



EVALUATION OF ANTIHYPERGLYCEMIC ACTIVITY OF *DODONAEA VISCOSA* LEAVES IN NORMAL AND STZ-DIABETIC RATS

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ABSTRACT

Dodonaea viscosa (Sapindaceae) leaves are traditionally used in the treatment of skin diseases, diarrhoea, colic pain etc. The ethyl acetate extract (DEA) and methanolic extract (DME) of *Dodonaea viscosa* leaves were administered orally at different doses (200 and 400mg/kg bw) to normal as well as STZ-diabetic rats. DME produced significant hypoglycemic effect in normal rats after 6h of administration. After acute treatment DME 400mg/kg produced marked fall (30.87%) after 6h of administration. DME 200mg/kg and 400mg/kg both showed improvement in glucose tolerance. Treatment of diabetic rats for 28 days with DME reduced the fasting glucose level by 43.81% than their pretreatment level. It brought about fall in level of total cholesterol by 36% and 38.89% and HbA1c by 29.44% and 35.6%. The increase in glycogen level was found to be 68.97% after treatment with DME 400mg/kg bw. It also normalized the elevated level of MDA in diabetic rats. DME 200mg/kg and 400mg/kg brought about the decreased level of GSH to near normal. The level of SGOT, SGPT were also found to be decreased which is comparable to the standard. These results clearly indicated that *Dodonaea viscosa* methanolic extract (DME) possess antidiabetic and antioxidant effect in diabetic rats.

Keywords: *Dodonaea viscosa*, Sapindaceae, Diabetes mellitus, Antihyperglycemic, Antioxidant

INTRODUCTION

Diabetes mellitus currently affects 143 million people worldwide and the number is growing rapidly. Recently, there has been increasing interest in the use of medicinal plants. The plant kingdom has become a target for the search by multinational drug and biologically active lead compounds¹. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes^{2,3}. The hypoglycaemic activity of a large number of these plants has been evaluated and confirmed in different animal models. *Dodonaea viscosa* is traditionally used in the treatment of rheumatism, skin infections, diarrhoeas, stomachaches, pains of hepatic or splenic origin, uterine colic and other disorders involving smooth muscles⁴. It is also used as an antipruritic in skin rashes and for the treatment of sore throat, dermatitis and hemorrhoids⁵. The literature survey reveals that the leaves of *Dodonaea viscosa* contain many important biological activities but no hypoglycemic activity has been mentioned. The present study was done to investigate whether the leaves of plant show any antihyperglycemic activity in STZ diabetic rats.

MATERIALS AND METHODS

Plant material and preparation of extract

The leaves of *Dodonaea viscosa* were collected from tractor training centre firm Hisar and were authenticated by Dr. H. B. Singh, Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), India. The leaves were shade dried and grounded into a granulated powder and defatted with petroleum ether. The hydroalcoholic extract was obtained extracting 3 kg of defatted powder with methanol (70%) at 60 °C for 72 h in Soxhlet followed by filtration and concentrated in rotaevaporator at 50 ± 5 °C to its one third volumes. The filtrate was partitioned with solvent ethyl acetate (Ethyl Acetate Extract; DEA) and evaporated on water bath till dryness. The extracts were stored at temperature below 10 °C and were freshly prepared with 0.2% agar for pharmacological experiments.

Preliminary Phytochemical screening

The extracts were tested phytochemically for the presence of alkaloids, tannins, carbohydrates, flavonoids etc. using various phytochemical tests.

Drug and chemicals

Glibenclamide was a generous gift from Torrent pharmaceuticals Ahmedabad, India. Streptozotocin and heparin were procured from SRL, India. EDTA was purchased from Hi-media Lab. Pvt Ltd., Mumbai. The chemicals were obtained from SD Fine chemicals Ltd., Mumbai and were of analytical grade.

Experimental animals and research protocol approval

Male Wistar rats (200-250g) were used for the study. They were procured from Disease Free Small Animal House, Chaudhary Charan Singh Haryana Agriculture University, Hisar (Haryana). The rats were housed in polycarbonate cages (29×22×14 cm) for a week under environmentally controlled conditions with a 10-14h light and dark cycle and maintained free access to food and water. The animals were kept fasted 2 h before and 2 h after drug administration. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India (Registration No. 0436).

Induction of experimental diabetes

Diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (60mg/kg bw) in 0.1M citrate buffer (pH 4.5) to a group of overnight fasted rats. Three days after injection, the rats with fasting blood glucose higher than 180 mg/dl were used for the experiments. Six rats were used in each experiment. Each animal was used once only in all of experiments. The food and water were removed from cages 12 h before testing.

The antihyperglycemic activity was assessed by two methods

1. By studying the hypoglycemic effect of the two extracts of *Dodonaea viscosa* leaves in normal healthy male rats
2. By studying antihyperglycemic effect of the extracts in STZ-diabetic rats using acute and chronic model.
3. The animals were divided into various groups
4. Group 1: Normal control Rats
5. Group 2 and 3: Normal rats treated were administered orally 200 mg/kg p.o. and 400 mg/kg p.o. residual methanolic extract DME of *Dodonaea viscosa* leaves respectively.

