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Medicago Sativa leaf extract supplementation corrects diabetes induced dyslipidemia, oxidative stress and hepatic renal functions and exerts antihyperglycaemic action as effective as Metformin

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ABSTRACT

The present study is designed to evaluate the holistic potential of an ethanolic leaf extract of Medicago sativa in combating diabetic hyperglycaemia as well as associated manifestations. Adult albino rats were made diabetic by using alloxan (120 mg / kg body weight). Both diabetic (D) and non diabetic (C) animals were treated with Medicago sativa (MSE) extract and metformin as a reference drug, for a period of 30 days. At the end of the treatment period, animals were sacrificed and various parameters of glycaemic status (Glucose, Insulin, GTT, IRT), serum lipids (CHO, TG, LDL, HDL, VLDL), oxidative stress (LPO, GPx, GSH, SOD and CAT), markers of renal and hepatic dysfunction (ALP, ACP, SGOT, SGPT, Urea, Creatinine), serum ionic levels and renal Na^+ - K^+ ATPase activity were evaluated. Results obtained indicate an effective glycaemic control in MSE treated D animals alongwith a bettered serum lipid profile. Diabetes induced increase in lipid peroxidation and renal and hepatic markers of dysfunction were normalized by MSE treatment and the effects were comparable to those seen with metformin treatment. Further, compromised levels of antioxidant enzymes and Na^+ - K^+ ATPase in diabetic animals, was also brought to C levels by MSE extract treatment. Both MSE and metformin treatment reversed the changes in the levels of serum electrolytes in D animals. Overall, this study provides evidence for further analysis of this plant in the development of a versatile multitargetted drug as a treatment for diabetes, with the results favouring better potency than even metformin.

Keywords: Diabetes, *Medicago sativa*, Oxidative stress, Metformin, Hyperglycaemia.

INTRODUCTION

Diabetes mellitus is a metabolic disorder with attendant altered metabolic profile that affects carbohydrates, lipids and proteins with a predisposition towards vascular disorders [1]. It is one of the commonest endocrine disorders and its prevalence is expected to intensify almost five times in another 10 years. Chronic hyperglycaemia leads to production of free radicals as a result of glucose oxidation , non enzymatic glycation of proteins and subsequent degradation of glycated proteins which in turn can lead to damage of cellular organelles and enzymes, all contributing to promotion and development of complications of diabetes mellitus [2].

Use of medicinal plants for amelioration of various metabolic disorders is finding favour with researches owing to their lesser side effects. There are several herbs, roots, fruits and other plant materials that are used for treating diabetes throughout the world with, a list of more than 100 such antidiabetic plants listed in the Indian traditional system of medicine as well [3].

Leaves of *Medicago sativa* have been used traditionally in South Africa for treating diabetes, used in the form of tea [4, 5]. Seeds of this plant have also been reported to have cholesterol lowering effects [6]. Owing to its rich source of Vitamins and phytoestrogens it is also used as a food additive in several developed countries [7, 8]. *Medicago sativa* is suspected to have an antihyperglycaemic property and insulin releasing action [9]. A best oral hypoglycaemic agent is expected to ameliorate all diabetes associated complications/manifestations besides exerting hypoglycaemic effect.

Thereby, the present study aims to evaluate the holistic potentials of *Medicago sativa* leaf extract to control glycaemia, lipidemia and oxidative stress in diabetic rats. The study also aims to explore the role of this plant in maintaining electrolyte balance and preventing hepatic and renal damage. The efficacy of this plant is also compared with that of a standard hypoglycaemic drug, Metformin.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Medicago sativa* were collected from Palanpur in Banaskantha district, Gujarat, India. The collected plant material was identified and its authentification was done by Prof.M.Daniel (Head, Department of Botany, The M.S.University of Baroda, Vadodara, India).

Preparation of plant extract

Leaves of *Medicago sativa* after collection were allowed to shade dry and the dried leaves were ground to a fine powder, which was then used for extraction in a soxhlet apparatus (Borosil Glass Works, Mumbai, India) for up to 10 hours using 95% ethanol at a boiling temperature of 60°C. The extract obtained from soxhlet extraction was allowed to cool and then filtered to remove the residue. The filtrate was then concentrated at 65°C by rotavapour to get a fine powder that was refrigerated at 4°C until further use [10]. The powder was diluted appropriately before use to obtain the desired concentration of the extract (MSE).

