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ANTIDIABETIC ACTIVITY OF DIFFERENT FRACTIONS OF HYDROALCOHOLIC EXTRACT OF *MNESITHEA GRANULARIS* IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder affecting a major proportion of the population worldwide. The present study was aimed to evaluate the antidiabetic effect of n-Hexane, Ethyl acetate, Ethyl acetate: Methanol (50:50) and Methanolic fractions from hydroalcoholic extract of *Mnesithea granularis* in streptozotocin induced diabetic rats. Among the fractions tested, methanolic fraction shown more significant antidiabetic activity and antidiabetic activity was in the order of MF>EMF>EAF>NHF. So MF was investigated further for its action on insulin, Hb, HbA1c, oxidative parameters, body weight and cell integrity of pancreas. Results indicated that animals treated with MF shown significant decrease in blood glucose, HbA1c, malondialdehyde levels and significant increase in insulin, Hb, SOD, catalase, reduced glutathione and body weight. It could be concluded that methanolic fraction of hydroalcoholic extract of *Mnesithea granularis* has favourable effect in bringing down the severity of diabetes however necessary studies are required on characterization of active principles.

KEY WORDS : *Mnesithea granularis* whole plant, Streptozotocin induced diabetes, Antidiabetic activity, Different fractions, Blood glucose.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder that is characterized by either the insufficient production or the lack of response to a key regulatory hormone of the body's metabolism, insulin[1]. Such a deficiency of insulin results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves. As the number of people with diabetes multiply worldwide, the disease takes an ever-increasing proportion of national and international health care budgets. It is projected to become one of the world's main disablers and killers within the next 25 years[2]. Regions with greatest potential are Asia and Africa, where DM rates could rise to two to three-folds than the present rates. The conventional therapies for diabetes have many shortcomings like side effects and high rate of secondary failure. On the other hand, plant derived products are expected to have similar efficacy without side effects as that of conventional drugs. According to the World Health Organization, more than 70% of the world's population must use traditional medicine to satisfy their principal health needs. A great number of medicinal plants used in the control of the diabetes mellitus have been reported[3]. There are various medicinal plants in the world, which are the potential sources of the drugs. In India, number of plants are mentioned in ancient literature (Ayurveda) for the cure of diabetic conditions known as madhumeha and some of them have been experimentally evaluated and the active principles were isolated[4]. One such plant that is being used by the traditional practitioners to treat diabetes is *Mnesithea granularis* belonging to the family *Poaceae*. Traditionally it is also being used in the treatment of digestive disorders, liver disorders and obesity. In our earlier work we investigated the antidiabetic activity of different solvent extracts of *Mnesithea granularis.* Of which, the most effective solvent extract found was hydroalcoholic extract (water: methanol 50:50). The constituents reported in this extract are carbohydrates, proteins, amino acids, anthraquinones, saponins, flavonoids, tannins, terpenoids and cardiac glycosides. Therefore after knowing the most effective solvent extract, isolating the active fraction from the most effective solvent extract would be useful in the development of new drugs from plants. The standard fraction of an active extract may prove better therapeutically, less toxic and inexpensive. Keeping these facts in mind, the present study was undertaken to identify the active antidiabetic fraction of the active extract.

MATERIALS AND METHODS

Plant Material

For the present study, *Mnesithea granularis* whole plant was collected from the forest area near to the Madanapalli, Chittoor district of Andhra Pradesh and the plant was botanically identified and authenticated by Dr. K. Madhava Chetty, Assistant professor, Department of Botany, S.V. University, Tirupati, A.P., India and a voucher specimen (RIPER/SN/001) was preserved in division of pharmacology, RIPER, Anantapur for further reference.

Preparation and Fractionation of Crude Extracts

Collected plant material was thoroughly examined for the foreign material, washed with water and shade dried for 21 days then made into powder by mechanical grinder. Extraction was carried out by cold maceration method using hydro alcohol (water: methanol 50:50) for 72 hours. Then the contents were filtered and the filtrates were concentrated using rotary flash evaporator. Thus the highly concentrated crude hydroalcoholic extract was obtained. It was then fractionated by column chromatography using n-Hexane, Ethyl acetate, Ethyl acetate : Methanol (50:50) and Methanol. The dried fractionated extracts were then preserved in the refrigerator for the experimental use.