6. Group 4 and 5: Normal rats treated were administered orally 200 mg/kg p.o. and 400 mg/kg p. o. ethyl acetate extract DEA of *Dodonaea viscosa* leaves respectively.
7. Group 6 (Diabetic control): Animals were administered vehicle only.
8. Group 7: Diabetic animals were administered Glibenclamide (600 µg/kg; p.o.) respectively.
9. Group 8 and 9: Diabetic animal were administered orally 200 mg/kg p. o. and 400 mg/kg p.o. residual methanolic extract DME of *Dodonaea viscosa* leaves respectively.
10. Group 10 and 11: Diabetic animals were treated with 200 mg/kg p.o. and 400 mg/kg p. o. ethyl acetate extract DEA of *Dodonaea viscosa* leaves respectively.

Acute study involved determination of serum glucose level after 0,2,4,6 and 24 hr after glibenclamide, DEA (200 mg/kg p.o. and 400 mg/kg p. o.) and DME (200 mg/kg p. o. and 400 mg/kg p. o.). chronic study involved repeated administration of glibenclamide, DME(200 & 400mg/kg b.w.) and DEA(200 & 400mg/kg b.w.) for 27 days once a day at predetermined time and serum glucose level was determined on 0, 7th, 14th and 21st and 28th day.

Oral acute toxicity study

Healthy Wistar rats were subjected to acute toxicity studies as per guideline (AOT no. 425) suggested by Organization for Economic Co-operation and Development (OECD) (2001). The rats were observed by housing them individually in the polypropylene metabolic cages continuously for 2 h for behavioral, neurological and autonomic profiles and for any lethality during next 48 h.

Effect of DME and DEA on glucose tolerance test

After 25 day treatments, on 26th day a fasting blood sample was collected from all the groups in heparinized micro-centrifuge tube from retro-orbital plexus. Blood samples were also collected at the time interval of 0, 30, 60, 90 and 120 min. after administration of glucose at a concentration of 2 g/kg of body weight.

Effect of DME and DEA on serum glucose and cholesterol

Serum cholesterol and glucose level were measured by commercial supplied biological kit Erba Glucose Kit (GOD-POD Method) and Erba Cholesterol Kit (CHOD-PAP Method) respectively using Chem 5 Plus-V₂ Auto-analyzer (Erba Mannheim Germany) in serum sample. Glucose and cholesterol values were calculated as mg/dl blood sample.

Estimation of glycosylated hemoglobin (Hb1Ac) and body wt

Glycosylated hemoglobin was measured using commercial supplied biological kit (Erba Diagnostic) in serum sample prepared as above

using Chem 5 Plus-V₂ Auto-analyser (Erba Mannheim Germany). Values are expressed as the percent of total hemoglobin. The body wt. of all the animals was estimated at start and end of the experiment.

Estimation of MDA level

Malondialdehyde (MDA), an index of free radical generation/lipid peroxidation, was determined as described by Okhawa *et al.*⁶. The absorbance was measured at 532 nm using double beam UV-Visible spectrophotometer (Systronics 2203, Bangalore, India) against a blank. MDA values are calculated using the extinction coefficient of MDA-thiobarbituric acid complex 1.56×10^5 l/mol \times cm and expressed as nmol/ml.

Estimation of serum reduced glutathione level

The reduced glutathione was estimated in serum by method⁷.

Estimation of liver glycogen content and SGOT, SGPT and other biochemical parameter

Liver glycogen estimation was done by the method as described by method⁸. Urea Nitrogen levels Liver glycogen levels were measured by the modified DAM method⁹. Creatinine levels were measured by the modified Jaffe's Kinetic method. SGOT and SGPT levels were measured by spectrophotometric method which involves NADH oxidation. Kidneys and retroperitoneal fat pads were removed, weighed and expressed as per cent body weight.

Data analysis

All the results were expressed as mean \pm standard error of mean (SEM). The data of all the groups were analyzed using one-way ANOVA followed by Dunnett's t-test using the software Instat. In all the tests, the criterion for statistical significance was $p < 0.05$.

RESULTS

Acute oral toxicity studies

Acute toxicity study revealed that both DEA and DME are safe upto 2000mg/kg bw. No lethality or toxic reaction was observed upto the end of study.

Effect of DME and DEA on normoglycemic animals

The effect of graded doses of *Dodonaea viscosa* methanolic extract DME and ethyl acetate extract DEA on blood glucose level of normal healthy rats are presented in table 1. DEA showed a significant hypoglycemic effect after 6h at 400mg/kg bw dose but at 200mg/kg no effect was observed. Further DME at 200mg/kg be and 400mg/kg produced a significant fall in blood glucose level which was found to be peak after 6h.