Experimental Animals

Female albino wistar rats weighing 200-250g were used for the experimentation. Animals were kept in the departmental animal house under a 12:12 light - dark cycle at an ambient temperature of 21 ± 2°C. Throughout the experimentation, food and water were provided *ad libitum* to the rats. Experiments were conducted according to the guidelines of CPCSEA and the proposal was approved by the Animal Ethical Committee of the Department of Zoology, The M.S.University of Baroda (Approval no 827/ac/04/CPCSEA). Induction of diabetes using Alloxan as a diabetogen was carried out according to the procedure described earlier [11, 12]. Body weight and food and water intake were recorded on a daily basis. While fasting blood glucose level was checked at regular intervals. Animals with blood glucose levels of 300mg/dl or more at the end of 7 days were considered diabetic and taken for experimentation.

Experimental Groups

Animals were divided into five groups of six rats each.
Group I: (C) Normal rats administered double distilled water for 30 days.
Group II: (C+MSE) Normal rats administered with the herbal extract (MSE) (500mg/kg of body weight) orally for 30 days.
Group III: (D) Diabetic rats administered with double distilled water as vehicle daily for 30 days.
Group IV: (D+ MSE) Diabetic rats administered with the extract (500mg/kg of body weight) orally for 30 days from day 7 of diabetic induction.
Group V: (D+Mt) Diabetic rats administered Metformin (500mg/kg of body weight) orally for 30 days from day 7 of diabetic induction.
Fasting blood glucose was checked at regular intervals and food and water intake were recorded daily.

Parameters evaluated:

Oral glucose tolerance test and insulin response test

Oral glucose tolerance test (GTT) was performed at the end of the experimentation (29th day) according to the procedure described by Singh *et al*. [12]. Response to insulin was checked in all the experimental groups on the 31st day according to the procedure explained earlier [12].

Biochemical Estimations

At the end of the experimentation, animals were deprived of food overnight and sacrificed by decapitation. Blood was collected prior to sacrifice from the jugular vein and serum was separated. Separated serum was then used for various biochemical estimations. Liver and kidneys were dissected out of the animals immediately and washed. Kidney and liver homogenates were prepared using 10% (PBS) for various estimations.

Serum glucose was estimated by the glucose oxidase method [13]. Insulin was assayed by an ELISA based kit (Rat Insulin ELISA from mercodia, Sweden). Serum markers of hepatic and renal function were estimated using a semi auto analyser using reagent kits. Serum enzymes like ALP, ACP, SGPT and SGOT were estimated using kits procured from Reckon diagnostic Pvt Ltd, Aspen Laboratories, Agappe diagnostics and Crest Biosystems respectively. Serum urea and creatinine were estimated using reagent kits from DiaSys Diagnostics and Nicholas Piramal India limited. Serum lipid profile was checked wherein total cholesterol, triglycerides, HDL

Cholesterol, LDL and VLDL cholesterol were assayed using kits obtained from Accurex Biomedical Pvt Ltd and Nicholas Piramal India Limited.

Serum levels of sodium, potassium and magnesium were estimated using a flame photometer. Calcium was assayed using kit obtained from Bio In-Vitro Diagnostics Pvt.Ltd. Na^+ - K^+ ATPase activity in the kidney was estimated by method of Floreani and Bonetti [14] and the resultant phosphate released was assayed by the method of Fiske and Subbarow, [15]. Tissue protein was estimated by method of Lowry *et al.* [16].

Tissue lipid peroxidation in liver and kidney was assayed by the method of Beuge and Aust [17]. Tissue enzymatic antioxidant like GPx, Catalase and SOD were assayed by the methods of Beutler *et al*. [18], Sinha [19] and Marklund and Marklund [20] respectively. Non-enzymatic antioxidant (GSH) level was estimated by the method of Rotruck *et al*. [21].

Statistical Analysis

All data are expressed as Mean±SE. The statistical significance was evaluated for all experimental groups by One Way ANOVA followed by Bonferoni Multiple comparison test using Graph Pad Prism, Version 3.0 software obtained from Graphpad softwares , San Diego, CA/USA.

RESULTS

Body and organ (Liver and kidney) weights and food and water intake

Table 1 depicts the changes in body weight, organ weight and food and water intake in all the experimental groups. There was significant decrement ($p<0.01$) in the body weight of diabetic rats compared to the non-diabetic control rats. Diabetic animals also showed a significant ($p<0.001$) increase in the food and water intake that were measured on a daily basis. The extract and metformin treated groups of diabetic rats showed increase in body weight similar to non-diabetic rats. Moreover, both extract and metformin treatments had a similar effect in lowering food intake in comparison to the diabetic control rats, while, metformin treatment was more effective in decreasing water intake compared to the extract administrated diabetic rats. Treatments with both extract and metformin showed a decrease in liver and kidney weights as against an increase rended for diabetic rats.