Preliminary Phytochemical Screening

Freshly prepared fractionated extracts were qualitatively tested for the presence of chemical constituents. Phytochemical screening was performed and these were identified by characteristic colour changes using standard procedure.

Drugs and Chemicals

Streptozotocin was procured from Sigma Aldrich Labs, Gliclazide was provided as a gift sample from Dr. Reddy Laboratories, Glucose kits were procured from Erba diagnostics.

Experimental Animals

Animals were housed in plastic cages (28 cm×43 cm×18 cm) and were maintained under conventional laboratory conditions (temperature 22±2^{0}C and humidity 50±15%) of temperature, with a regular 12-h light/12-h dark cycle throughout the study. They were fed standard pellet chow and were allowed water *ad libitum*. Wistar rats of both sexes weighing 150-200gm were used for study. All protocols were performed in accordance with the Institutional Animal Ethical Committee (IAEC) of RIPER as per the directions of the CPCSEA (Committee for the purpose of Control and Supervision on Experiments on Animals).

Induction of Diabetes

After fasting for 18 h, diabetes was induced by intraperitoneal injection of streptozotocin dissolved in 0.1 M cold sodium citrate buffer, pH 4.5, at a dose of 55 mg/kg [5]. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycaemia [6]. After 72 h, rats with marked hyperglycemia (FBG ≥250 mg/dl) were selected and used for the study. All the animals were allowed access to tap water and pellet diet and maintained at room temperature. .

Experimental Design

Effect of different fractions of hydroalcoholic extract of Mnesithea granularis (HAMG) on serum glucose levels in streptozotocin induced diabetic rats

The animals were divided into seven groups and each group consisted of six rats.

Group I: Untreated normal rats

Group II: Untreated diabetic rats

Group III: Diabetic rats treated with Gliclazide 4.5mg/kg, p.o. for 30 days

Group IV: Diabetic rats treated with n-Hexane fraction of HAMG (NHF) 50mg/kg, p.o. for 30 days

Group V: Diabetic rats treated with Ethyl acetate fraction of HAMG (EAF) 50mg/kg, p.o. for 30 days

Group VI: Diabetic rats treated with Ethyl acetate: Methanol (50:50) fraction of HAMG (EMF) 50mg/kg, p.o. for 30 days

Group VII: Diabetic rats treated with Methanolic fraction of HAMG (MF) 50mg/kg, p.o. for 30 days

Blood samples were collected on 1st, 10th, 20th and 30th day of study from retro orbital venous plexus following the technique described by Coccheto and Bjornsson[7], allowed to clot and were centrifuged at 3000 rpm for 20 min. The serum was separated and used for the estimation of glucose levels[8].

Effect of MF on serum insulin levels

On 31st day of experiment, blood samples were collected by cardiac puncture from group I, II, III and VII and allowed to clot and were centrifuged at 3000 rpm for 20 min. The serum was separated and used for the estimation of insulin[9] levels.

Effect of MF on blood Hb and glycosylated haemoglobin (HbA1c) levels

On 31st day of experiment, blood samples were collected by cardiac puncture from group I, II, III and VII and used for the estimation of Hb and HBA1c [10] levels.

Effect of MF on serum oxidative parameters

On 31st day of experiment, blood samples were collected by cardiac puncture from group I, II, III and VII and allowed to clot and were centrifuged at 3000 rpm for 20 min. The serum was separated and used for the estimation of SOD[11], catalase, reduced glutathione[12] and malondialdehyde[13] (MDA).

Effect of MF on body weight

Change in body weight of animals from group I, II, III and VII was determined by weighing the animals on day 1 and 30.

Histopathological Procedures

On 31st day group I, II, III and VII rats were sacrificed under anaesthesia, pancreas were immediately excised, fixed in 10% solution of formaldehyde and histopathological studies were carried out at Star diagnostic laboratories, Anantapuramu, A.P., India.

STATISTICAL ANALYSIS

Data were expressed as mean ± standard error of the mean (S.E.M); and comparison between the different treatments was carried out using analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test using computerized Graph Pad Prism, version 4.5 software (Graph Pad Software Inc).