Table 1: Hypoglycemic effect of *Dodonaea viscosa* on normal rats

Treatment	Blood glucose levels(mg/dl) at time(h)				
	0	2	4	6	24
Vehicle	86.8 \pm 3.9	87.2 \pm 3.2	86.6 \pm 4.6	85.5 \pm 3.8	83.8 \pm 4.2
DEA 200mg/kg	81.36 \pm 1.69	80.33 \pm 1.89	79.5 \pm 2.01	78 \pm 2.19	84 \pm 1.18
DEA 400mg/kg	81.4 \pm 4.6	80.2 \pm 4.2	72.2 \pm 4.8*	65.8 \pm 3.7*	82.6 \pm 4.5
DME 200mg/kg	85.8 \pm 4.2	79.6 \pm 3.8	69.2 \pm 4.1*	61.2 \pm 4.8*	86.1 \pm 4
DME 400mg/kg	87.6 \pm 3.2	79.4 \pm 4.4	64.1 \pm 4.6*	56.8 \pm 5.1*	72.2 \pm 3.8

DEA *Dodonaea viscosa* ethyl acetate extract; DME *Dodonaea viscosa* methanolic extract Values are presented as mean \pm S.E.M.; n = 6 in each group. One way ANOVA followed by Dunnett's test * $p < 0.01$ compared to 0 value;

Effect of DME and DEA on acute hyperglycemia

The effect of *Dodonaea viscosa* leaves extracts i.e. *Dodonaea viscosa* ethyl acetate extract (DEA) and *Dodonaea viscosa* methanolic extract (DME) in STZ diabetic rats on acute hyperglycemic model is depicted in table 2. The ethyl acetate fraction of *Dodonaea viscosa* (DEA) showed a glucose lowering effect only after 6h at 400mg/kg bw p.o.

DEA 200mg/kg bw p.o. did not show any significant glucose lowering effect On the other hand *Dodonaea viscosa* methanolic

extract showed a marked fall in blood glucose level at both 200mg/kg bw and 400mg/kg bw after 2h compared to its initial value. Glibenclamide 600µg/kg bw showed marked decrease in blood glucose starting from 2h and remained till 24h.

Effect of DME and DEA on chronic hyperglycemia

After chronic administration of *Dodonaea viscosa* seed extract for 27 days to type 1 diabetic rats. *Dodonaea viscosa* ethyl acetate extract (DEA) 200mg/kg bw p.o. did not show any significant glucose

lowering effect but DEA 400mg/kg bw showed a significant blood glucose lowering effect after 14 days of administration (table 3).

On the other hand DME at both 200mg/kg bw and 400mg/kg bw showed more decrease in blood glucose level after 7 days of

administration. Higher dose 400mg/kg bw showed more decrease in blood glucose level as compared to initial '0' value. The antihyperglycemic effect of DME remained till 28 days.

The results of DME are comparable to that of glibenclamide.

Table 2: Effect of *Dodonaea viscosa* on STZ diabetic rats on acute antihyperglycemic model

Treatment	Blood glucose level (mg/dl) at corresponding time				
	0h	2h	4h	6h	24h
Diabetic control	390.10±7.65	385.78±8.12	376.21±8.85	380.12±7.84	398.7±6.46
DEA 200mg/kg	383±3.89	399.50±2.12	376.67±4.37	371±4.01	395.5±4.39
DEA 400mg/kg	406.67±2.70	400.5±0.99	406.5±4.60	358±2.67*	395.83±2.65
DME200mg/kg	377.67±3.39	361.5±4.39**	314.83±4.85*	275±3.32*	368.67±5.11
DME400mg/kg	405.33±2.24	398.33±1.68**	305.17±1.60*	280.17±2.78	400.83±2.19
Glibenclamide	437.33±3.22	347.17±3.21*	213.67±3.92*	191.17±1.93*	232.17±6.7*

DEA *Dodonaea viscosa* ethyl acetate extract; DME *Dodonaea viscosa* methanolic extract Values are presented as mean ± S.E.M.; n = 6 in each group. One way ANOVA followed by Dunnett's test * p <0.01, ** p<0.05 compared to 0 value; glibenclamide (600µg/kg bw p.o.)

Table 3: Effect of *Dodonaea viscosa* on STZ diabetic rats on chronic antihyperglycemic model

Treatment	Blood glucose level (mg/dl) at corresponding day				
	0	7	14	21	28
Diabetic control	359±3.57	363.83±1.9	367.67±2.04	372.50±2.55	383.50±4.06
DEA 200mg/kg	352±2.14	360.67±1.35	364±3.5	368.5±3.13	375.50±6.43
DEA 400mg/kg	354±2.56	356.33±2.04	341.33±3.18*	313.67±4.21*	307.83±7.63
DME200mg/kg	350±2.46	298±3.34*	273.67±2.26*	255.83±5.41*	210±5.89*
DME400mg/kg	339.67±2.78	297.83±3.65*	259±9.79*	223.67±7.47*	190.83±3.01*
Glibenclamide	350±2.12	291.83±3.83*	269±6.61*	203.83±5.76*	179.33±2.49*

DEA *Dodonaea viscosa* ethyl acetate extract ; DME *Dodonaea viscosa* methanolic extract Values are presented as mean ± S.E.M.; n = 6 in each group. One way ANOVA followed by Dunnett's test * p <0.01 compared to 0 value; glibenclamide (600µg/kg bw p.o.)

