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EVALUATION OF ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF *FICUS HISPIDA* ON STREPTOZOTOCIN INDUCED RATS

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ABSTRACT

Diabetes mellitus is a disorder with increased concentration of blood glucose level because of dearrangement in carbohydrate, protein and fat metabolism due to defective secretion or defective action of insulin. WHO estimated that, in 1995 that the number of people with diabetes anticipates rising from current estimate of 150-220 million in 2010 and 300 million in 2025 in that India is the leading country with more number of peoples suffer with diabetes. It has been reported that approximately 58 million people would suffer with diabetes by the year of 2025 in India. Some common therapies used in the treatment of diabetes includes insulin, sulfonylureas, biguanides and thiazolidinediones. Although , oral hypoglycemic agents/insulin are the major drugs used for the treatment of diabetes and are effective in controlling hyperglycemia, they have prominent side effects and fail to alter the course of diabetic complications. The purpose of present study was to investigate the antidiabetic and antioxidant activity of methanolic extract of leaves of *Ficus hispida* (MEFH) on STZ induced rats. MEFH was subjected to preliminary phytochemical screening, acute oral toxicity study. Effect of MEFH on SGOT, SGPT, total cholesterol, HDL, TG, LDL, VLDL, serum total protein, serum creatinine, serum urea and glycogen content were studied. Antioxidant enzymes like LPO, SOD, CAT and GPx were also studied and histopathology studies were carried out.

Key Words:- *Ficus hispida*, Streptazotocin (STZ), Antioxidant, Diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a serious complex chronic condition that is a major source of ill health worldwide. This metabolic disorder is characterised by hyperglycaemia and disturbances of carbohydrate, protein and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin. It is mainly classified in to type I diabetes and type II diabetes. Type I diabetes is due to cell destruction, usually leading to absolute insulin deficiency, Type II diabetes predominantly due to insulin resistance with relative insulin deficiency (Alberti and Zimmet, 1998). Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Oxidative stress has been shown to have a significant effect in the causation of diabetes as well as diabetes related complications in human beings (Adam D Timmis, 2001). Reactive oxygen species (ROS) plays major role in the pathogenesis of diabetes and its complications, free radicals have toxic effect on tissues and increase in the blood glucose molecules also in certain condition induces free radical generations. These free radicals by non-enzymatic glycosylation of proteins and polyol pathway change the oxidative stress in diabetes (Wilson, 1998). Oxidative stress may have significant effect in the glucose transport protein (GLUT) or at insulin receptor (Jacqueline *et al*., 1997). *Ficus hispida* is used by the maba (indigenous medicine-man of Manipur) in the treatment of Diabetes Mellitus it is also useful in ulcers, psoriasis, anemia, piles,

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jaundice, haemorrhage of the nose and mouth. Its leaves are of particular interest from a medicinal point of view as an antidiarrheal, hepatoprotective, and antitussive, antipyretic, astringent, anti-inflammatory, vulnerary, haemostatic and antiulcer (Ripu *et al*., 2006).

MATERIALS AND METHODS

Preparation of plant extract:

The leaves of *Ficus hispida Linn* (Moraceae) were collected from local source, Tamil Nadu (Nagapattinam) in March. The plant was identified and authenticated by Dr.Sasikala Ethirajulu, Assistant Director, Central Research Institute for Siddha, Arumbakkam, and Chennai 600 106 Tamil Nadu. The fresh leaf of *Ficus hispida Linn* leaves were extracted with methanol in Soxhlet extractor at room temperature for 24 hours. The extract was stored at 0-4°C (Subhash *et al*.,2000). The yield of the methanol extract was 14.43% .