RESULTS

Percentage yield, colour and nature of different fractions of hydroalcoholic extract of Mnesithea granularis

The results are tabulated in table number 1. For the present study fractionation was carried out by column chromatography using n-Hexane, Ethyl acetate, Ethyl acetate : Methanol (50:50) and Methanol and all the fractionated extracts were evaluated for their percentage yield, colour and nature.

Table 1
Percentage yield, colour and nature of different fractions of hydroalcoholic extract of Mnesithea granularis

S.NO.	FRACTION	% YIELD	COLOUR	TEXTURE
1.	NHF	4%	Greenish brown	Semisolid
2.	EAF	5.5%	Greenish brown	Semisolid
3.	EMF	5%	Dark brown	Semisolid
4.	MF	8%	Dark brown	Semisolid

Preliminary phytochemical screening

The results are tabulated in table number 2. n-Hexane fraction revealed the presence of terpenoids. Ethyl acetate fraction revealed the presence of flavonoids, tannins and terpenoids. Ethyl acetate : methanol fraction revealed the presence of anthraquinones, saponins, flavonoids, tannins and cardiac glycosides. Methanolic fraction revealed the presence of carbohydrates, proteins, amino acids, anthraquinones, saponins, flavonoids, tannins and cardiac glycosides.

Table 2
Preliminary phytochemical screening

S.NO	CONSTITUENTS	NHF	EAF	EMF	MF
1.	Carbohydrates	□ ve	□ ve	□ ve	+ ve
2.	Proteins	□ ve	□ ve	□ ve	+ ve
3.	Amino acids	□ ve	□ ve	□ ve	+ ve
4.	Anthraquinones	□ ve	□ ve	+ ve	+ ve
5.	Saponins	□ ve	□ ve	+ ve	+ ve
6.	Flavonoids	□ ve	+ ve	+ ve	+ ve
7.	Tannins	□ ve	+ ve	+ ve	+ ve
8.	Terpenoids	+ ve	+ ve	□ ve	- ve
9.	Cardiac glycosides	□ ve	□ ve	+ ve	+ ve

Effect of different fractions of hydroalcoholic extract of Mnesithea granularis on serum glucose levels in streptozotocin induced diabetic rats

The results are tabulated in table number 3. All fractionated extracts under study shown significant decrease in serum glucose levels and antidiabetic potency was in the order of MF>EMF>EAF>NHF. So based upon the yield and effect on serum glucose levels, MF was selected for further investigations.

Table 3
Effect of different fractions of hydroalcoholic extract of Mnesithea granularis on serum glucose levels in streptozotocin induced diabetic rats

S.NO	GROUP	SERUM GLUCOSE LEVELS (mg/dL)			
		1st DAY	10th DAY	20th DAY	30th DAY
1.	Normal	91.50 ± 3.01	89.0 ± 3.1	87.0 ± 5.0	85.0 ± 4.20
2.	Diabetic control	369.8 ± 11#	426.5 ± 8.38#	450.5 ± 4.85#	477.5 ± 5.66#
3.	Gliclazide	348 ± 5.35ns	173 ± 6.01*	135.8 ± 5.63*	90.5 ± 5.62*
4.	NHF	365.3 ± 7.86 ns	424.5 ± 3.5 ns	424.3 ± 3.11 ns	309.5 ± 11.35*
5.	EAF	365.5 ± 8.05 ns	401.8 ± 3.7 ns	288.8 ± 8.26*	186.5 ± 7.8*
6.	EMF	380.3 ± 6.53 ns	320.5 ± 4.03*	251.5 ± 7.5*	150 ± 6.16*
7.	MF	382.0 ± 5.71 ns	181.5 ± 5.69*	139 ± 5.0*	90.5 ± 5.188*