Effect of DME on oral glucose tolerance test

Oral glucose tolerance test was done only on *Dodonaea viscosa* methanolic extract (DME) treated rats because DEA did not give any significant glucose lowering effect during acute and chronic study. An increase in rate of clearance of glucose was found in case of DME treated diabetic rats when compared to diabetic control. DME 400mg/kg bw p.o. also showed much improvement in glucose tolerance as compared to diabetic control (fig 1)

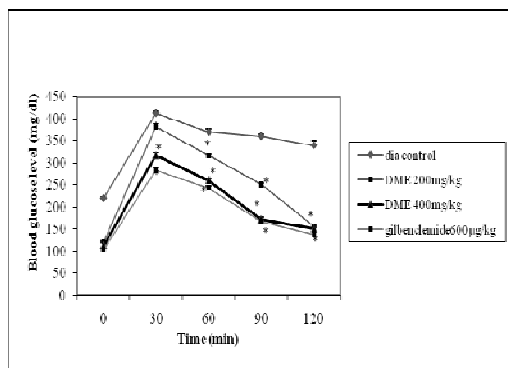


Fig. 1: Effect of *Dodonaea viscosa* methanolic extract on oral glucose tolerance test

Values are presented as mean ± S.E.M.; n = 6 in each group. One way ANOVA followed by Dunnett's test * p <0.01 compared to diabetic control ; glibenclamide(600µg/kg bw p.o.)

Effect of DME on body wt. and glycosylated hemoglobin HbA_{1c}

Fig. 2 and fig 3 represents effect of *Dodonaea viscosa* methanolic extract on body wt. and glycosylated haemoglobin in STZ diabetic

rats. There was a significant decrease in body wt. of diabetic rats as compared to normal rats.

Oral administration of DME at doses 200mg/kg bw and 400mg/kg bw p.o. for 28 days significantly increased the body wt. of treated diabetic rats to 231.11±1.69(p<0.01) and 245.41±41(p<0.01) correspondingly as compared to diabetic control 193.6±3.21.

Glycosylated hemoglobin level was found to be increased 12.16±1.01(p<0.01) in STZ diabetic rats as compared to normal rats. Oral administration of 200mg/kg bw and 400mg/kg bw doses of DME significantly decreased the HbA_{1c} level of diabetic rats to 8.58±1.08(p<0.05) and 7.83±0.83(p<0.05). Glibenclamide 600µg/kg bw significantly decreased the HbA_{1c} 7.33±1.08(p<0.01) as compared to diabetic control.

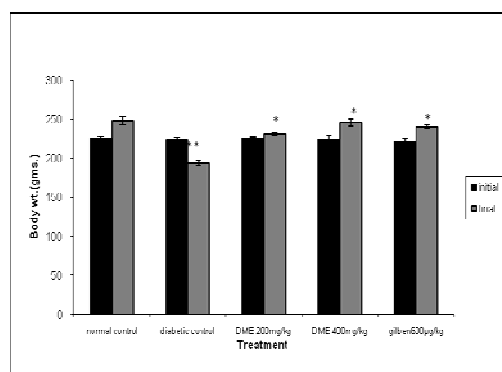


Fig. 2: Effect of *Dodonaea viscosa* methanolic extract on body wt

Values are presented as mean ± S.E.M.; n = 6 in each group. One way ANOVA followed by Dunnett's test * p <0.01 compared to diabetic control; glibenclamide (600µg/kg bw p.o.)

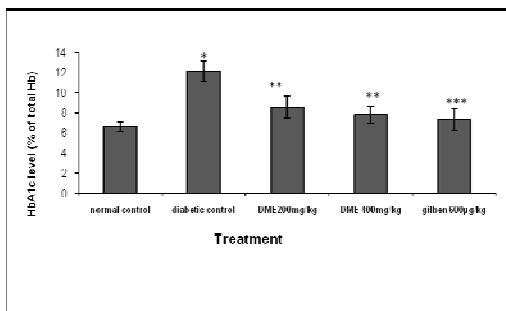


Fig. 3: Effect of *Dodonaea viscosa* methanolic extract on HbA_{1c}

Values are presented as mean ± S.E.M.; n = 6 in each group. One way ANOVA followed by Dunnett's test * p <0.01 compared to normal ; **p<0.05 compared to diabetic control; ***p<0.01 compared to diabetic control; glibenclamide(600µg/kg bw p.o.)