Experimental Animals:

Adult Female Wistar rats of weighing 250-300 gms were used for this study. The inbred animals were procured from the animal house of C.L. Baid Metha College of Pharmacy, Thoraipakkam, and Chennai- 97. They were housed five per cage under standard laboratory conditions at a room temperature at 22 ± 2^0 C with 12 hr light/dark cycle. The animals were acclimatized to laboratory conditions one week and provided with standard pellet chow and water *ad libitum*. Ethical committee clearance was obtained from IAEC (IAEC – CPCSEA XXXIV/02/dated 7/12/2011)

Experimental procedure:

Preliminary Phytochemical analysis of MEFH:

The MEFH was subjected to preliminary phytochemical screening for the presence or absence of phytoconstituents such as alkaloids, carbohydrates, flavonoids, gums and mucilage, tannins, phenols, saponins and terpenes, proteins, steroids, glycosides and sterols (Peraza *et al*., 2002).

Acute Oral Toxicity Study:

The Acute Oral Toxicity Study was done according to the OECD guidelines 423. A single administration of 2000 mg/kg b.w /p.o of the MEFH for three days and observed for 14 days (Manoj Gandhi and Ramesh lal, 1988)

Effect of MEFH on normoglycemic rats:

Overnight fasted normal rats were randomly divided into 4 groups (n=6), Group I are normal rats, Group II were treated with MEFH 200mg/kg b.w/ p.o., Group III were treated with high dose of MEFH 400mg/kg b.w/p.o., Group IV were treated with glibenclamide 3mg/kg b.w/p.o.

Effect MEFH on blood glucose level on Streptozotocin (STZ) induced diabetic rats

The animals were divided in to 5 groups each constituting 6 rats. Group I are normal rats, all other groups were treated with single dose of STZ (55 mg/kg b.w., i.p), STZ induced diabetic rats of Group III were treated with MEFH 200mg/kg b.w/ p.o,), STZ induced diabetic rats of Group IV were treated with MEFH 400mg/kg b.w/ p.o), and STZ induced diabetic rats of Group V were treated with Glibenclamide 3mg/kg b.w/p.o. for 21 days (Girija *et al*., 2011).

Biochemical and histopathology analysis:

At the end of 21 days study, all the animals were sacrificed under light ether anaesthesia. Rats were sacrificed by decapitation and blood was collected from all the groups and serum was separated to study the biochemical parameters. Effect of MEFH on blood glucose, SGOT, SGPT, total cholesterol, HDL, TG, LDL, VLDL, total protein, creatinine, urea and glycogen were estimated (Lowry *et al*., 1951). LPO, SOD, CAT and GPx in liver were studied by using standard procedure (Sirisha and Sreenivasalu, 2010) and Histopathology studies of liver and pancreas were carried out by using standard procedure (Ecobichon, 1997).

RESULTS

The MEFH showed presence of alkaloids, carbohydrates, flavonoids, gums and mucilage's, tannins, phenols, saponins and terpenes, proteins, steroids and glycosides and absence of sterols. (Table 1)

MEFH did not exhibit mortality or any profound toxic reactions at a dose of 2000mg/kg/p.o. According to the (OECD) 423 guidelines (Table 2).

The blood glucose levels of Group II in normoglycaemic and glucose loaded hyperglycaemic rats(NG-OGTT) were decreased(ns) when compared with Group I. Group II and Group III showed significant reduction ($p<0.001$) in blood glucose levels when compared with Group I. (Table 3) Effect of MEFH on blood glucose level in STZ induced diabetic rats for 21 days was found to be increased significantly in Group II when compared with Group I. Group III did not show significant decrease in the blood glucose level after second week. Group IV showed significant decrease ($p<0.01$) at first week, which further reduced in the second and third weeks ($p<0.001$) respectively. Group V produced a significant ($p<0.001$) decrease for three weeks. (Table 4).

Total cholesterol level, serum LDL, VLDL, triglyceride, creatinine, urea level significantly increased ($p<0.001$) in Group II when compared with Group I. Group III and Group IV showed significant decrease in Total cholesterol level (ns,$p<0.01$), serum LDL ($p<0.05$), ($p<0.001$), VLDL ($p<0.05$), ($p<0.001$), triglyceride ($p<0.05$), ($p<0.001$), creatinine ($p<0.01$), ($p<0.001$), urea level ($p<0.01$), ($p<0.001$)) when compared with Group II. Group V also showed a significant ($p<0.001$) decrease in all above parameters when compared with Group II. The serum HDL-cholesterol and total protein level was significantly decreased in Group II when compared with Group I. Both Group III and IV showed significant increase ($p<0.01$), ($p<0.001$) when compared with Group II. Group IV showed significant ($p<0.001$) increase in both when compared to Group II. (Table 5) Group II showed significant ($p<0.001$) increase in SGOT and SGPT level when compared to Group I. Group III and IV showed significant decrease in SGOT ($p<0.05$), ($p<0.001$) and SGPT ($p<0.01$),($p<0.001$) levels when compared to Group II. Group V showed significant ($p<0.001$) decrease respectively in both the levels when compared to Group II. (Table 6)