Blood glucose and insulin levels

There was a steep increase in the fasting blood glucose level in diabetic rats as seen from table 2, with a corresponding decrease in circulating insulin titre. Treatment with MSE showed a significant time dependent decrement in blood glucose level and was very much comparable with that obtained with metformin treatment. The serum insulin level was significantly higher in MSE administered diabetic rats much closer to the diabetic level. Metformin was also effective but not as effective as MSE.

GTT and IRT

Treatment with MSE had no significant effect in non-diabetic animals (Fig 1,2). However MSE and metformin treatments of diabetic rats showed considerably bettered GTT curves with, lowered positioning of the curves in comparison to diabetic rats. Out of the two treatment

schedules, metformin registered relatively more lowered positioning of glucose tolerance curve compared to the extract treated rats. Insulin response curves of both MSE and metformin treated diabetic rats showed a bettered response to insulin in comparison to the diabetic rats (Fig 3, 4).

Serum lipid profile:
Serum lipid profile of diabetic rats showed a significant increment (P<0.001) in serum total, LDL and VLDL cholesterol levels along with TG and a corresponding decrement in HDL cholesterol (Table 3). Both MSE and metformin treatments showed significant (p<0.001) decrement in cholesterol and TG levels with HDL, LDL and VLDL levels being in the range of non-diabetic rats.

Serum markers of hepatic and renal dysfunction
The changes in the marker enzymes of hepatic and renal dysfunction are as shown in Table 4. In diabetic rats, the levels of all four hepatic enzymes were significantly (P<0.01) elevated. Similarly, the levels of urea and creatinine, markers of renal function were also increased. Both MSE and metformin were effective in preventing these increases, though MSE being more effective in maintaining hepatic markers and metformin being effective in maintaining renal markers.

Serum levels of Na^+, K^+, Mg^{++} and Ca^{++} and, renal Na^+-K^+ ATPase activity
Serum ionic status and Na^+-K^+ ATPase activity are depicted in table 5. Diabetic animals showed significant increment in serum sodium, potassium and calcium levels alongwith a significant decrement in the magnesium ion concentration. Apart from this ionic dysregulation, diabetic animals also showed significant decrement in renal Na^+-K^+ ATPase activity. Treatment with both MSE and metformin was effective in preventing the changes induced by diabetes with metformin being relatively more effective with respect to Na^+ and Mg^{++} and MSE for all other parameters.

Hepatic and renal Lipid peroxidation levels
Diabetic animals showed a significant increment in hepatic and renal levels of LPO (Table 6 and 7). Both MSE and metformin afforded near complete protection against diabetes induced increase in LPO.

Table 1: Changes in body weight, tissue weight and food and water intake

GROUPS	INITIAL WEIGHT(g)	FINAL WEIGHT(g)	FOOD INTAKE (g/animal/day)	WATER INTAKE (ml/animal/day)	LIVER WEIGHT (g/100gbw)	KIDNEY WEIGHT (g/100gbw)
C	246.33 ± 15.91	270.33 ± 7.27	18.15 ± 0.01	37.56 ± 1.15	2.18 ± 0.37	0.53 ± 0.05
C + MSE	220 ± 5.01	242.5 ± 2.50 [a]	15.43 ± 0.28 [c]	38.31 ± 0.14	2.20 ± 0.01	0.48 ± 0.11
D	236.25 ± 2.39	215 ± 4.56 [b]	33.93 ± 0.03 [c]	112.11± 0.10 [c]	3.44± 0.14 [c]	0.88 ± 0.05 [c]
D + MSE	210 ± 7.35	225 ± 7.35	28.46 ± 0.29 [e]	100.60± 0.35 [e]	3.02 ± 0.11	0.56 ± 0.07
D + Mt	220 ± 10.02	242.5 ± 17.55	24.4± 0.19 [e]	55.56 ± 0.27 [e]	3.13 ±0.05	0.57 ± 0.11 [e]

Data are expressed as Mean±SE [a] p<0.05, [b] p<0.01, [c] p< 0.001 when compared with Normal * p< 0.05, • p< 0.01, [e] p< 0.001 when compared to Diabetic Control

Hepatic and renal antioxidant status and protein contents.
Diabetic animals showed a significant decrement in tissue protein and Glutathione contents and activity levels of GPx, CAT and SOD (Table 6). Both MSE and metformin maintained the levels of all parameters in the non-diabetic range, offsetting the effects of diabetes. On a comparative

basis, MSE was found to be relatively more effective than metformin in maintaining the levels of tissue protein and endogenous anti-oxidant levels.

