International Journal of Research in Pharmacology & Pharmacotherapeutics

ISSN Print: 2278-2648 IJRPP |Vol.9 | Issue 2 | Apr - Jun - 2020
ISSN Online: 2278-2656 Journal Home page: www.ijrpp.com

Research article Open Access

# Antidiabetic activity of alcoholic fruit extract of *mallotus philippensis* muell.arg. in streptozotocin induced diabetic rats

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## ABSTRACT

Herbal drugs are frequently considered to be less toxic and also free from side effects, than synthetic ones. In the present work the antidiabetic activity of the *Mallotus philippensis* was evaluated. The alcoholic fruit extract showed significant decrease in blood glucose level, glycosylated haemoglobin level and significant increase in plasma insulin level when administered orally for 21 days to STZ induced diabetic rats at a dose of 200 and 400 mg/kg. The extract showed significant decrease in serum parameters such as total cholesterol level, ALT and AST levels. In antioxidant study, the extract showed significant activities in both enzymic antioxidant parameters like SOD, CAT, GPx and non ezymic antioxidant parameters like GSH, Vit C, Vit E. These results demonstrate the antidiabetic potential of alcoholic fruit extracts of *Mallotus philippensis* and suggests that *M.philippensis* fruit may have the therapeutic value in diabetes and related complications.

**Keywords:** Mallotus philippensis, Antidiabetic, Diabetes mellitus, STZ Induced rats

## INTRODUCTION

Diabetes mellitus commonly referred as Diabetes, it is a group of metabolic diseases in which there is high blood sugar levels over a prolonged period [1]. Symptoms of high blood sugar include frequent urination, increased thrist, and increased hunger. If left untreated, diabetes can cause many complications [2]. Acute complications can include diabetic ketoacidosis, non-ketotic hyperosmolar coma, or death [3]. Serious long term complications include heart disease, stroke, chronic kidney failure, foot ulcers, and damage to eyes [2]. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced [4]. There are four types of diabetes mellitus:

- Type 1 diabetes results from the pancreas‘s failure to produce enough insulin. This form referred as “insulin dependent diabetes mellitus” (IDDM) or “juvenile diabetes”. The cause is unknown [2].
- Type 2 diabetes begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progress a lack of insulin may also develop. This form referred as “non insulin dependent diabetes mellitus”

(NIDDM) or "adult onset diabetes". The primary cause is excessive body weight and not enough exercise [2].

- Gestational diabetes is the third main form and occurs when pregnant women without a previous history of diabetes develop high blood sugar levels. They prone to diabetes in future [5].
- **MODY** Maturity Onset Diabetes of the Young is the fourth type of diabetes. Specific monogenetic defects of the beta-cells have been identified and usually give rise to maturity onset diabetes of the young (MODY). MODY is defined as a genetic defect in beta-cell function [6].

Prevention and treatment involve maintaining a healthy diet, regular physical exercise, a normal body weight, and avoiding use of tobacco. Control of blood pressure and maintaining proper foot care are important for people with the disease. Type 1 DM must be managed with insulin injections. Type 2 DM may be treated with medications with or without insulin [7]. Insulin and some oral medications can cause low blood sugar [8]. Weight loss surgery in those with obesity is sometimes an effective measure in those with type 2 DM [9] gestational diabetes usually resolves after the birth of the baby. Mody type diabetes managed by sulfonylureas treatment [6] as of 2015, an estimated 415 million people had diabetes worldwide, with type 2 DM making up about 90% of the cases. This represents 8.3% of the adult population, with equal rates in women and men. As of 2014, trends suggested the rate would continue to rise. Diabetes at least doubles a person's risk of early death. From 2012 to 2015, approximately 1.5 to 5.0 million deaths each year resulted from diabetes [10]. Mallotus philippinensis Muell. Arg (Euphorbiaceae) are widely distributed perennial shrub or small tree in tropical and subtropical region in outer Himalayas regions with an altitude below 1,000 m and are reported to have wide range of pharmacological activities. Mallotus philippinensis species are known to contain different natural compounds, mainly phenols, diterpenoids, steroids, flavonoids, cardenolides, triterpenoids, coumarins, isocoumarins, and many more especially phenols; that is, bergenin, mallotophilippinens, rottlerin, and isorottlerin have been isolated, identified, and reported interesting biological activities such as antimicrobial, antioxidant, antiviral, cytotoxicity, antioxidant, anti-inflammatory, immunoregulatory activity protein inhibition against cancer cell [11]

