *Clin Chim Acta*, 276, 163–172.
11. Manjulata Kumawat, Tarun Kumar Sharma. (2013). Antioxidant Enzymes and Lipid Peroxidation in Type 2 Diabetes Mellitus Patients with and without Nephropathy. *J Med Sci*. 5(3), 213–219.
12. Tortora GJ, Derrickson B. (2006). Principal of anatomy and physiology, 11th edition. United State. John Wiley & Sons.Inc, 669.
13. Parthiban R, *et al.* (2012). Antidiabetic activity of kovaikizhanguchooranam in Alloxan induced diabetic rats. *International journal of life science and pharm research*, 2(4), 68.
14. King H, Herman W. (1998). Global Burden of Diabetes, 1995–2025: prevalence, numerical estimates, and projections. *Diabetes Care*, 21(9), 1414–1431.
15. Ahmed D, Sharma M, Mukerjee A, Ramteke PW, Kumar V. (2015). Improved glycemic control, pancreas protective and hepatoprotective effect by traditional poly-herbal formulation "QursTabasheer" in streptozotocin induced diabetic rats. *BMC Complement Altern Med*, 1, 24.
16. Meenakshi P, Bhuvaneshwari R, Rathi MA, Thirumoorthi L, Guravaiah DC, Jiji MJ, *et al*. (2010). Antidiabetic activity of ethanolic extract of Zaleya decandra in alloxan-induced diabetic rats. *ApplBiochemBiotechnol*, 162(4), 1153-1159.
17. Satyavati GV and Gupta AK, Shankar TNB, Shanta NV, Ramesh HP, Murthy IAS, Murthy VS. (1987). Medicinal plants of India eds Indian Council. *Ind. J. Exp. Biol*. 18(2), 1987, 18-73.
18. Shankar TNB, Shanta NV, Ramesh HP, Murthy IAS, Murthy VS. (1980). Toxicity Studies onTurmeric (Cuecuma longa): Acute Toxicity studies in rats, Guineapigs& Monkeys. *Ind. J. Exp. Bio*, 18(1), 73-75.
19. Holman RR and Turner RC. Oral agents and insulin in the treatment of NIDDM. Text Book of Diabetes, J. Pickup and G.Williams, Blackwell,Oxford, UK, 1991, 467–469.
20. Kameswara Rao B. (2001). Antihyperglycemic activity of Momordica cymbalaria in alloxan diabetic rats. *Journal of Ethno pharmacology*, 78(1), 2001, 67–71.
21. Kameswrarao B. (1997). Herbalmedine in The Management of Diabetes Mellitus in Developing Countries. Interprintby Indigenous Resources, New Delhi, 375–377.
22. Indian Spices. (2013). Turmeric processing".kaubic.in, 07.
23. Chattopadhyay, Ishita, Kaushik Biswas, Uday Bandyopadhyay; Ranajit K Banerjee. (2004). Turmeric and curcumin, Biological actions and medicinal applications. *Current Science*, 87(1), 44–53.
24. Tayyem RF, Heath DD, Al-Delaimy WK, Rock CL. (2006). Curcumin content of turmeric and curry powders. *Nutr Cancer*, 55(2), 126–131.
25. Nagpal M, Sood S. (2013). Role of curcumin in systemic and oral health: An overview. *J Nat Sci Biol Med*, 4(1), 3–7.

26. Tayyem RF, Heath DD, Al-Delaimy WK, Rock CL. (2006). Curcumin content of turmeric and curry powders. *Nutr Cancer*, 55(2), 126–131.
27. Nagpal M, Sood S. (2013). Role of curcumin in systemic and oral health: An overview. *J Nat SciBiol Med* 4 (1), 3–7.
28. Dunn JS, Sheehan HL, Mcletchie NGB. (1943). Necrosis of islets of Langerhans produced experimentally. *Lancet*, 241, 484-487.
29. OECD/OCDE. (2000). OECD Guidelines for the testing of chemicals, revised draft guidelines 423: Acute Oral toxicity-Acute toxic class method, revised document, CPCSEA, Ministry of Social Justice and Empowerment, New Delhi: Government of India.
30. Semwal C, Shah K, Chauhan NS, Badhe R, Divakar K. (2008). Anti-diabetic activity of stem bark of Berberis aristata. in alloxan induced diabetic rats. *The International Journal of Pharmacology*, 6-1.
31. Federiuk IF, Casey HM, Quinn MJ, Wood MD, Ward WK. (2004). Induction of type 1 diabetes mellitus in laboratory rats by use of alloxan; route of administration, pitfalls, and insulin treatment. *Comprehensive Med*, 54, 252-257.
32. Ohkawa H, Ohishi N and Yagi K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal.Biochem*, 95, 351.
33. Devasagayan TPA, Bbloor KB. (2003). Methods for estimating lipid peroxidation : an analysis of merits and demerits. *Indian journal of Biochemistry and Biophysics*, 40, 300-308.