Table 2 : Changes in blood glucose level during the entire four week treatment period

GROUPS	BLOOD GLUCOSE (mg/dl)						INSULIN
	0 DAY	15 DAYS AFTER ALLOXAN INJECTION	I WEEK (AFTER TREATMENT)	II WEEK	III WEEK	IV WEEK	
C	85±2.56	98±3.01	89±4.13	95±4.87	97±6.2	95.43±1.15	0.37±0.01
C + MSE	88±3.22	94±2.25	98.25±4.48	98±8.79	93.45±10.33	86.83± 4.19	0.39±0.02[b]
D	89±5.31	394±18.25 [c]	399±22.4 [c]	402±28.54 [c]	415±36.55 [c]	431.33± 6.93 [c]	0.15±0.01[C]
D + MSE	85±4.32	420±15.24	346±23.12[•]	297±32.45 [e]	263±17.22 [e]	243.83± 10.10 [e]	0.31±0.01 [e]
D + Mt	85±2.11	331±10.7	308±21.32[•]	257±15.25 [e]	222±19.78 [e]	206.50± 72.68 [e]	0.22±0.02

Data are expressed as Mean±SE[a] p<0.05, [b] p<0.01, [c] p< 0.001 when compared with Normal ***** p< 0.05, [•] p< 0.01, [e] p< 0.001 when compared to Diabetic Control

Table 3: Serum lipid profile of extract and metformin treated diabetic and non-diabetic rats

GROUPS	CHO (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
C	80.00±1.16	70.27±1.22	53.33±1.20	15.67±0.88	13.00±0.58
C + MSE	80.33±2.73	64.33±1.77	54.67±3.48	14.33±0.88	12.67±0.88
D	103.33±3.53 [c]	141.33±2.03 [c]	46.33±0.88	30.67±0.88 [c]	24.33±0.88 [c]
D + MSE	76.67±2.97 [e]	102.67±5.46 [e]	55.33±2.34[•]	18.67±0.67 [e]	18.33±0.33 [e]
D + Mt	78.67±2.34 [e]	111.33±5.21 [e]	60.67±2.97	25.00±0.58 [e]	17.67±0.88 [e]

Data are expressed as Mean±SE[a] p<0.05, [b] p<0.01, [c] p< 0.001 when compared with Normal ***** p< 0.05, [•] p< 0.01, [e] p< 0.001 when compared to Diabetic Control

Table 4: Serum hepatic and renal marker enzymes in control and treated diabetic and non- diabetic rats

GROUPS	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	ACP (U/L)	UREA (mg/dl)	CREATININE (mg/dl)
C	51 ± 6.35	82 ± 4.04	197.5 ± 11.21	10.8 ± 1.32	69.66 ± 28.27	0.6 ± 0.10
C + MSE	50.5 ± 2.02	75 ± 4.11	187.5 ± 8.95 [b]	9.85 ± 0.08	54.5 ± 0.86 [b]	0.61 ± 0.008
D	83.75 ± 0.43 [c]	104.5 ± 0.86 [c]	289.5 ± 0.28 [c]	21.05 ± 0.028 [c]	142.5 ± 6.64 [c]	0.8 ± 0.05 [c]
D + MSE	52.5 ± 3.75 [e]	94.5 ± 2.02[•]	189 ± 26.01 [e]	10.55 ± 0.14	87± 0.57 [e]	0.65 ± 0.02[•]
D + Mt	67 ± 0.57 [e]	99.75 ± 0.14 [e]	211.5 ± 0.86 [e]	18.35 ± 0.20 [e]	80.65 ± 0.20 [e]	0.55 ± 0.02 [e]

Data are expressed as Mean±SE[a] p<0.05, [b] p<0.01, [c] p< 0.001 when compared with Normal ***** p< 0.05, [•] p< 0.01, [e] p< 0.001 when compared to Diabetic Control

Table 5: Changing in serum Na, K, Mg, and Ca and renal Na^+ - K^+ ATPase activity in extract or metformin treated non-diabetic and diabetic rats

GROUPS	SERUM SODIUM (m eq/L)	SERUM POTASSIUM (m eq/L)	SERUM MAGNESIUM (m eq/L)	SERUM CALCIUM (mg/dl)	RENAL Na+K+ATPase (nM of Pi/ min/ mg protein)
C	128.66 ± 0.88	6 ± 0.55	2.46 ± 0.08	12.06 ± 0.24	32 ± .12
C + MSE	119.33 ± 4.91	5.8 ± 0.32	2.1 ± 0.05 [a]	12.83 ± 0.76	35 ± 1.21
D	132.33 ± 0.88	7.16 ± 0.59	1.96 ± 0.08 [c]	10.23 ± 0.49	23 ± 2.11 [c]
D + MSE	120.33 ± 1.85*****	5.76 ± 0.29	2.6 ± 0.05	14.86 ± 1.61*****	30 ± 1.08[•]
D + Mt	106.6 ± 1.20 [e]	5.83 ± 0.12 [e]	2.16± 0.08 [e]	24.63 ± 0.91 [e]	28 ± 0.9

