

Attenuation of Diabetic Conditions by *Sida rhombifolia* in Moderately Diabetic Rats and Inability to Produce Similar Effects in Severely Diabetic in Rats

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Key Words

blood glucose, diabetes, dyslipidemia, oral glucose tolerance, oxidative stress

Abstract

Objectives: This study was done out to evaluate the effects of *Sida rhombifolia* methanol extract (SRM) on diabetes in moderately diabetic (MD) and severely diabetic (SD) Sprague-Dawley rats.

Methods: SRM was prepared by soaking the powdered plant material in 70% methanol and rota evaporating the methanol from the extract. Effective hypoglycemic doses were established by performing oral glucose tolerance tests (OGTTs) in normal rats. Hourly effects of SRM on glucose were observed in the MD and the SD rats. Rats were grouped, five rats to a group, into normal control 1 (NC1), MD control 1 (MDC1), MD experimental 1 (MDE1), SD control 1 (SDC1), and SD experimental 1 (SDE1) groups. All rats in the control groups were administered 1 mL of distilled water (DW). The rats in the MDE1 and the SDE1 groups were administered SRM orally at 200 and 300 mg/kg body weight (BW), respectively, dissolved in 1 mL of DW. Blood was collected initially and at intervals of 1 hour for 6 hours to measure blood glucose. A similar experimental design was followed for the 30-day long-term trial. Finally, rats were sacrificed, and blood was collected to measure blood glucose, lipid profiles, thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH).

Results: OGTTs indicated that two doses (200 and 300 mg/kg BW) were effective hypoglycemic doses in normal rats. Both doses reduced glucose levels after 1 hour in the MDE1 and the SDE1 groups. A long-term trial of SRM in the MD group showed a reduced glucose level, a normal lipid profile, and normal GSH and TBARS levels. In SD rats, SRM had no statistically significant effects on these parameters. Normal weight was achieved in the MD rats, but the SD rats showed reduced BW.

Conclusion: The study demonstrates that SRM has potential to alleviate the conditions of moderate diabetic, but not severe diabetes.

1. Introduction

Diabetes is a chronic disorder of carbohydrate metabolism due either to the relative or the absolute lack of insulin being produced by the beta cells or to insulin resistance. Insulin resistance develops as a result of the over production of insulin as in obese patients. Persistent hyperglycemia is the key feature of diabetes that leads to glycosylation of macromolecules. Glycosylated macromolecules, especially proteins, behave as free radicals and generate oxidative stress, which is a plausible factor for the development of diabetic complications [1]. During a pre-diabetic condition, hyperglycemia also plays a significant role in the destruction of beta cells by imparting oxidative stress because of glycosylation processes [2].

Treatment of chronic diseases with modern medicines is directed to the symptoms and not the root

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cause of the disease. Such treatments create other physiological disorders [3]. This is also the case with the treatment/management of diabetes. In such a chaotic situation, a need exists for a more effective approach that can prevent/manage the disease with fewer or no side effects. Treatment of diabetes with botanicals is focused on the holistic approach and has fewer or no side effects compared to treatment with synthetic drugs. Botanicals prevent and cure the disease and help the individual to achieve his/her natural body rhythm. Many botanicals are used for the management of diabetes, but their effects and mechanisms are all different. They also differ in terms of efficiency. Hence, a need exists to standardize the available herbal medicines in terms of their efficacy and mechanism of action. We have already published from our laboratory reports on several plants with anti-diabetic effects [4, 5]. The next plant in the series is *Sida rhombifolia* (SR). Water extract of the aerial part of SR has been reported by our laboratory to have hypoglycemic and hypolipidemic properties in diabetic rats [6].