## MATERIALS AND METHODS

### Collection and authentication, shade drying and granulation of plant material

The Fruits of *Mallotus Philippensis* Muell.Arg. were collected in the month of December from, Cherpulassery, Palakkad (Dist), Kerala South India. The plant material was taxonomically identified and authenticated by Dr.A.Balasubramanian, Director, ABS Botanical conservation, Research & Training center, Kaaripatti, Salem.

### Preparation of extract

*Mallotus Philippensis* Muell.Arg fruits were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No 40 and stored in an airtight container for further use.

### Extraction procedure

The coarse Fruit powder was extracted with ethanol by continuous hot percolation using soxhlet apparatus. After completion of extraction, extract was filtered and the solvent was removed by under reduced pressure. The dried extract was stored in desiccators.

### Animals

Adult Wistar albino rats of either sex weighing 150-160 gm were procured from the animal house of Kings Institute, guindy, Chennai, Tamilnadu, India used. The animals were maintained on the suitable nutritional and environmental condition throughout the experiment. The animals were housed in polypropylene cages with paddy house bedding under standard laboratory conditions for an acclimatization periods of 7 days prior to performing the experiment. The animals had access to laboratory chow *ad libitum* (H.G.Vogel, 2002). The Institutional Animal Ethics Committee approved the experimental protocol and the conditions in the animal house approved by Committee for Supervision on Experiments on Animals. The study was conducted in accordance with IAEC guidelines (**IAEC approval No:**

**IAEC/XLVIII/04/CLBMCP/2016 dated on 04/05/2016**).

## Acute oral toxicity

Acute oral toxicity refers to those adverse effects that occur following oral administration of a single dose of a substance or multiple doses given within 24 hours [12].

### *$LD_{50}$ (median lethal oral dose)*

$LD_{50}$ (median lethal oral dose) is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. The $LD_{50}$ value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

## Experimental induction of diabetes

A freshly prepared solution of STZ (45 mg/kg in 0.1 M citrate buffer, pH 4.5) was injected intraperitoneally to overnight-fasted rats The rats exhibited hyperglycaemia within 48 h of STZ administration. The rats having fasting blood glucose (FBG) values of 250 mg/dl or above were considered for the study [13]. The antidiabetic activity was tested on a total of 30 rats (24 diabetic rats and 6 normal rats) and they were divided into five groups and each group consists of 6 animals as follows,

**Group I-** Served as control, received 0.5% CMC (1ml/kg; p.o) for 21 days.

**Group II-** Diabetic control received single streptozocin injection (45mg/kg; b.wt; i.p) freshly prepared in citrate buffer on Day 1 and received citrate buffer for 21 days.

**Group III-** STZ+ plant extract low dose (200mg/kg, b.wt; p.o) suspended in 0.5% CMC for 21days

**Group IV-** STZ+ plant extract high dose (400mg/kg, b.wt; p.o) suspended in 0.5% CMC for 21days

**Group V-** STZ+ Standard Glibenclamide (600 µg/kg, b.wt; p.o) dissolved in 5% CMC for 21days

The study involved repeated administration of *EEMP* for 21 days at a prefixed times and blood glucose levels were estimated in samples withdrawn after 1st day, 7, 14 and 21st day. The animals had free access to food and water during this period.

Blood samples from the experimental rats were collected from the tail by using pricking lancet. The collected blood samples were analyzed for blood glucose levels by the glucometer using strip technique and blood glucose levels were expressed in mg/dl. Fasting blood glucose of all rats was determined before the start of the experiment. On day 21st the blood was collected by retro orbital under mild ether anaesthesia from overnight fasted rats, into tubes containing potassium oxalate and sodium fluoride as anticoagulant for estimation of fasting plasma glucose. Plasma and serum were separated by centrifugation. After centrifugation at 2,000 rpm for 10 minutes, the clear supernatant was used for the analysis of various biochemical parameters. After collection of blood, all the treated animals were sacrificed the pancreas and liver tissues were isolated and rinsed in ice- cold saline and kept in formalin solution (10%) for further histopathological studies.