Data are expressed as Mean±SE[a] p<0.05, [b] p<0.01, [c] p< 0.001 when compared with Normal ***** p< 0.05, [•] p< 0.01, [e] p< 0.001 when compared to Diabetic Control

Table 6 : Changes in hepatic protein and lipid peroxidation and antioxidant status in control and treated diabetic and non-diabetic rats

GROUPS	PROTEIN (mg/100mg tissue)	LPO (nM of MDA /100g tissue)	GSH (mg of GSH /min/100g tissue)	GPx (µgof GSH/min/mg protein)	CATALASE (µM of H2O2 decomposed/mg protein/min)	SOD (U/mg protein)
C	17.37±0.71	41.32± 1.18	35.55±2.34	4.63±0.21	54.89±2.14	9.16±0.59
C + MSE	18.73±0.40	27.13± 1.53 ^{c}	37.48±1.67	4.65±0.29	52.64±2.87	9.85±0.87
D	13.22±0.48 ^{b}	63.61± 1.27 ^{c}	10.86±0.69 ^{c}	2.64±0.21 ^{c}	22.13±1.72 ^{c}	5.94±0.64 ^{c}
D + MSE	14.86±0.81	30.09± 1.89 ^{e}	34.90±0.78 ^{e}	4.21±0.25 ^{e}	44.41±3.16 ^{e}	7.30±0.26$^{\bullet}$
D + Mt	13.08±1.05	28.92± 0.27 ^{e}	32.86±2.88 ^{e}	4.13±0.15 ^{e}	38.76±2.02 ^{e}	6.88±0.42*

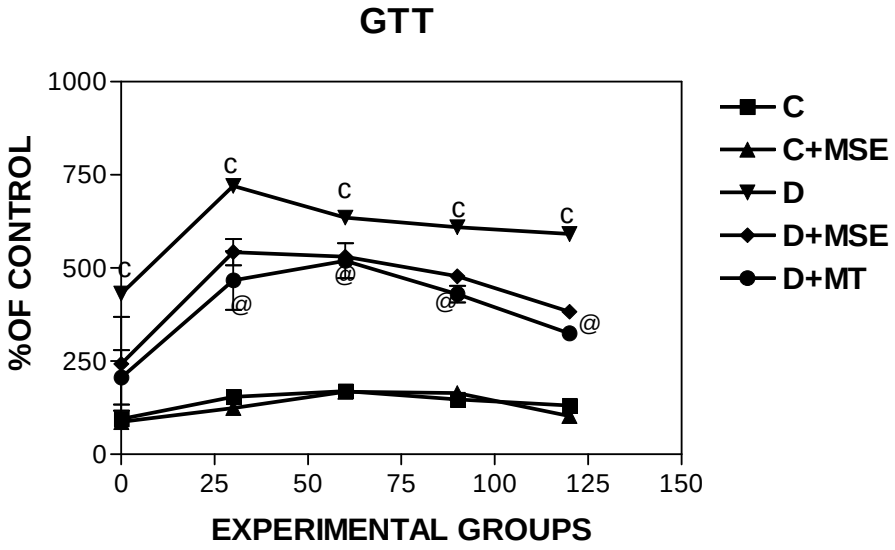
Data are expressed as Mean±SE ^{a} p<0.05, ^{b} p<0.01, ^{c} p< 0.001 when compared with Normal * p< 0.05, $^{\bullet}$ p< 0.01, ^{e} p< 0.001 when compared to Diabetic Control

Table 7: Changes in renal protein and lipid peroxidation and antioxidant status in control and treated diabetic and non-diabetic rats

GROUPS	PROTEIN (mg/100mg tissue)	LPO (nM of MDA /100g tissue)	GSH (mg of GSH /min/100g tissue)	GPx (µgof GSH/min/mg protein)	CATALASE (µM of H2O2 decomposed/mg protein/min)	SOD (U/mg protein)
C	10.48±0.36	47.45±1.45	24.39±0.67	2.50±0.19	26.73±0.19	5.72±0.36
C + MSE	12.52±0.54	41.66±4.61	29.33±1.35 ^{a}	2.96±0.29	25.71±2.67	5.89±0.04
D	8.41±0.29	71.63±1.45 ^{c}	13.12±0.28 ^{c}	1.30±0.10 ^{a}	12.29±1.27 ^{c}	3.79±0.14 ^{c}
D + MSE	13.74±1.22 ^{e}	37.73±0.95 ^{e}	24.76±0.81 ^{e}	4.08±0.44 ^{e}	22.92±2.34$^{\bullet}$	4.54±0.46$^{\bullet}$
D + Mt	11.29±0.56	33.06±1.28 ^{e}	22.39±1.48 ^{e}	2.85±0.16$^{\bullet}$	24.60±2.45$^{\bullet}$	3.54±0.46