SR is a perennial plant and belongs to the family *Malvaceae*. It is widely distributed in Southern Africa, including Botswana. In the ayurvedic system of medicine, SR is used to build immunity, well being, and vitality for the entire system. It has also been reported to have anti-inflammatory and anti-arthritis effects, to be effective as a treatment for gonorrhoea, and to have hepatoprotective properties [7]. The research finding of Dhalwal *et al* [8] showed mild hypoglycemic and hypolipidemic effects of the water extracts of leaves of SR in severely diabetic (SD) rats. An alcohol extract of SR has been reported to have hypoglycemic, hypolipidemic and anti-oxidant properties in diabetic rats [9]. Because water extracts of SR have shown significant hypoglycemic effects in moderately diabetic (MD) rats [6] and mild hypoglycemic effects in SD rats [8] and because no reports have been published on the effects of methanol extracts of SR on diabetes, the present study was designed to test the response to treatment with methanol extract of the aerial plant of SR of MD and SD rats by looking at their glucose levels, lipid profiles and anti-oxidant status.

2. Materials and Methods

The aerial part of SR (authentication voucher no. 006) used in this study was collected from the university of Botswana campus in the month of February in 2012. The plant parts were washed, chopped into small pieces, and shade dried. After that, they were crushed to powder and soaked in 70% methanol for cold extraction. After seven days, the methanol extract of SR (SRM) was filtered and then made solvent free by evaporation under reduced pressure. The yield of the extract was 9.8% weight/weight (wt/wt).

Male albino SD rats, each weighing approximately 250 g, were used for all the experiments. Following the guidelines of the university's Animal Use and Care Committee (Ref no: UBR/RES/ACUC/001), the animals had free access to water and food *ad libitum*. The food for the animals diet was purchased from Epol, South Africa, and consisted of the following: 12.1% (wt/wt) crude protein, 0.4% (wt/wt) methionine, 4.1% (wt/wt) fat, 40% (wt/wt) maize flour,

4.0% (wt/wt) salt mix, 2.2% (wt/wt) vitamin mix and essential fatty acids, 3.0% (wt/wt) bran, and 0.2% (wt/wt) moisture. As percentages, the total calories were 18% protein, 72% carbohydrate, and 10% fat.

The blood glucose and the lipid profile were measured by using kits from Aggape Diagnostics, Switzerland. Other chemicals were bought from local suppliers and were of analytical reagent (AR) grade. Mild diabetes was induced in rats by injecting alloxan monohydrate (50 mg/kg body weight (BW)) intraperitoneally for two consecutive days. After one week, the blood glucose was measured to confirm diabetes. After the confirmation, rats were kept in the diabetic condition for a week. To induce severe diabetes, we injected 120 mg/kg of alloxan monohydrate one week later. Rats having glucose levels between 125 and 175 mg/dL once and then checked the glucose level were considered mildly/MD rats while rats having glucose levels higher than 175 mg/dL were considered SD rats.

In the first experiment, we investigated the effect of SRM on the oral glucose tolerance tests (OGTTs) in normal rats. For this experiment, 20 male rats were selected and divided into 4 groups of five each: one control group and three experimental groups (EX1, EX2, and EX3). All the rats were denied food and water overnight (for 18 hours). Blood samples were taken from the rats in all four groups before treatment, and their fasting glucose levels were measured with a glucometer. Control rats were administered glucose (2 g/kg BW), and the rats in three EX groups were administered 100, 200 and 300 mg/kg BW of the extract along with glucose at 2 g/kg BW, respectively. All the administrations were oral and done with the help of a syringe and a tube. After the doses had been administered, blood samples were taken at 1 hour intervals for 3 hours, and their blood glucose levels were estimated.

In the second experiment, we investigated the hourly effect of SRM on the glucose levels in diabetic rats. For this experiment, 25 rats (5 normal rats + 10 MD and 10 SD rats) were selected and grouped as follows: The normal control 1 (NC1) group had 5 rats that were administered orally 1 mL of distilled water (DW). The MD control 1 (MDC1) group had 5 rats that were administered orally 1 mL of DW. The MD experimental 1 (MDE1) group had 5 rats that were administered orally 200 mg/kg BW of SRM in 1 mL of DW. The SD control 1 (SDC1) group had 5 rats that were administered orally 1 mL of DW. Finally, the SD experimental 1 (SDE1) group had 5 rats that were administered orally 300 mg/kg bw of SRM in 1 mL of DW. All rats were kept fasting overnight, and blood samples were taken the next day (0 hour), after which the rats were administered extract, followed by blood samples being taken and glucose levels being measured every hour for 6 hours.