A significant ($p<0.001$) decrease in the liver SOD, CAT, GPx was observed in Group III when compared with Group I. Group III and IV showed significant increase in SOD (NS), ($p<0.01$), CAT(ns), ($p<0.001$), GPx($p<0.01$), ($p<0.001$) when compared with Group II, whereas 200mg/kg/p.o did not show any significant increase. Group IV showed significant ($p<0.001$) increase in SOD when compared STZ induced diabetic animals. A significant ($p<0.001$) increase in the LPO was observed in Group II when compared to Group I. Group III and IV showed significant ($p<0.01$), ($p<0.001$) decrease when compared to Group II. Group IV showed significant ($p<0.001$) decrease in LPO when compared to Group II. (Table 7).

Histopathology of liver and pancreas

Histopathological examinations of diabetic animals showed centrilobular necrosis accompanied by fatty changes and ballooning degeneration were observed in the remaining heptocytes in the liver of rats treated with STZ were much of intensity and which was recovered with the treatments MEFH (Figure 1). Pancreas of Group I showed normal islets and acini whereas diabetic control showed damages and atrophy islets with acini. Group IV showed preserved normal islets in pancreas, Group III showed small pancreatic islet and Group IV showed hyperplastic islets with acini. (Figure 2).

Table 1. Phytochemical screening of MEFH

S.No.	Constituents	MEFH
1.	Alkaloids, Carbohydrates, flavonoids, gums and mucilage's, tannins, phenols, saponins and terpenes, proteins, steroids and glycosides	+
2.	Sterols	_

Table 2. Acute oral toxicity studies of MEFH (OECD 423 guideline)

Si. No.	Treatment group	Dose	Weight of animal in gms		Signs of toxicity	Onset of toxicity	Reversible or irreversible	Duration
			Before test	After test				
1.	MEFH	2g/kg	225	230	No signs of toxicity	Nil	Nil	14 days
2.	MEFH	2g/kg	250	260	No signs of toxicity	Nil	Nil	14 days
3.	MEFH	2g/kg	250	260	No signs of toxicity	Nil	Nil	14 days

Table 3. Effect of MEFH blood glucose levels in normoglycaemic and glucose loaded hyperglycaemic rats (NG-OGTT)

Group	Blood glucose levels (mg/dl)				
	0 min	30 min	60 min	90 min	120 min
Group I	88.6±2.530	92.5± 2.340	110.2± 3.010	105.8± 2.610	97.5± 3.420
Group II	90.1± 2.600	89.70± 1.964a ^{ns}	102.5± 2.630a^{ns}	94.4± 2.215a*	89.1± 2.604a^{ns}
Group III	92.4± 2.812	88.2± 3.400a ^{ns}	86.6± 2.540a^{ns}	85.6± 2.550a***	79.3± 3.150a***
Group IV	93.2± 2.70	85.7± 1.85a^{ns}	81.1± 2.61a***	74.3± 3.12a***	68.40± 3.62a***

Comparisons were made between: a- Group I vs. II, III, IV. Statistical Significance test for comparison was done by one way ANOVA followed by Dunnet's't' test. *p< 0.05, **p< 0.01, ***P<0.001, ns-non significant. Values are expressed as mean ± SEM of 6 animals.