Data are expressed as Mean±SE ^{a} p<0.05, ^{b} p<0.01, ^{c} p< 0.001 when compared with Normal * p< 0.05, $^{\bullet}$ p< 0.01, ^{e} p< 0.001 when compared to Diabetic Control

Fig 1: Glucose tolerance curves of non-diabetic and diabetic rats treated with extract or metformin



Data are expressed as Mean±SE ^{a} p<0.05, ^{b} p<0.01, ^{c} p< 0.001 when compared with Normal * p< 0.05, $^{\bullet}$ p< 0.01, ^{e} p< 0.001 when compared to Diabetic Control

Fig 2 : Showing area under curve (AUC) of non-diabetic and diabetic rats treated with extract or metformin

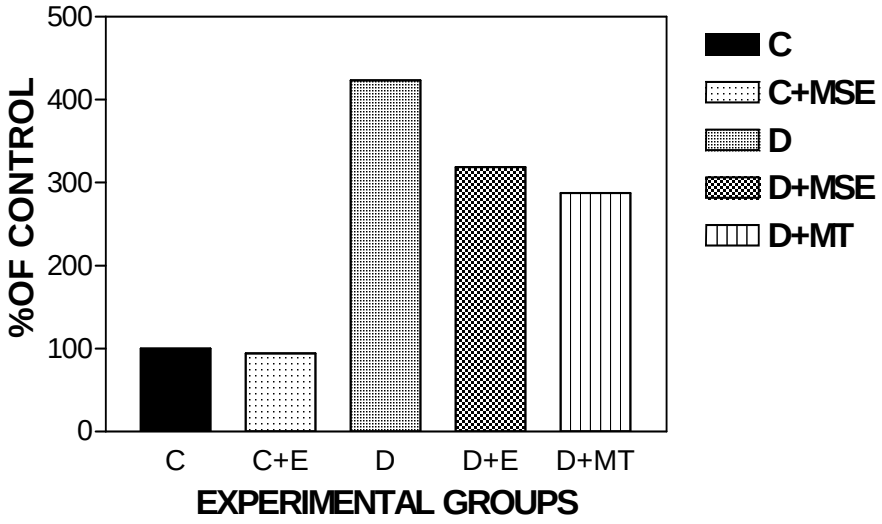
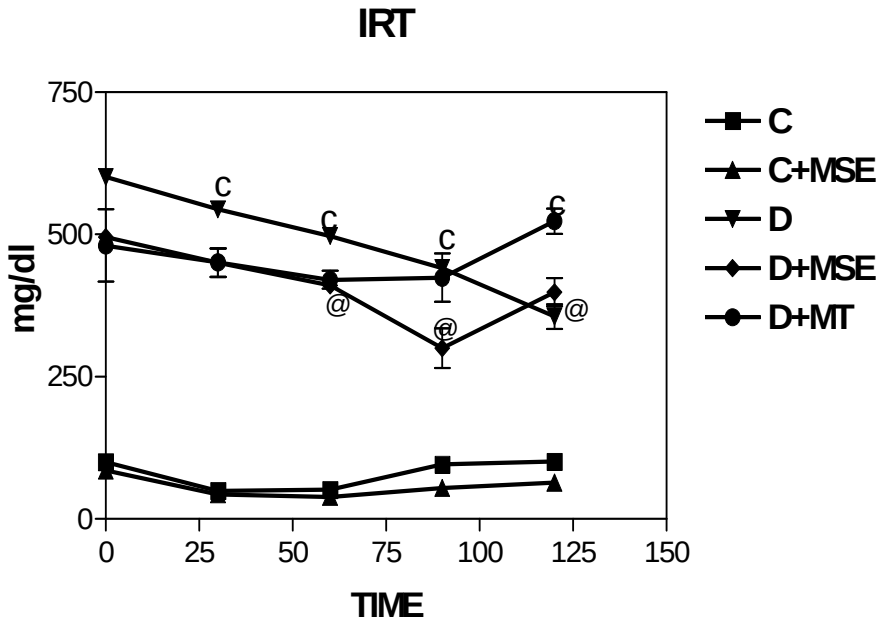
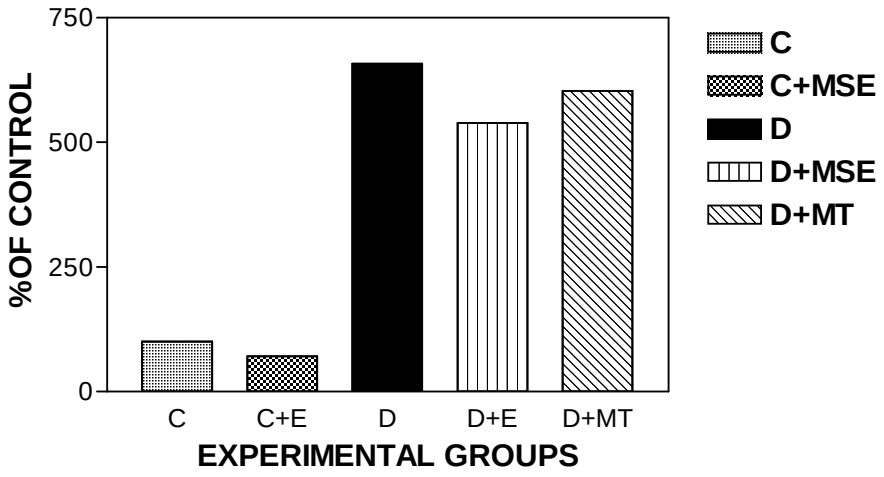