The liver was quickly removed and perfuse immediately with ice-cold saline (0.9% NaCl). A portion of the kidney was homogenized in chilled Tris-HCl buffer (0.025 M, pH 7.4) using a homogenizer. The homogenate obtained was centrifuged at 5000 rpm for 10 min, supernatant was collected and used for various biochemical assays.

The Blood glucose levels were estimated by Hexokinase method [14]. The glycosylated haemoglobin levels were estimated by "Tina-quant" (turbidimetric inhibition immunoassay" [15]. The total cholesterol levels were estimated by cholesterol oxidize- Peroxidase method. [16] Estimation of Aspartate aminotransferase (AST, Serum Glutamic-Oxaloacetic Transaminase) and Alanine aminotransferase (ALT, Serum Glutamic-Pyruvic Transaminase) by enzyme catalyzes the reaction [17, 18]. Lipid peroxidation (LPO) was assayed by the method of Ohkawa et al. [19] Estimation Superoxide dismutase (SOD) was assayed by the method of Misra and Fridovich [20]. Estimation of Catalase (CAT) was assayed by the method of Sinha [21]. Estimation of Glutathione peroxidase (GPx) was assayed by the method of Rotruck et al. [22]. The total reduced glutathione (GSH) was determined according to the method of Ellman. [23] The level of vitamin C was estimated by the method of Omaye et al. [24] Vitamin E content was estimated by the method of Palan et al. [25] Insulin was assayed in plasma using a commercial kit by enzyme linked immunosorbant assay (ELISA) technique. [26]

## Histopathology

A bit of tissue from pancreas was cut and fixed in bouin's fluid immediately after removal from the animal body. The tissues were fixed in bouin's fluid for about 24 hours. The tissues were then taken and washed in glass distilled water for a day to remove excess of picric acid. The sections were stained with hematoxylin and eosin (H & E) stain, which were used to demonstrate different structures of the tissue

## Statistical analysis

The data of all the results were represented as Mean ± S.E.M. on statistically analysed by one-way ANOVA followed by Tukey's multiple comparison test was used for statistical analysis $p<0.05$ was considered significant.