In the third experiment, we investigated the effect of long-term administration of SRM in MD and SD rats. A SRM dose of 200 mg/kg of BW was selected to investigate its effect in MD rats, and a dose of 300 mg/kg BW was selected to investigate the effect in SD rats. For this experiment, 30 (5 normal + 10 MD and 15 SD) rats were taken and divided into 5 groups as follows: The normal control (NC, n = 5), the MD control (MDC, n = 5), and the SD control (SDC, n = 10) groups were administered DW every day. The MD experimental (MDE) group was administered SRM at a dose

Table 1 Effects of SRM on the glucose levels in MD and SD rats observed for 6 hours after its administration

Groups	Glucose levels (mg/dL) at 1 hour intervals (0 to 6 hours)						
	0	1	2	3	4	5	6
NC1	107.25 ± 4.76	110.38 ± 4.33	105.12 ± 5.17	100.13 ± 6.15	95.12 ± 5.13	90.62 ± 3.62	86.32 ± 3.19
MDC1	173.44 ± 4.31	179.34 ± 3.19	167.23 ± 4.98	171.43 ± 3.09	171.98 ± 5.32	178.43 ± 5.87	169.34 ± 4.01
MDE1	163.35 ± 5.87	150.53 ± 4.98	135.98* ± 3.11	123.65* ± 3.98	120.34† ± 5.41	121.65† ± 4.89	118.40* ± 3.65
SDC1	359.76 ± 5.23	360.83 ± 5.21	351.98 ± 4.33	348.31 ± 3.97	341.08 ± 5.81	347.11 ± 4.92	340.89 ± 6.43
SDE1	396.81 ± 8.13	375.43 ± 7.16	358.45 ± 9.7	349.73* ± 8.14	343.37* ± 5.93	347.40* ± 6.58	339.68* ± 7.33

All the control groups were administered distilled water. * $P < 0.05$ and † $P < 0.01$ when compared with their baseline values.

SRM, *Sida rhombifolia* methanol extract; MD, moderately diabetic; SD, severely diabetic; NC1, normal control 1; MDC1, moderately diabetic control 1; MDE1, moderately diabetic experimental 1 administered SRM (200 mg/kg body weight); SDC1, severely diabetic control 1 administered SRM (300 mg/kg body weight); SDE1, severely diabetic experimental 1.

of 200 mg/kg BW, $n = 5$) while the SD experimental (SDE) group was administered SRM at a dose of 300 mg/kg BW, $n = 5$). The experiment lasted for 30 days. At the end of the experiment, rats were sacrificed, and blood was collected from the brachial artery into heparinized vials. Blood was centrifuged, and plasma was separated and kept at -70°C for biochemical estimates.

Triglycerides (TGs), cholesterol, low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL) were measured by using kits bought from Aggape Diagnostic, Switzerland. Methods described in the kits were followed. Thiobarbituric acid reactive substances (TBARS) were estimated by using the method described by Sushmakumari *et al* [10]. The measurements were made in terms of malonyldialdehyde (MDA), which is formed after the breakdown of fatty acids. It serves as a convenient index of lipid peroxidation with thiobarbituric acid (TBA) to give red species. Ethoxypropane was used as the standard. A mixture of 1.2 mL of trichloroacetic acid (10% tricyclic antidepressant (TCA)), TBA and hydrochloric acid (HCL) (1 : 1 : 1) was added to 0.8 mL of plasma. The reaction mixture was allowed to stand for 10 minutes in boiling water, after which it was cooled and 2 mL of freshly-prepared sodium hydroxide was added. The absorbance was measured at 535 nm.

Reduced glutathione (GSH) was estimated by using the method of Ellman [11]. 5,5-dithiobis 2-nitrobenzoic acid (DTNB) is reduced by the free sulfhydryl group (-SH) of GSH to form one mole of 2-nitro-s-mercaptobenzoic acid per mole of -SH. The released nitrobenzoic acid anion has an intense yellow color and can be used to measure -SH groups by investigating the absorption maximum at 412 nm. The reaction mixture contained 50 μL of serum, 950 μL of DW and 1.5 mL of the precipitating solution. One mL of the mixture was mixed with 4 mL of phosphate solution and 0.5 mL of DTNB. After 5 minutes of standing, the absorbance was measured at 412 nm.