Fig 3 : Insulin response curves of non-diabetic and diabetic rats treated with extract or metformin



Data are expressed as Mean±SE[a] p<0.05, [b] p<0.01, [c] p< 0.001 when compared with Normal * p< 0.05, * p< 0.01, [e] p< 0.001 when compared to Diabetic Control

Fig 4 : Showing area under curve (AUC) of non-diabetic and diabetic rats treated with extract or metformin



DISCUSSION

Diabetes is one such disorder of its type that has constantly demanded researchers to work for novel therapeutics that have the potency to combat not only hyperglycaemia but also target the associated complications. Thus, a constant search is on to identify such drugs that would have multitarget actions. Herbal preparations have become the current choice of research to this end, owing to their effectiveness and lesser side effects. The present study is one such attempt to explore on a holistic basis the potentials of *Medicago sativa* in combating hyperglycaemia, dyslipidemia, hepatic and renal dysfunctions and oxidative stress in diabetic animals.

As evident from the present as well as many other studies, alloxan induced diabetic animals manifest increased daily food and water, considered as key symptoms associated with this disorder [22]. Apart from polydipsia and polyphagia there is also marked decrement in body weight as a characteristic feature of their disorder [23]. Loss of structural proteins of organs as observed herein could be attributed to this loss in body weight and also supported by the observations of Rajkumar *et al*. [24]. Both MSE and metformin treatments have recorded significant decrement in diabetics induced increase in food and water intake. Prevention of diabetic manifestation by both MSE and metformin is indicated by an actual increase in body weight well supported by increase in hepatic and renal protein contents.

In the present study supplementation MSE is found to be very effective in lowering diabetic hyperglycaemia in four weeks by about 42% which is better than the 37% antihyperglycaemia shown by Mt treatment. An earlier study by Swanston-flatt *et al*.[9] had indicated the antihyperglycaemic efficacy of *Medicago sativa* in streptozotocin induced diabetic rats when given as dietary supplement or as infusion in drinking water. Further reduction in postprandial blood glucose level in Type II diabetic rats treated with 1g/kg of aqueous extract of Lucerne (*Medicago sativa*) was thought to be possibly through increased insulin secretion [25]. The observed antihyperglycaemic potency of this plant seems to be essentially due to promotion of insulin secretion as the insulin titre in D+MSE animals is found to be lower by only 16% as against a 60% deficit in D animals and 41% deficit in D+Mt animals. Our observation of

increased insulin secretion is well supported by the reported threefold increase in insulin secretion from BRIN-BD11 pancreatic beta cell line in presence of an aqueous extract of *Medicago sativa* [26, 5]. Apparently, MSE has potential in lowering diabetic hyperglycaemia by way of increased insulin secretion, which is even better than the antihyperglycaemic and insulin releasing ability of metformin.

The anti-hyperglycaemic effect of both MSE and Mt is again well reflected in the observed glucose tolerance and insulin response curves, which show a position lower than those of diabetic animals. The area under the curves which is significantly high in diabetic animals shows significant reduction in the case of both MSE and Mt treated groups of animals. The improvement in glucose disposal as attested to by the improvement in GTT and IRT, can be accredited to increased glucose oxidation and incorporation of glucose into glycogen as observed for the abdominal muscle of diabetic mouse treated with a dietary supplement of Lucerne [5].

Apart from the promotion of increased insulin secretion and action, MSE might also have other extra pancreatic mechanisms of action in mediating its antihyperglycaemic effect. The extra pancreatic mechanisms of glucose homeostasis might involve enhanced peripheral glucose transport and metabolism as potent as insulin even in absence of insulin, suggesting the competence of MSE to act through terminal pathways of insulin signalling. [27, 28, 5]. The mechanisms of MSE dependent glucose homeostasis might also be attributed to its potential to lower glucose absorption [29].