# RESULTS AND DISCUSSION

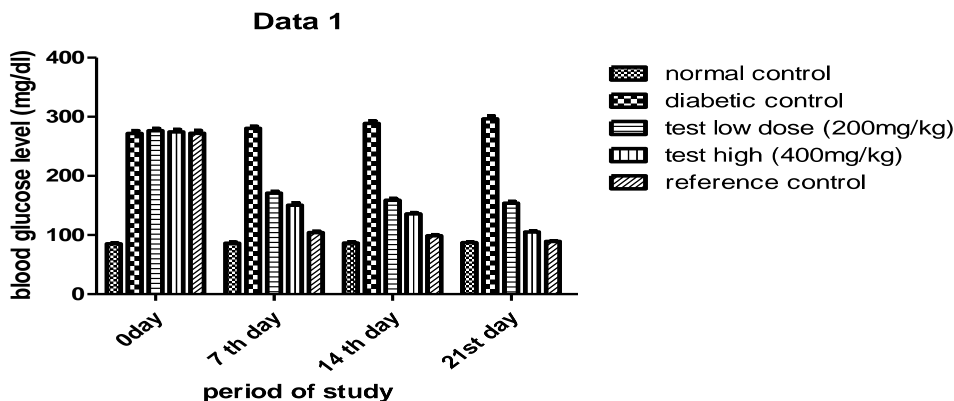


**Fig.1. Shows Blood glucose level of Groups-I to V**

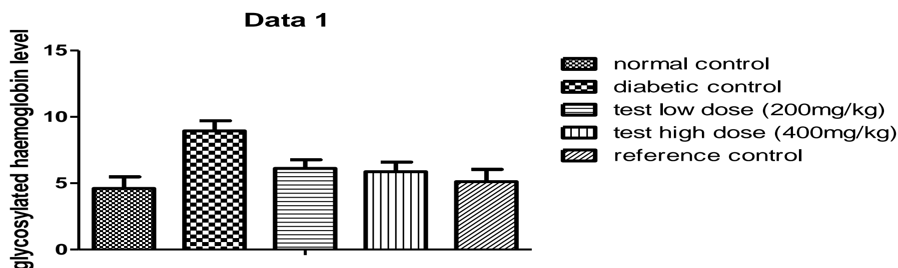


**Fig.2. Shows glycosylated Hb level of Groups-I to V**

**Table.1 shows Total cholesterol level of Groups-I to V**

| Groups | Total cholesterol level |
|---|---|
| Control 0.5% CMC (1ml/kg; p.o) | 65.88±1.203 |
| STZ (45mg/kg; b.wt; i.p) | 140.38±1.244* |
| STZ + Plant extract LD (200mg/kg, b.wt; p.o) | 77±2.399** |
| STZ+ Plant extract HD (400mg/kg, b.wt; p.o) | 72.06±2.033** |
| STZ+ Glibenclamide (600 µg/kg, b.wt; p.o) | 69.48±2.333** |

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).
*= when compared to the control group.
** = when compared to the STZ treated group.
Data was analysed by one-way ANOVA followed by Tukey's multiple comparison test.