The program used for the data analysis was Sigma Stat (3.1 version, Systat Software, San Jose, CA, U.S.A.). Data were subjected to descriptive statistics, after which the differences among the groups were analyzed by using the one-way analysis of variance (ANOVA), followed by the Holm-Sidak test for comparisons.

3. Results

The effect of the administrations of the three doses of SRM on the OGTTs in normal rats is demonstrated in Fig. 1. The result shows that all three doses affected the OGTT curve, but the doses of 200 and 300 mg/kg BW had more pronounced and significant effects than the dose in the EX1 group. In all cases, the glucose levels peaked after 1 hour. Changes were noted in the recovery phases. In the control groups, glucose levels were normalized after the 3 hours, although after the second, the glucose level was starting to move towards normalization. In the EX groups, the patterns of recovery were similar, but the glucose level was lower after the 3 hours. Both the 200 and the 300 mg/kg BW doses showed significant effects on the OGTTs in normal rats. The normalization was quicker as compared to the EX1 and the normal control (NC) groups. Because of their significant effects on the OGTTs, the dose of 200 mg/kg BW was selected for MD rats, and the dose of 300 mg/kg BW was selected for SD rats.

The results for the hourly effects of SRM on SD and MD rats are presented in Table 1. The table shows that both the 200 and the 300 mg/kg BW doses had significant ($P < 0.05$) effects on glucose levels when the EX groups were compared to the NC, SDC, and MDC groups. In both cases, SRM started reducing the glucose level one hour after administration, and a significant reduction ($P < 0.05$) was noted at 3 hours. The reduced glucose level persisted up to 6 hours, the last observation.

The results of long-term administration of the two doses of SRM on the glucose levels in MD and SD rats are presented in Fig. 2. The results show that SRM extract had significant effects on both the MD ($P < 0.05$) and the SD rats ($P < 0.05$) at their respective doses when compared with their baseline levels and the respective control levels. SRM brought the elevated glucose level to normal at the end of the treatment in MD rats. The glucose level (106.22 ± 4.8 mg/dL) in the MDE group at day 31 differed significantly ($P < 0.05$) from the level on day 1 and from the levels in the SDC group (186.92 ± 3.4 mg/dL on day 30). In the SDE group, a significant reduction in the level of glucose was observed on day 30 as compared to the levels on day 1 (P

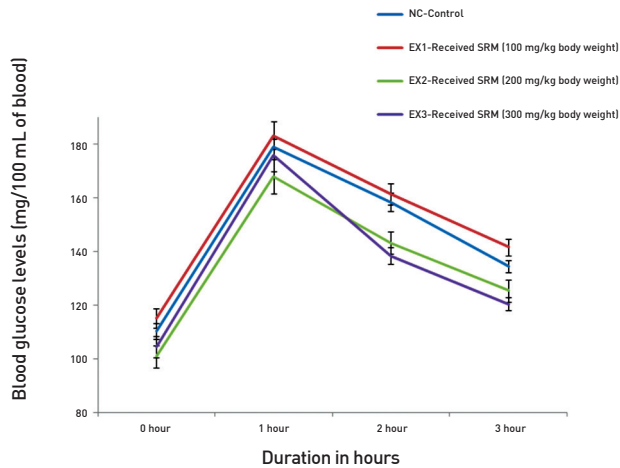


Figure 1 Effects of different doses of SRM on oral glucose tolerance in normal rats.

SRM, *Sida rhombifolia* methanol extract; NC, normal control; EX1, EX2, EX3, experimental groups.