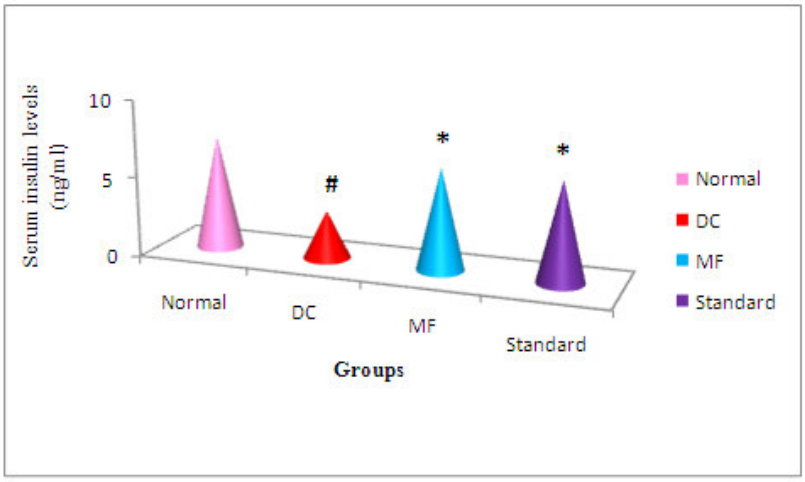
Values are expressed as mean ± S.E.M, n=6 in each group ns- Non significant
#p <0.001when compared to normal, *p<0.001 when compared to diabetic control.

Effect of MF on serum insulin levels

The results are graphically illustrated in Fig.1.Streptozotocin significantly decreased serum insulin levels when compared to normal group. Administration of MF significantly increased serum insulin levels when compared to diabetic group.

Graph 1

Effect of MF on serum insulin levels



Values are expressed as mean ± S.E.M, n=6 in each group

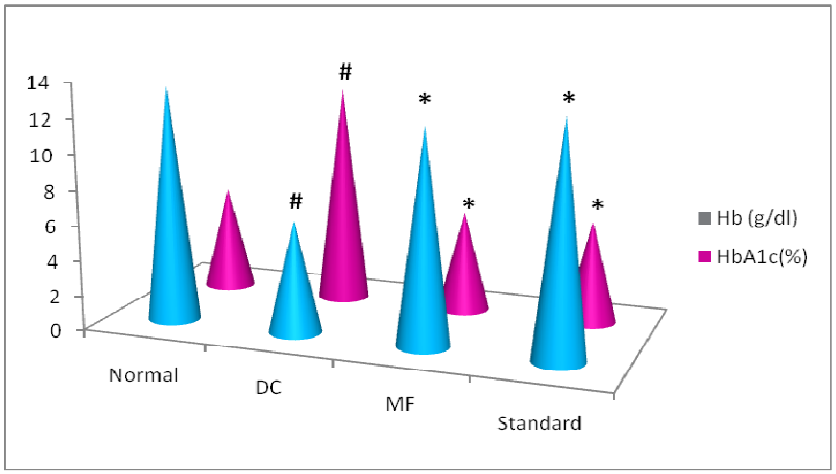
#p <0.001 when compared to normal, *p< 0.001 when compared to diabetic control.

Effect of MF on blood Hb and glycosylated haemoglobin (HbA1c) levels

The results are graphically illustrated in Fig.2. Streptozotocin significantly decreased blood Hb levels and increased HbA1c levels when compared to normal group. Administration of MF significantly increased blood Hb levels and decreased HbA1c levels when compared to diabetic group.

Graph 2

Effect of MF on blood Hb and glycosylated haemoglobin (HbA1c) levels



Values are expressed as mean ± S.E.M, n=6 in each group

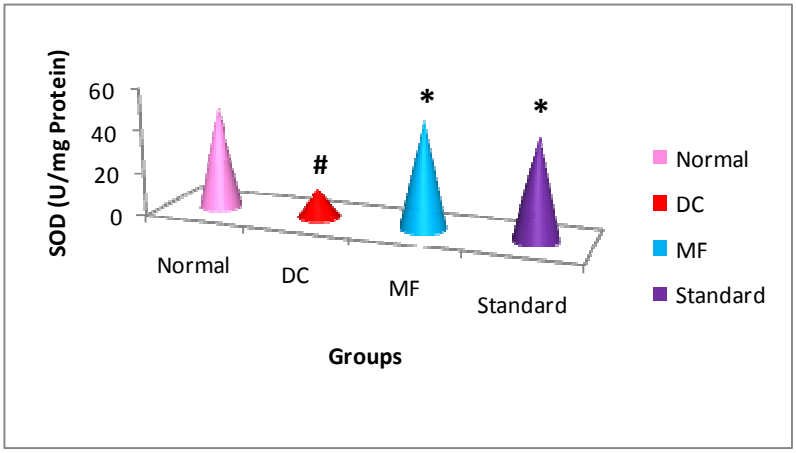
#p <0.001 when compared to normal, *p< 0.001 when compared to diabetic control.