Table 4. Effect of MEFH on blood glucose level on STZ induced diabetic rats for 21 days

Group	Blood glucose level(mg/dl)			
	Day – 1	Day – 7	Day – 14	Day – 21
Group I	78.0± 1.82	83.33± 1.43	86.55± 1.32	90.01± 0.85
Group II	286.6± 3.58	292.16± 3.620a***	298.32± 2.34a***	307.33± 3.23a***
Group III	267.33±2.88	235.33± 3.030a*b**	187.22± .110a**b**	135.65± 2.28a**b**
Group IV	273.83± 2.74	215.56± 2.34a**b**	163.12± 2.44a***b**	127.12± 1.43a***b**
Group V	281.00±3.66	196.76±2.75a***b**	148.50±1.72a***b**	95.15±1.05a***b**

Table 5. Effect of MEFH Total cholesterol, HDL, LDL, VLDL, TG and total protein on STZ induced diabetic rats

Group	Total Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	VLDL Cholesterol (mg/dl)	TG (mg/dl)	Total Protein(mg/dl)
Group I	60.82± 1.70	50.80± 2.24	34.97±1.80	23.98±1.32	53.30± 1.18	7.86± 0.20
Group II	85.80± 2.89a***	24.58± 1.20a***	87.80±2.24 a***	47.70± 1.72a***	92.98± 1.01a***	3.85± 0.10a***
Group III	78.30± 2.20a^{ns}b^{ns}	31.09±1.40a**b**	78.98±1.70a*b**	41.59± 1.13a*b**	86.74± 1.66a*b**	4.62 ±0.11a**b**
Group IV	71.90± 1.50 a**b**	42.98± 1.04a***b**	52.57±1.20a***b**	35.60± 1.11 a***b**	70.15± 1.38 a***b**	6.72± 6.72 ***b**
Group V	67.50± 1.23a***b**	49.30± 0.94a**b**	46.40±1.43a***b^{b}	29.95± 1.34 a***b**	63.40± 1.40a***b**	7.10± 0.10***b**

Table 6. Effect of MEFH on Serum creatinine, urea, SGOT and SGPT on STZ induced diabetic rats

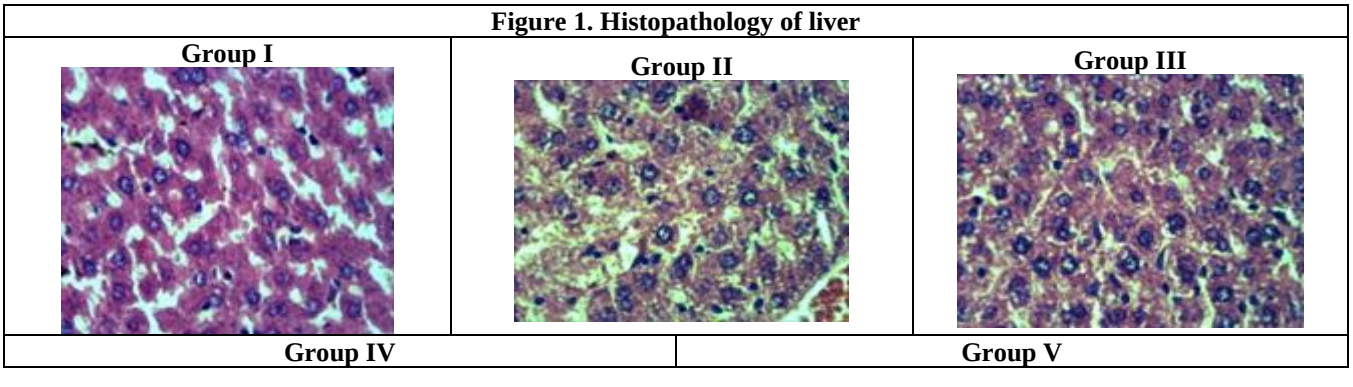
Group	Serum Creatinine (mg/dl)	Serum Urea(mg/dl)	SGOT (mg/dl)	SGPT (mg/dl)
Group I	0.52± 0.04a	41.33± 1.80	38.35± 1.21	34.98± 1.58
Group II	1.56± 0.03a***	73.00± 1.34a***	71.35± 1.34a***	67.35± 1.87a***
Group III	1.30± 0.05 a**b**	65.17± 1.66 a**b**	66.10± 1.28 a*b**	57.46± 1.82a**b**
Group IV	1.01± 0.07 a***b**	52.00± 1.50 a***b**	44.66± 1.10a***b**	47.21± 1.39 a***b**
Group V	0.76± 0.04 a***b**	49.48± 1.14 a***b**	41.93± 1.30 a***b**	41.99± 1.51a***b**