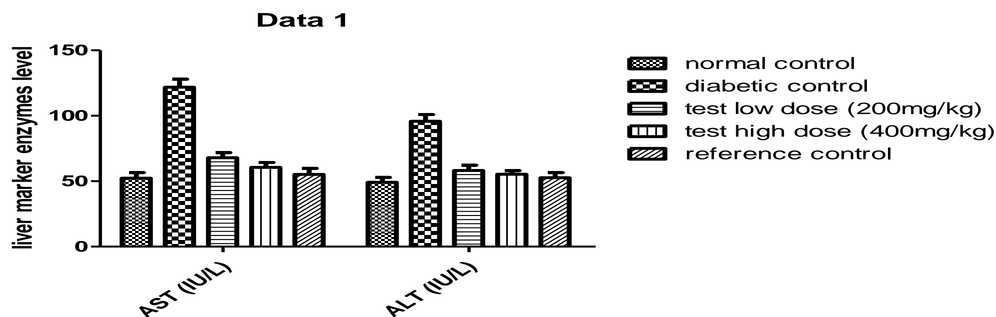


**Fig.4 Shows Liver marker enzymes (AST & ALT) level of Groups-I to V**

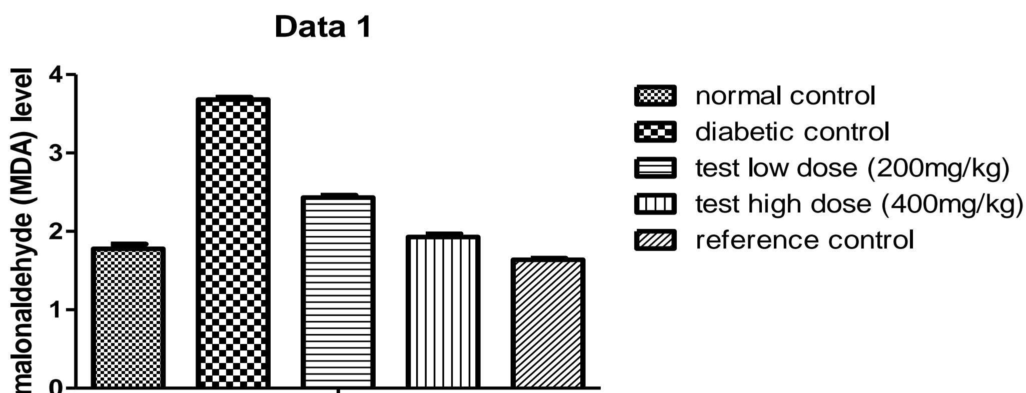


**Fig.5 Shows levels of MDA of Groups-I to V**

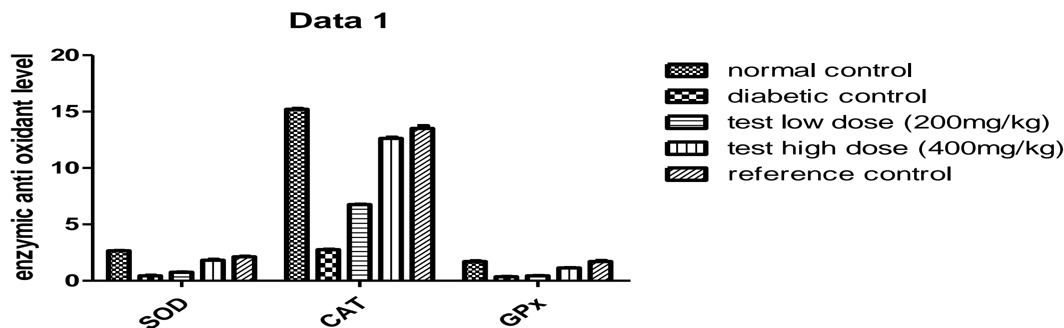


**Fig.6 Shows SOD, CAT & GPx level of Groups-I to V**

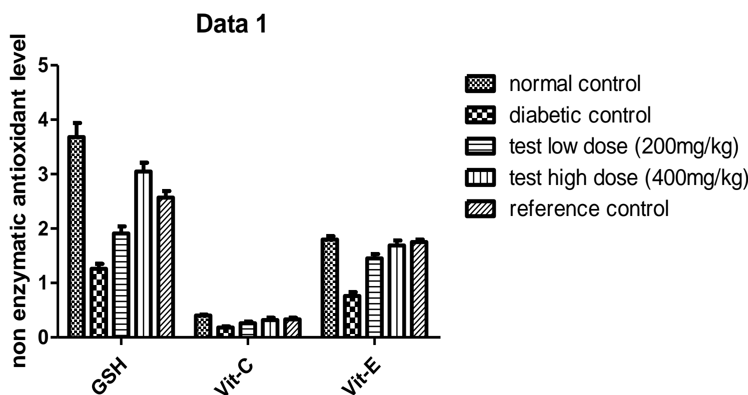


**Fig.7 Shows GSH, Vit.C & Vit E level of Groups-I to V**

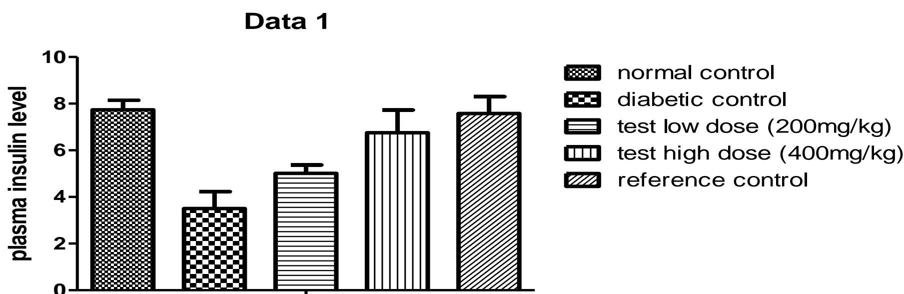


**Fig.8 Shows Plasma Insulin level Groups-I to V**

Diabetes mellitus, a chronic metabolic disease characterized by a deficiency in the pancreas insulin production and/or by peripheral insulin resistance. The management of diabetes without any side effects is still a challenge to the medical system. Herbal drugs are prescribed widely because of their effectiveness, fewer side effects and relatively low cost. Wide array of plant derived active principles have demonstrated anti-diabetic activity. The adverse effects of hypoglycaemic drugs and insulin and the excessive cost of these medications can be mentioned as some disadvantages regarding the diabetes treatment, which stimulate the search for new therapeutic agents that present safety, effectiveness and low cost. Nowadays there is growing trend towards using herbal preparations and/or derivatives in traditional and complementary medicines to treat symptoms. [27] In this way, it has been cresent the

interest of current ethnopharmacological research to investigate the plants species with antihyperglycemic effect, focusing in the evaluation of the efficacy and safety of plant preparations for diabetic treatment.

The fundamental mechanism underlying hyperglycemia in diabetes involves over production (excessive glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues. The metabolism of glucose, proteins and lipids is abnormal in diabetes due to insulin secretion defect, leading to various metabolic disorders. Herbal drugs may act on blood glucose through different mechanism, some of them may have insulin like substances. Stimulation of beta cells to produce more insulin and others may increase cells in the pancreas by activating regeneration of pancreatic cell.