Effect of DME on serum total cholesterol and liver glycogen and other biochemical parameters

Fig 4 and Fig 5 mentions the effect of *Dodonaea viscosa* methanolic extract on serum total cholesterol and liver glycogen content. Total cholesterol was significantly higher (120±2.97) in diabetic control as compared to normal healthy control (65±2.72). However total cholesterol significantly decreased to (76±2.78, p<0.01) and (73.33±1.97, p<0.01) after 28 days treatment with the 200mg/kg bw and 400mg/kg bw p.o. of *Dodonaea viscosa* extract.

The glycogen content was found to be very low in case of diabetic control as compared to healthy control. In case of DME treated diabetic rats the glycogen content was found to be significantly increased (49±2.86) and (60.83±2.13) at 200mg/kg bw p.o. and 400mg/kg bw p.o. respectively. The urea, creatinine and SGPT levels were reduced by the treatment with the extract (Table 4).

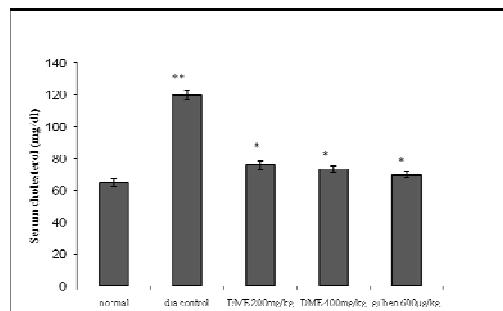


Fig. 4: Effect of *Dodonaea viscosa* methanolic extract on total cholesterol

Values are presented as mean ± S.E.M.; n = 6 in each group. One way ANOVA followed by Dunnett's test ** p <0.01 compared to normal ; *p<0.01 compared to diabetic control

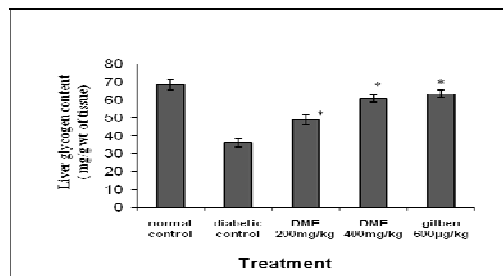


Fig. 5: Effect of *Dodonaea viscosa* methanolic extract on glycogen content

Values are presented as mean ± S.E.M.; n = 6 in each group. One way ANOVA followed by Dunnett's test ** p <0.01 compared to normal ; *p<0.01 compared to diabetic control

Table 4: Effect of DME on Creatinine, Urea nitrogen, SGOT and SGPT Level in STZ diabetic rats

Groups	Creatinine (mg/ dL)	Urea nitrogen (mg/ dL)	SGOT (U/ L)	SGPT (U/L)
Normal control	0.30 ± 0.01	12.12 ±0.87	138.00 ±2.48	58.75 ± 4.53
Diabetic control	1.08 ±0.08	33.92 ±6.07	157.75 ±28.23	122.00 ± 8.53
DME (200 mg/Kg)	0.40 ± 0.08**	15.74 ±0.52*	110.12 ±4.44	77.75 ± 5.46**
DME (400 mg/Kg)	0.41 ±0.08**	16.94 ±0.80*	121.00 ±7.31	85.75 ± 2.86**
Glibenclamide (600µg/Kg)	0.30± 0.01**	13.67±0.95*	129.25±6.40	73.00 ± 5.58*

Values are presented as mean ± S.E.M.; n = 6 in each group. One way ANOVA followed by Dunnett's test ** p <0.01 compared to normal ; *p<0.01 compared to diabetic control

Effect of DME on antioxidant parameter

Serum MDA level was found to be increased significantly as compared to healthy control (fig 6).

In diabetic rats treated with DME 200mg/kg bw and 400mg/kg bw p.o. there was a significant reduction (p<0.01) of MDA level i.e. (2.83±0.06) and (2.03±0.1) respectively as compared to diabetic control (4.98±0.08).

Fig 7 depicts the effect of *Dodonaea viscosa* methanolic extract on serum glutathione level. The serum glutathione (GSH) level was significantly decreased in diabetic rats compared to control animals.

The level of glutathione was returned to near normal range in STZ diabetic rats treated with DME and diabetic rats treated with glibenclamide. Higher dose of DME showed more significant results.

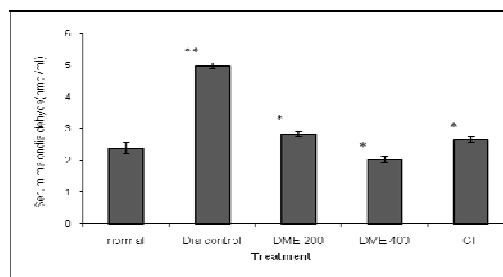


Fig. 6: Effect of *Dodonaea viscosa* methanolic extract on MDA

Values are presented as mean ± S.E.M.; n = 6 in each group. One way ANOVA followed by Dunnett's test **p<0.01 compared to normal control; *p<0.01 compared to diabetic control; DME *Dodonaea viscosa* methanolic extract; glibenclamide 600µg/kg