Table 7. Effect of MEFH on SOD, CAT, LPO and GSH-Px on STZ induced diabetic rats

Group	SOD (µmol/mg protein)	CAT (µmol H_2O_2 consumed/min per mg protein)	LPO (nmol MDA/mg protein)	GSH-Px (nmol/g tissue)
Group I	7.65± 0.30	3.81± 0.189	15.84± 0.34	21.11± 0.03
Group II	3.51± 0.19a***	2.01± 0.130a***	36.77± 0.454 a***	9.09± 0.02 a***
Group III	3.94± 0.31a^{ns}b^{ns}	2.30± 0.131a^{ns}b^{ns}	28.58± 0.301 a**b**	11.82± 0.10a**b**
Group IV	4.89± 0.24a**b**	3.32± 0.215 a***b**	18.25± 0.32 a***b**	15.27± 0.13a***b**
Group V	5.24± 0.21a***b**	3.362± 0.156 a***b**	17.55± 0.24a***b**	18.12± 0.03a***b**

MDA- Malondialdehyde.

Figure 1. Histopathology of liver



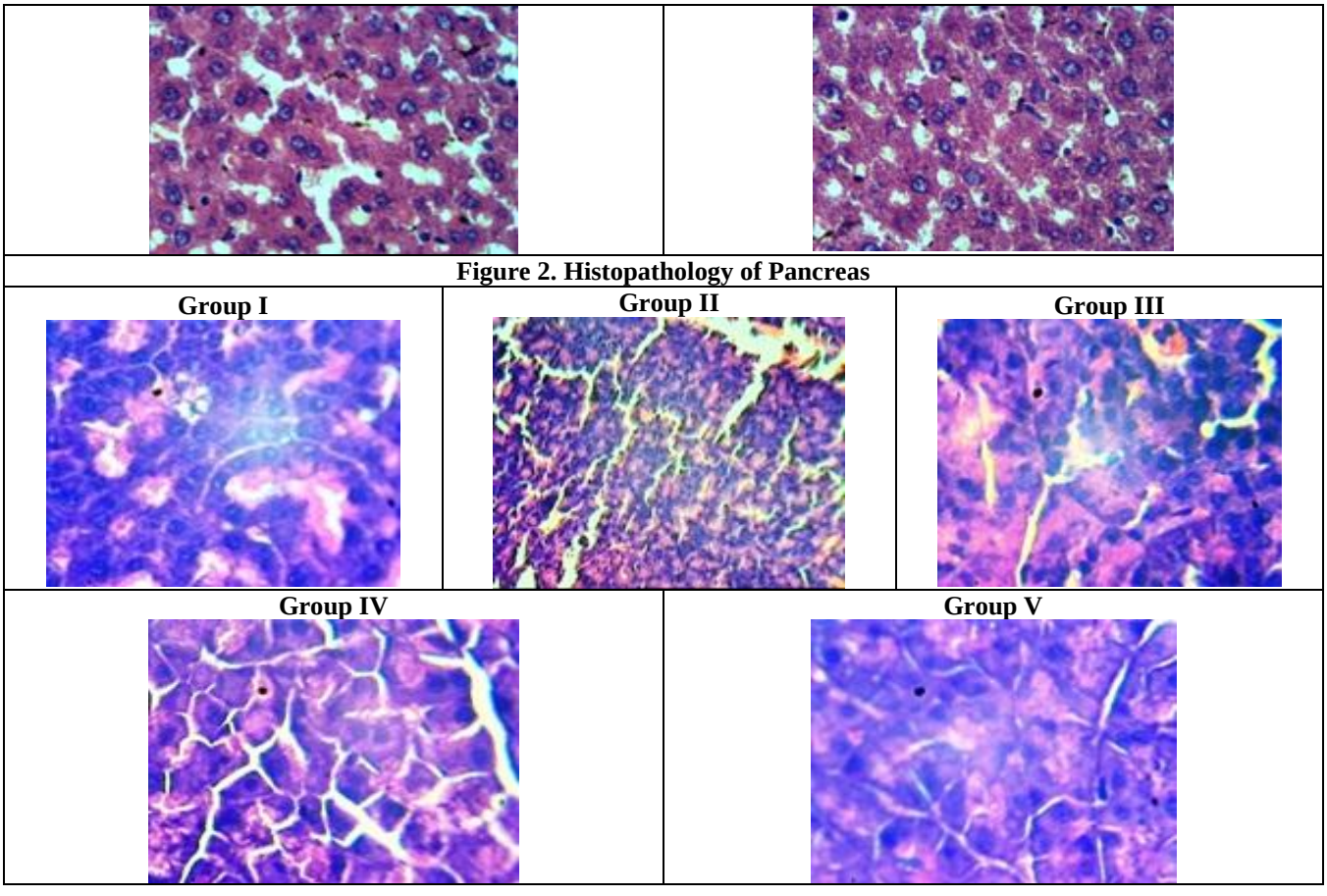


Figure 2. Histopathology of Pancreas

DISCUSSIONS AND CONCLUSION

STZ is an alkylating agent which causes DNA damage which results in the activation of Poly (ADP-ribose) synthetase that leads to the depletion of NAD and ATP virtually causes β cell necrosis in the experimental rats (Csaba Szabo, 2005). It leads to a reduction in insulin release there by a drastic reduction in plasma insulin concentration leading to stable hyperglycemic state (Didem *et al*., 2005). Experimental induction of diabetes with low dose of STZ is associated with the characteristic loss of body weight which is due to increased muscle wasting and due to loss of tissue protein. Diabetic rats treated with MEFH protective effect in controlling muscle wasting i.e reversal of gluconeogenesis and may also be to the improvement in insulin secretion and glycemic control (Shirwaikar *et al*., 2006).

Group III and Group IV significantly reduced blood glucose level The possible mechanism involved may be due to the reduced glucose transport or absorption from the gut. Extra pancreatic action probably by stimulation of glucose utilization and increase in glycogenic or glycolytic enzyme activities in peripheral tissues, decrease in the secretion of counter-regulatory hormones like glucagon and growth hormones (Cisse *et al*., 2005).