Effect of MF on serum oxidative parameters

The results are graphically illustrated in Fig. 3, 4, 5 and 6. Streptozotocin significantly decreased serum SOD, catalase, GSH and significantly increased malondialdehyde levels when compared to

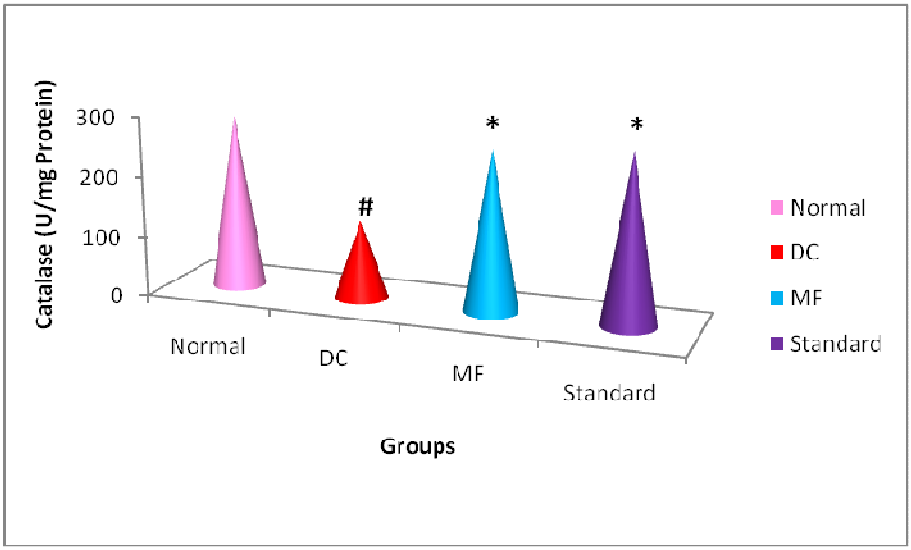
normal group. Administration of MF significantly increased serum SOD, catalase, GSH and significantly decreased malondialdehyde levels when compared to diabetic group.

Graph 3
Effect of MF on SOD



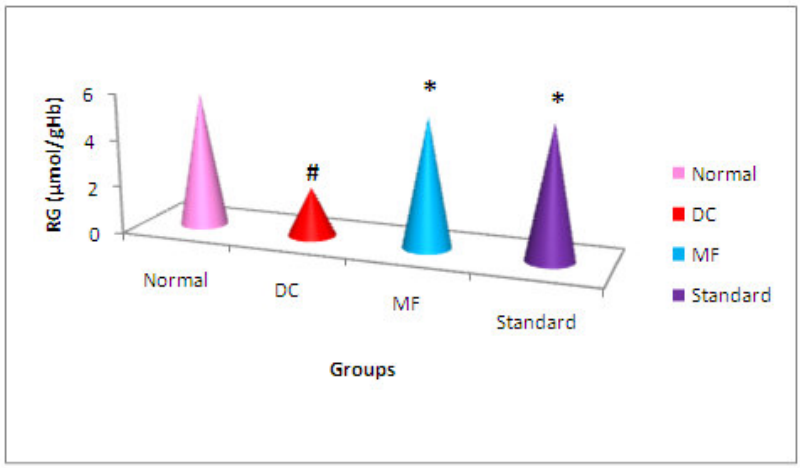
Values are expressed as mean ± S.E.M, n=6 in each group
#p <0.01 when compared to normal, *p< 0.01 when compared to diabetic control.