The *Mallotus Phililppensis* Muell.Arg. Fruit had been claimed for its anti-diabetic activity and there is no degree of research work which has not been done but, claiming *Mallotus philippensis* Fruits have therapeutic use on blood glucose levels. [11] Hence, project on *Mallotus Phililppensis* Muell. Arg. Fruit was carried out to provide scientific validation on anti-diabetic activity.The preliminary phytochemical analysis of *EEMP* revealed the presence of carbohydrates, flavanoids, terpenoids, glycosides, proteins, tannins, steroids and phenols. Mainly flavanoids which may be responsible for its anti-diabetic properties [18]. Acute toxicity studies revealed the non-toxic nature of the *EEMP* There was no lethality or any toxic reactions found with high dose (2000 mg/kg body weight) till the end of the study. According to the OECD 423 guidelines (Acute Oral Toxicity: Acute Toxic Classic Method), an $LD_{50}$ dose of 2000 mg/kg and above was considered as unclassified so the *EEMP* was found to be safe.

Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared streptozotocin (STZ-45mg/kg body weight) in 0.1M citrate buffer (pH 4.5) in a volume of 1ml/kg body weight. [18] The diabetic group showed marked increase of glucose level as compared to the normal group. The oral administration of *EEMP* reversed the blood glucose level in which the action was through potentiating of pancreatic secretion of insulin from islets beta cells or due to enhanced transport of blood glucose to the peripheral tissue. There was a significant decrease in blood glucose level in the extract treated groups.

Glycosylated haemoglobin concentrations are helpful and solid tool for the appraisal of glycemic control in diabetics as suggested by the international diabetes federation. [28] Treatment groups (*EEMP* test low dose, *EEMP* test high dose and Glibenclamide) of diabetic rats unquestionably decrease the level of glycosylated haemoglobin. A noteworthy decrease of glycosylated haemoglobin showed the ability of the extract in the control of diabetes. [29]

Hypercholesteremia are primary factor involved in the development of atherosclerosis and coronary heart diseases which are the secondary complications of diabetes. [30] EEMP significantly reduced total cholesterol in STZ-diabetic rats. Thus, it is reasonable to conclude that EEMP, could modulate blood lipid abnormalities. The injection of STZ induces a hepatocellular damage, which is indicated by significant increase in AST and ALT in diabetic group as compare to control group. Furthermore STZ induces hepatocellular damage, which results in leakage of AST and ALT from liver systole to the blood stream and/or may change the permeability of liver cell membrane [31] in the present study, *EEMP* significantly decreased AST and ALT enzyme activities in diabetic rats. The improvements in the levels of the enzyme are a consequence of an improvement in the carbohydrate, fat and protein metabolism. The restoration of AST and ALT after treatment also indicates a revival of insulin secretion. [32] The results from present study indicates that EEMP may reduce the level of serum cholesterol, SGOT and SGPT. It confirms that functions are on the protection of vital tissues Pancreas, thereby reducing the causation of diabetes in experimental animals.

Lipid peroxidation eventually leads to extensive membrane damage and disfunction. [33] Decreased lipid peroxidation and improved antioxidant status may be one of the mechanism by which drug treatment could contribute to the prevention of diabetic complications. [34] In our study, *EEMP* significantly attenuated the increased lipid peroxidation which could be due to the antioxidant effect of flavanoids, detected in the preliminary phytochemical screening of the extract.

Oxidative stress is a condition of reduction in antioxidative enzymes like SOD, CAT, GPx [35]. Superoxide dismutase (SOD) is an important defence enzyme which catalyses the dismutation of superoxide radicals, which scavenges the superoxide ions by catalyzing its dismutation. Catalase (CAT), a heme enzyme which removes hydrogen peroxide. [36] The decreased activities of CAT and SOD thereby result in the increased production of hydrogen peroxide and oxygen by auto oxidation of glucose and non-enzymatic glycation, [37] Which is known to occur during diabetes.