One of the associated complications of diabetes is dyslipidemia marked by hypertriglyceridemia and hypercholesterolemia, known to be consequences of Type I diabetes than can also lead to the development of Type II diabetes. Hence, correcting the associated dyslipidemia is of prime importance when evaluating any antihyperglycaemic or anti-diabetic therapeutic. In the present study diabetic dyslipidemia is evident from the significantly increased levels of CHO, TG, LDL and VLDL in the alloxanized diabetic rats. Supplementation of diabetic rats with MSE extract significantly decreases the CHO, TG, LDL and VLDL levels and with no previous reports on the hypolipidemic actions of Lucerne (*Medicago sativa*) in diabetic rats, this evaluations stands as an important observation in this context. The hypotriglyceridemic and hypocholesterolemic effects along with LDL and VLDL lowering are clearly more potent in MSE supplemented diabetic rats even better than treatment with metformin. Further, the recovery in HDL level, significantly lowered in diabetic animals, is also very well marked in MSE supplemented rats, again bettering the effect of metformin. Apparently, ethanolic MSE is potent in correcting diabetic dyslipidemia, which adds to its efficacy as an antidiabetic therapeutic. In support of our finding in the recent report of Asgary *et al*. [30] of the efficacy of Alfa alfa (*Medicago sativa*) on lipoproteins and fatty streak formation in diet induced atherosclerotic hypercholesterolemic rabbits.

Oxidative stress and diabetes has an interesting dual relationship as a cause of diabetes as well as its effect which can then lead to many secondary complications [31, 2]. Thus in order to evaluate the holistic efficacy of *Medicago sativa*, there is need to critically evaluate diabetic oxidative stress and its amelioration by the plant. In the present study diabetic rats are characterised by increased oxidative stress as marked by significantly increased Lipid peroxidation and decreased levels of both non-enzymatic and enzymatic antioxidants in both liver and kidney. Supplementation of diabetic rats with MSE does not only effectively curtail lipid peroxidation

levels but also restored the levels of endogenous antioxidants. On a comparative basis these effects of MSE in combating oxidative stress seems more potent than Mt adding to its therapeutic value. All these changes could in part be an indirect effect mediated through lowered glucose level and bettered insulin titre or even a direct action mediated through effect on endogenous antioxidant system. In all, the present observations provide support to the efficacy of MSE in combating diabetic manifestation by amelioration of oxidative stress, even better than the antidiabetic drug, metformin.

It is very much likely that the dysregulation in carbohydrate, lipid and protein metabolisms alongwith increased oxidative stress can affect the hepatic and renal functions in severe diabetic state. To this end diabetic rats showed an increase in the serum levels of markers of hepatic and renal function indicating functional dysregulation caused in these organs as a result of diabetes. Increased gluconeogenesis and ketogenesis during diabetes have been related with increased in activities of SGPT and SGOT while, increased tissue proteolysis and decreased protein synthesis have been related with increased serum urea and creatinine levels [32, 33]. Apart from these changes there is also noticeable increase in ALP and ACP activities correlatable with liver dysfunction [34]. Supplementation with MSE has been noted to significantly lower the serum levels of hepatic and renal markers. These ameliorative changes induced by MSE are more potent than with metformin especially with reference to hepatic dysfunction, providing a basis for the contention that the extract has both hepato and reno protective potentials.

While considering the diabetes associated complications there is also need to look into the delicate systemic ionic balance that otherwise can be a cause of several complications like hypertension and nephropathy. The analysis of serum levels of sodium, potassium, calcium and magnesium and renal Na^+ - K^+ ATPase activity carried out in this behest, have shown significant increment in serum levels of sodium, potassium and calcium with a decrease in magnesium and reduced Na^+ - K^+ ATPase activity in diabetic animals. Both, MSE supplementation and Mt treatment are found to be effective in reversing these changes in diabetic animals with the potency of MSE being equal or even better.

In conclusion, the present study on a holistic evaluation of antidiabetic efficacy of the plant *Medicago sativa*, in combating all possible diabetic complications has revealed a very potent therapeutic potential even bettering the antidiabetic drug in vogue, metformin. This study is the only one of its type so far that provides a platform for further detailed analysis and research on this plant in the development of a versatile multitargeted drug that could not only effect glycaemic regulation but also target all associated diabetic complications. It is likely that the holistic potential of this plant could be related with its content of conmestrol (phytoestrogen) and flavanoids [35, 36, 37, 38, 39]. saponins [40] and also vitamins like A,B,C and K [30].

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