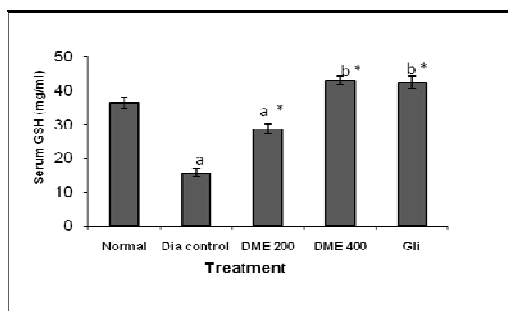


Fig. 7: Effect of *Dodonaea viscosa* methanolic extract on GSH

Values are presented as mean \pm S.E.M.; n = 6 in each group. One way ANOVA followed by *Dunnett's* test a $p < 0.01$ compared to normal control; b $p < 0.05$ compared to normal * $p < 0.01$ compared to diabetic control; DME *Dodonaea viscosa* methanolic extract;

DISCUSSION AND CONCLUSION

The aim of the present study was to evaluate the antihyperglycemic and hypolipidemic effects of leaves of *Dodonaea viscosa* in STZ-induced diabetic rats. Diabetes mellitus causes a disturbance in the uptake of glucose as well as glucose metabolism. The use of a lower dose of STZ (60 mg/kg) produced an incomplete destruction of pancreatic β -cells even though the rats become permanently diabetic. After treatment with a low dose of STZ there should be many surviving β -cells, and regeneration is also possible¹⁰. Hyperglycemia generates abnormally high levels of free radicals by autooxidation of glucose and protein glycation, and oxidative stress has been reported to be a causal factor of cardiovascular complications in STZ-induced diabetes mellitus¹¹. Hyperglycemia is associated with the generation of reactive oxygen species (ROS) causing oxidative damage particularly to heart, kidney, eyes, nerves, liver, small and large blood vessels and gastrointestinal system¹². The serum glucose lowering activity of the plant extracts were compared with glibenclamide, a standard hypoglycemic drug. Glibenclamide has been used for many years to treat diabetes, to stimulate insulin secretion from pancreatic β -cells¹³. From the results of the present study, it appears that still insulin producing cells are functioning and the stimulation of insulin release could be responsible for most of the metabolic effects. It may be suggested that the mechanism of action of *Dodonaea viscosa* is similar to glibenclamide. This is the first report that demonstrates antidiabetic properties for *Dodonaea viscosa* leaves.

The present study investigated the effect of crude extracts of *Dodonaea viscosa* leaves (residual methanolic DME and ethyl acetate DEA) on glycemia of STZ-diabetic and normal rats. Streptozotocin (60mg/kg bw) administered *i.p.* in rats, effectively induced diabetes, as observed by the elevation of serum glucose level and loss of body wt. of the animals as compared to non-diabetic rats. DEA did not produce any hypoglycemic effect in normal rats. The diabetic rats treated with DEA produced a glucose lowering effect after completion of 6h at 400mg/kg bw *p.o.* but at 200mg/kg bw *p.o.* did not show any significant glucose lowering effect. The normal rats treated with DME were found to be hypoglycemic after completion of 4hr. Further the extract showed peak antihyperglycemic effect at 6h in diabetic rats indicating a lag period of 5-6 h before the peak effect was reached. The effect waned at 24h.

The chronic study indicated that a period of 7 days is required for attaining antihyperglycemic effect. DEA at 200mg/kg bw did not show antihyperglycemic effect; however at 400mg/kg bw reduction in serum glucose level was observed after 14 days, which remained till 21 days. Induction of diabetes with STZ is associated with the characteristic loss of body weight, which is due to increased muscle wasting¹⁴ and due to the loss of tissue proteins¹⁵. The chronic treatment for 27 days with DME and glibenclamide in the tested doses brought about improvement in body weights of STZ treated diabetic rats indicating its beneficial effect in preventing loss of body wt. in diabetic rats. An increase in the body weight of diabetic rats

might be due to an improvement in insulin secretion and glycemic control¹⁶.

Diabetic animals have impaired glucose tolerance. Additional load of glucose was found to impair the tolerance further. From the data obtained in OGTT, it may be suggested that DME acts by increasing peripheral utilization of glucose. Glibenclamide was more effective in showing antihyperglycemic effect as well as glucose tolerance. DEA did not give satisfactory results during acute and chronic study, therefore further study was continued only with DME.

The increased glycosylated hemoglobin in the diabetic control rats indicated that erythrocytes were more prone to oxidative stress in diabetes¹⁷. Glycosylated hemoglobin had been found to increase in patients with diabetes mellitus¹⁸. Oral administration of *Dodonaea viscosa* methanolic extract decreased hyperglycemia and therefore the level of HbA_{1c} decreased. Glucose synthesis in the rat liver and skeletal muscles was impaired during diabetes¹⁹ hence glycogen content of skeletal muscle and liver markedly decreased in diabetes²⁰. Oral administration of DME significantly increased tissue glycogen levels in STZ-diabetic rats. This prevention of depletion of glycogen in the liver is possibly due to stimulation of insulin release from β -cells²¹.