Glutathione peroxidise (GPx) is an antioxidant enzyme, catalyses the scavenging and inactivation of hydrogen and lipid peroxidise [38]. The results showed that hepatic activity of catalase, superoxide dismutase and glutathione peroxidase decreased significantly in STZ induced diabetic group (Group II). The normal control group maintained optimal value for activity of antioxidants. *EEMP* treatment in diabetic rats significantly increased the antioxidant enzyme activities and reversed them to their normal values. The same phenomenon was seen in the results of glibenclamide treated groups. An array of non-enzymatic antioxidant like GSH, Vitamin C and Vitamin E are involved in scavenging free radicals *in vivo.*

Reduced glutathione (GSH), a tripeptide present in the all cells, is an important antioxidant [39]. It is essential to maintain structural and functional integrity of cells. Hyperglycemia can increase oxidative stress and change the redox potential of glutathione [40]. Decreased levels of GSH in liver of diabetic rats may increase their susceptibility to oxidative injury.

Vitamin C is an excellent hydrophilic, dietary antioxidant and it readily scavenges ROS and peroxyl radicals [41]. It also acts as co-antioxidant by generating Vitamin A, E and GSH from radicals. A decrease in the level of Vitamin C was observed in liver of diabetic rats. Such a fall in level of Vitamin C could be due to the increased utilization of Vitamin C in the deactivation of increased level of ROS or due to decrease in GSH level, since GSH is required in recycling of Vitamin C. [42] Another possibility is that hyperglycemia inhibits ascorbic acid and its cellular transport.

Vitamin E is an antioxidant, a substance that helps prevent damage to the body’s cells. [43] Streptozotocin induced diabetic rats were found to have decreased GSH, Vitamin C and Vitamin E levels in liver as compared to control rats. Treatment with *EEMP* and the standard drug, glibenclamide produced significant increase in the levels of these non-enzymatic antioxidants.

The serum insulin level decreased in diabetic rats, whereas *EEMP* extract, brought about a marked increase in serum insulin in streptozotocin-induced diabetic rats. This increase may be a consequence of the stimulation of insulin synthesis and secretion [44].

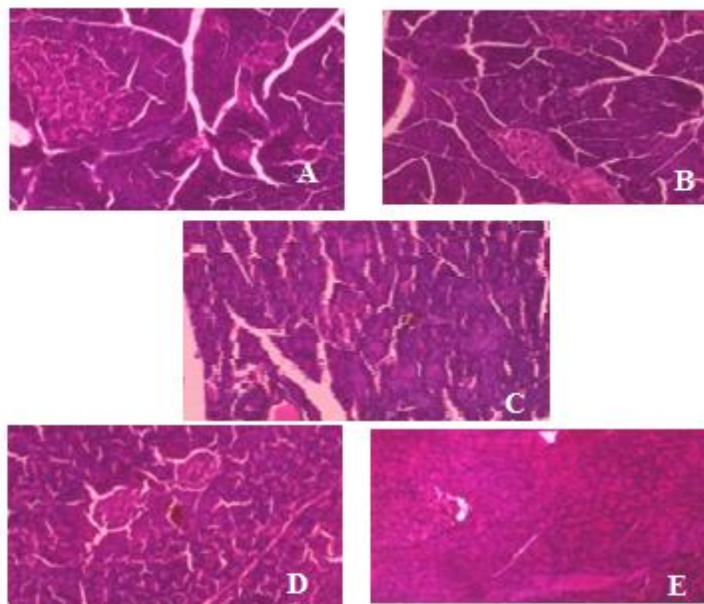


**Fig.9 Shows histopathology of pancreas of a) Group I normal control; b) STZ induced diabetes control; c) STZ + *EEMP* (200 mg/kg); STZ + *EEMP* (400 mg/kg) and Standard Glibenclamide (600 µg/kg)**

The histopathological investigation along with the biochemical evaluation suggests the possibility of the islets regeneration and recovery of normal carbohydrate metabolism in treated group *EEMP*. The regenerative effect of the pancreatic cells by *Mallotus philippensis* via exocrine cells of pancreas may enlighten the positive effects of these agents on the production of insulin. Reports on histopathological analysis of pancreas of the *Mallotus philippensis* alone treated rats showed results that were very similar as that of the control group. Based on the above results, it was concluded that *Mallotus Phililppensis* Muell.Arg. exerted statistically significant anti-diabetic activity against STZ induced diabetic rats.

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