Graph 4
Effect of MF on Catalase



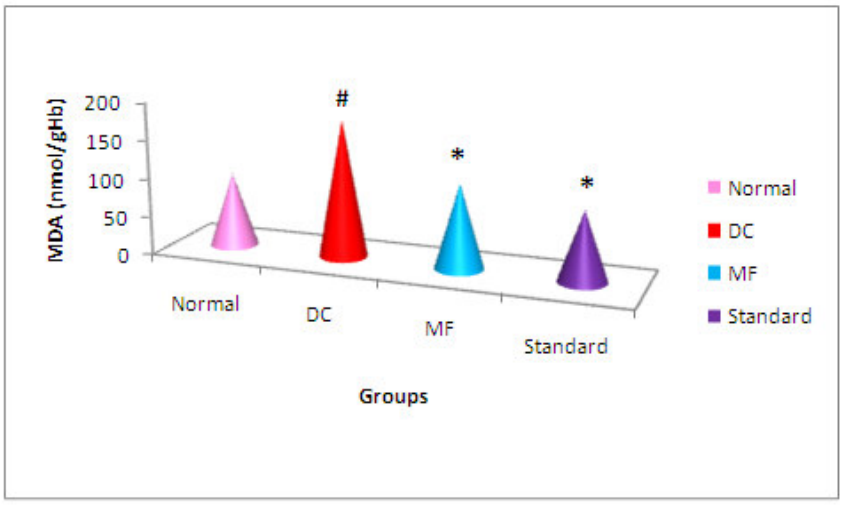
Values are expressed as mean ± S.E.M, n=6 in each group
#p <0.001 when compared to normal, *p< 0.001 when compared to diabetic control.

Graph 5
Effect of MF on Reduced glutathione



Values are expressed as mean ± S.E.M, n=6 in each group
#p <0.001 when compared to normal, *p< 0.01 when compared to diabetic control.

Graph 6
Effect of MF on lipid peroxidation

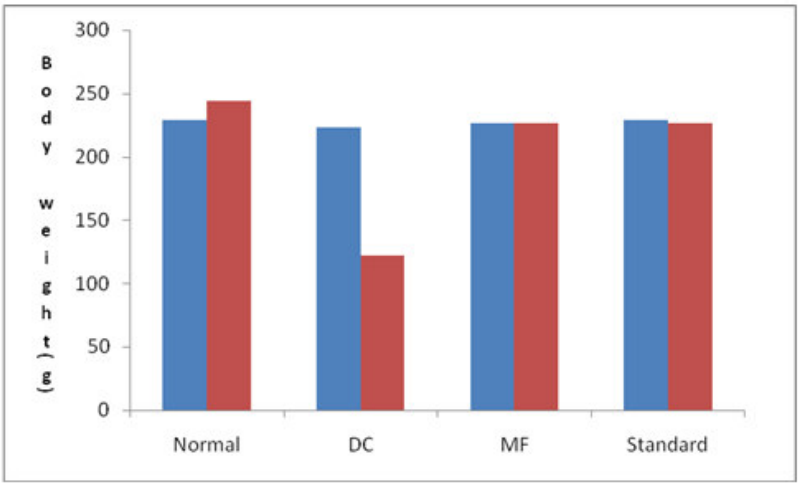


Values are expressed as mean ± S.E.M, n=6 in each group
#p <0.001 when compared to normal, *p< 0.001 when compared to diabetic control.