Abnormalities in lipid profile are one of the most common complications in diabetes mellitus found in 40% of diabetic cases²². Diabetes causes an increase in the cholesterol, triglycerides, LDL and VLDL²³. High levels of total cholesterol in blood are major coronary risk factors. The abnormal high concentration of serum lipids in the diabetic subject is due mainly to the increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. The administration of *Dodonaea viscosa* methanolic extract caused a significant reduction in total cholesterol 36% and 38.89% at 200mg/kg and 400mg/kg bw respectively.

Hyperglycemia results in the generation of free radical which can exhaust antioxidant defense thereby leading to the disruption of cellular function, oxidative damage to membrane and enhance the susceptibility to lipid peroxidation²⁴ and diffuse from the site of tissue damage which is measured by malondialdehyde level. Serum malondialdehyde was found to be increased in diabetic rats (4.98 \pm 0.08) as compared to normal rats (2.38 \pm 0.18). Decrease in the level of glutathione (GSH) in diabetic rats (15.66 \pm 1.14 mg/dl) compared to normal rats (36.16 \pm 1.60) give evidences for altered antioxidant system during diabetes²⁵. Decreased level of glutathione in serum of STZ diabetic rats was partly due to its utilization by the tissue to compromise the deleterious effect of lipid peroxidation²⁶. *Dodonaea viscosa* extract (400 mg/kg *p. o.*) increased the level of GSH to (42.83 \pm 1.35) in STZ-diabetic rats, which is an indication of its antioxidant properties²⁷.

In methanol extract treated diabetic rats the reduction in urea level indicated reduced proteolysis²⁸ and this might be the reason for the increase in the body weight of the animals. The entry of renal glucose is not dependent on action of insulin and therefore in the event of hyperglycemia there is an increase in the entry of glucose²⁹. This has been postulated to cause increased intra-renal glycogen deposition which leads to glycosylation of basement membrane collagen in kidney. Therefore the weight of kidneys increases in diabetic condition³⁰. The reduction of the kidney weight in the extract treated animals indicates that the treatment could prevent the onset of macrovascular complications. Likewise, the reduction in urea and creatinine levels indicates the renoprotective effect of the extract.

The preliminary phytochemical analysis of DME showed presence of carbohydrates, flavonoids, steroids, fixed oils, tannins and saponins. Flavonoids are potent antioxidants and are known to modulate the activities of various enzymes due to their interaction with various biomolecules. Chakravarthy *et al.* (1980) have reported that flavonoids regenerate the damaged β -cells in the STZ diabetic rats. From our experimental findings it is possible to conclude that DME exhibited promising antidiabetic activity in STZ diabetic rats. Flavonoids are responsible for increase in insulin secretion and peripheral glucose utilization which are abundant in DME extract.

The mechanism of action of antihyperglycemic action may be attributed to involvement of DME in improved insulin secretion, peripheral glucose utilization and reduced gastrointestinal absorption of glucose.

Its antihyperlipidaemic effect could represent a protective mechanism against atherosclerosis, especially in diabetic condition. Finally, it can be considered that DME is safe for oral consumption and elicits promising hypoglycemic activity in animal experiments. Hence, it may be pursued for its clinical usefulness in the management of diabetes mellitus.