Hyperlipidemia is pathological state observed in the DM, elevated serum total cholesterol, TG, LDL-cholesterol, VLDL- cholesterol and reduced serum HDL level consequently increases the risk of diabetic complications and atherosclerosis. In the present study treatment with Group III and Group IV significantly ($p<0.001$) reduced the total cholesterol, TG, LDL-cholesterol, VLDL- cholesterol and significantly increased the HDL levels. This effect may not only due to better glycaemic control but also due to its action on the lipid metabolic pathway (Annie Shirwaikar *et al*., 2005).

In type II diabetes there is a risk of elevated production of ROS due to increased concentration of lipid peroxidation its end product (lipid radical and lipid peroxide) are harmful to the cells in the body (Chang *et al*., 2005). Super oxide dismutase and catalase is the most important enzyme that scavenge the toxic free radicals and form the major anti oxidant system. SOD and catalase

catalyzes the decomposition of hydrogen peroxidation to water and oxygen thus protecting the cell from the oxidative damage. Treatment with Group III, IV and V significantly increased the enzyme activity. Group IV exhibit pharmacological response related to glibenclamide. The reduction of hydrogen peroxidation is catalyzed by glutathione peroxidase into water and oxygen at the expense of GSH. GP_x activity is also reduced in diabetic condition. This may be due to inactivation of the enzyme involved in disposal of O_2 species and also insufficient availability of GSH. Histopathological studies of Group III and Group IV showed prominent islet cell, hyperplasia and regeneration of islet cells (Bonner weir, 1988). It acts as a proof for possible anti-hyperglycemic property of the MEFH.

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