Effect of MF on body weight

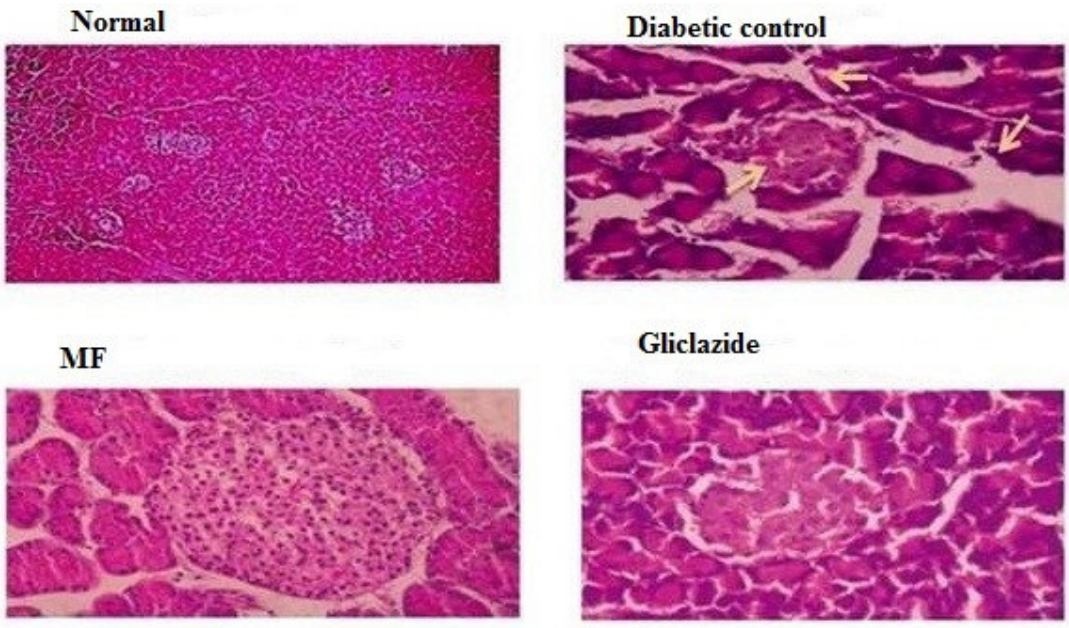
The results are graphically illustrated in Fig.4. Administration of MF to diabetic rats resulted in increased body weight compared to untreated diabetic rats.

Graph 8
Effect of MF on body weight



Histopathological Studies

Figure 1
Histology slides



Diabetic control rats showed complete destruction of pancreatic β-cells. Administration of MF and gliclazide showed an increase in β-cell count and the remodelling of the structure of pancreas.

DISCUSSION

The present study was undertaken to examine the antidiabetic activity of various fractionated extracts of hydroalcoholic extract of *Mnesithea granularis* and to find out the active antihyperglycemic fraction of the active extract of this plant. In the present study, streptozotocin was chosen to induce experimental diabetes in rats, since it causes alterations similar to those found in diabetic human[14]. Findings of the present investigation revealed that STZ induced diabetes resulted in a significant increase in serum glucose

level, decrease in serum insulin level, decrease in Hb levels and elevation in glycosylated haemoglobin (HbA1c) level. Furthermore STZ significantly increased oxidative stress indicated by decrease in blood SOD, Catalase, GSH and increase in serum MDA levels. Data of current study showed that different fractionated extracts exhibited antihyperglycemic activity but MF was shown more significant effect on blood glucose levels. Hence, it was selected for further investigation on other biochemical parameters. Data of present investigation revealed that daily administration of MF for 30 days reduced hyperglycemia which is evidenced by significant reduction in glucose levels; serum HbA1c levels as well as significant rise in serum insulin and serum Hb levels. Furthermore significant increase in blood SOD, catalase, GSH and significant decrease in serum MDA levels were observed in animals treated with MF for 30 days when compared to diabetic control group. The more significant antidiabetic activity of MF may be due to the presence of bitter principles, flavonoids and saponins. Literature showed that bitter principles, flavonoids and saponins are good antidiabetic metabolites[15]. The hypoglycemic effect of bitter principles may be due to astringent properties or absorptive capacity of glucose is being modified from the gut either due to gut hormones or plasma insulin response. Different actions of flavonoids[16] include reduction of aldose reductase, regeneration of pancreatic cells and enhanced release of insulin. Other fractions lacked these metabolites and this may account for their non-significant antidiabetic activity. Glycosides and tannins have similarly been implicated in the antidiabetic activities of plant[17,18].

CONCLUSION

From the present study it could be concluded that MF decreased STZ induced hyperglycemia and ameliorated oxidative stress. Its antidiabetic activity may be related to insulinomimetic and antioxidant action. This research supports the inclusion of this plant in traditional antidiabetic preparations and the formulations made using these identified effective extracts and fraction of this plant could serve the purpose better than the existing formulations with crude extract. We need further study to determine the mechanism of action and to isolate the active principles responsible for antidiabetic activity.

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CONFLICT OF INTEREST

Conflict of interest declared none

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