REFERENCES

- Evans, W.C., Trease and Evan's Pharmacognosy, WB Saunders, London, 1996, 14th ed., p. 780
- Ajgaonkar, S.S., Herbal drugs in the treatment of diabetes, a review. IDF Bulletin 24 1979., 10-17.
- Alarcon-Aguilar, F.J., Roman-Ramos, R., Perez-Gutierrez, S., Aguilar-Contreras, A., Contreras-Weber, C.C., Flores-Saenz, J.L. Study of the antihyperglycemic effect of plants used as antidiabetics, Journal of Ethnopharmacology 1998;61: 101-110.
- Rojas, A., S. Cruz, H. Ponce-Monter, and R. Mata, Smooth muscle relaxing compounds from *Dodonaea viscosa*. Planta Medica, 1996; 62:154-159.
- Rojas A, Hernandez L, Pereda MR, Mata R., Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants J. Ethnopharmacol., 1992; 35:275-283.
- Okhawa H, Ohishi N, Yagi K., Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal Biochem. 1979., 95:351, 8
- Sedlak J, Lindsay RH., Estimation of total protein bound and non protein bound sulfhydryl group in tissue with ellman's reagent. Anal Biochem., 1968;15: 192-205
- Carroll, N.V., Longley, R.W., Roe, J.H.,. The determination of glycogen in liver and muscle
- by use of anthrone reagent. The Journal of Biological Chemistry 1956; 220: 583 - 593.
- Wybenga, D.R., Di Giorgio, J., Pileggi, V.J.,. Manual and automated methods for urea nitrogen measurement in whole serum. Clinical Chemistry 1971; 17: 891 - 895
- I Ahmed; MS Lakhani; M Gillet; H Raja. Anti-hyperglycemic and antioxidant activity of stem bark of *Polyalthia longifolia* var. *angustifolia* Diabetes Res. Clin. Pract., 2001, 51, 151
- Sangameswaran B., Ilango K. Antihyperglycemic and antihyperlipidaemic effects of extracts of *Ipomoea reniformis* Chios on Alloxan Induced Diabetic Rats. Scholars Research Library, Annals of Biological Research, 2010;1 (1) : 157-163
- Tunali S and Yanardag R, Effect of vanadyl sulfate on the status of lipid parameters and on stomach and spleen tissues of streptozotocin-induced diabetic rats. Pharmacol Res, 2006, 53: 271-277
- Tian J, Atkinson MA, Clare-Salzler M, Herschenfeld A, Forsthuber T, Lehmann PV, Kaufman DL, Insulin-Dependent Diabetes Mellitus, J Exp Med 1996; 183:1561-1567
- S.K. Swanston-Flat, C. Day, C.J. Bailey, P.R. Flatt., Traditional plant treatment for diabetes: studies in normal and Streptozotocin diabetic mice Diabetologia 1990; 33:462-464
- Chatterjee S, Zareena N, Gautam S, Adhikari S, Variyar PS, Sharma A., Antioxidant activity of some phenolic constituent from green pepper (*Piper nigrum* L.) and fresh nutmeg mace (*Myristica fragrans*), Food chem 2007 ; 101(2):515-23
- Del Bosque-Plata L et al., Analysis of the glucokinase gene in Mexican families displaying early-onset non-insulin dependent diabetes mellitus including MODY families J Pancreas , 2005; 6(3),238-245
- Frederick Madison Allen, Current opinion in endocrinology, The endocrinologist 2009;19:3,93
- K. Baskaran, B.K. Ahmath, K.R. Shanmugasundaram, E.R.B. Shanmugasundaram, Antidiabetic effect of leaf extracts from *Gymnema sylvestre* in non-insulin dependent diabetes mellitus patients. J. Ethnopharmacol 1990;30: 295-305
- D.F. Hwang, Y.S. Lai, M.T. Chang., Toxic effects of grass carp, snake venom and chicken bile juices in rats. Toxicol. Lett. 1997 85, 85-92
- Goutam Ghosh et al., Biological action and medicinal properties of various constituent of *Azadirachta indica* (Meliaceae)" an Overview. Scholars Research Library, Der Pharmacia Lettre, 2010, 2(2), 206-216
- S.S. Rathi, A study on drug-drug interaction between anti-hypertensive drug (propranolol) and anti-diabetic drug (glipizide), Phytotherapy Research 2002, 16:8, 774-777.
- K. Ravi, S. Rajasekaran, S. Subramanian, Antihyperglycemic effect of *Eugenia jambolana* seed kernel on streptozotocin-induced diabetes in rats, Food Chem. Toxicol. 2005, 43, 1433-1439
- Jasmine R. et al., hypoglycemic and hypolipidaemic activity of *Eugenia jambolana* in STZ diabetic rats, Asian journal of Biochemistry 2007; 2(4): 269-273.
- Van Dum PS, Van Asbeck BS, Erkelens DW, Marx JJM, Gispen WH, Bravenboer B., Estimation of liver glycogen, Diabetes Metabolism Rev. 1995 ;11:181-192.
- Chakravarthy B.K., Gupta S., Gambir S.S., Gode K.D., Pancreatic beta cell regeneration. A novel antidiabetic mechanism of *Pterocarpus* and *Pterocarpus marsupium* Linn, in normal and alloxanised diabetic rats, Indian Journal of Pharmacology 1980;12:123-127
- Matcovis B, Varga SI, Szaluo L, Witsas H., The effect of diabetes on the activities of the peroxide metabolic enzymes, Horm Metab Res. 1994; 14:77, 9
- M.N. Chatterjea, R. Shinde, Text Book of Medical Biochemistry, Jaypee Brothers Medical Publishers, New Delhi, 2002., 317
- Oliveira, H.C., Dos Santos, M.P., Grigulo, R., Lima, L.L., Martins, D.T.O., Lima, J.C.S., Stoppiglia, L.F., Lopes, C.F., Kawashita, Hypoglycemic and antihyperglycemic effect of *Begonia malabarica* in STZ rats, N.F. Journal of Ethnopharmacology 2008; 115: 515 -519.
- Belfiore, F., Rabuazzo, A.M., Iannello, S., 1986. Anabolic response of some tissues to diabetes. Biochemistry Medicine Metabolism and Biology 35, 149-155
- Raju, J., Gupta, D., Rao, A.R., Yadava, P.K., Baquer, N.Z. *Trigonella foenum graecum* (fenugreek) seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes. Molecular and Cellular Biochemistry., 2001; 224: 45 - 51.