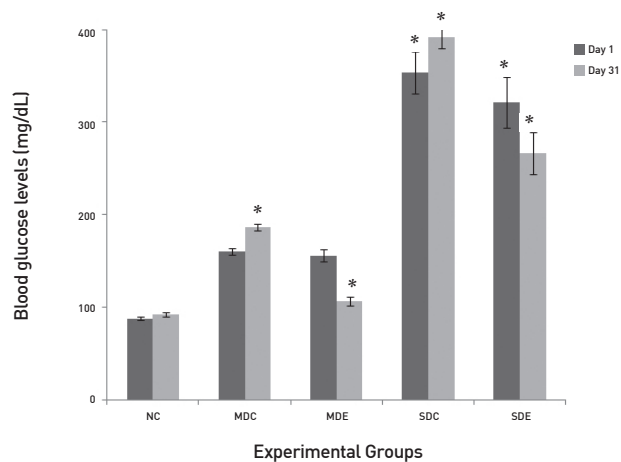


Figure 2 Blood glucose levels, expressed as means \pm SEMs, $n = 5$ for each treatment ($P < 0.05$, $\dagger P < 0.001$), in rats treated with SRM compared with their baseline values and the values in normal control rats on the corresponding day.

NC, normal control; MDC, moderately diabetic control; MDE, moderately diabetic experimental; SDC, severely diabetic control; SDE, severely diabetic experimental, SEM, standard errors of the mean; SRM, *Sida rhombifolia* methanol extract.

< 0.05), but the blood glucose levels were still very high and indicated a diabetic condition. Thus, although SRM reduced the glucose level, it failed to bring the elevated glucose levels to normal in the SD rats.

The results of daily administration of SRM on the lipid profile in MD and SD rats are presented in Figs. 3, 4, 5, 6. Fig. 3 shows that the TG levels were significantly high ($P <$

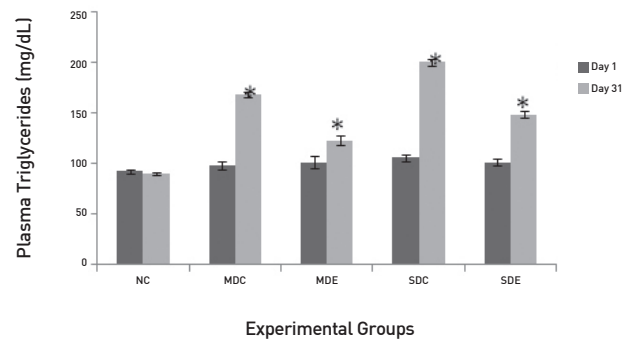


Figure 3 Plasma triglycerides levels, expressed as means \pm SEMs, $n = 5$ for each treatment ($P < 0.05$, $\dagger P < 0.001$), in rats treated with SRM compared with their baseline values and the values in the normal control rats on the corresponding day.

NC, normal control; MDC, moderately diabetic control; MDE, moderately diabetic experimental; SDC, severely diabetic control; SDE, severely diabetic experimental, SEM, standard errors of the mean; SRM, *Sida rhombifolia* methanol extract.

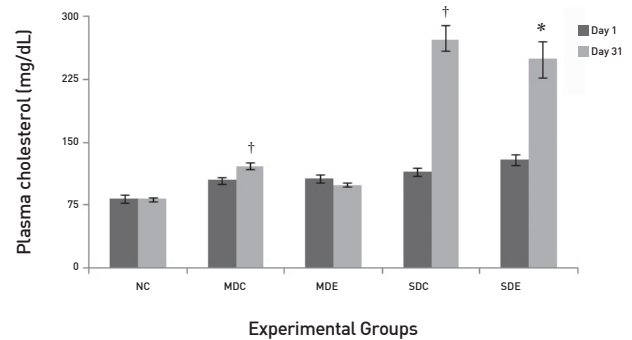


Figure 4 Plasma cholesterol levels, expressed as means \pm SEMs, $n = 5$ for each treatment ($P < 0.05$, $\dagger P < 0.001$), in rats treated with SRM compared with their baseline values and the values in the normal control rats on the corresponding day.

NC, normal control; MDC, moderately diabetic control; MDE, moderately diabetic experimental; SDC, severely diabetic control; SDE, severely diabetic experimental, SEM, standard errors of the mean; SRM, *Sida rhombifolia* methanol extract.

0.05) in both the control groups, MDC and SDC, as compared to the corresponding EX groups, MDE and SDE. The level in the MDE group on day 30 did not show a significant difference from the level on day 1 or from the level in the NC group. TG levels in the SDE group had increased significantly from its baseline value on day 1 ($P < 0.05$), but had not reached the higher level, as in the SDC group, on day 30. The TG level in the SDE rats differed significantly from that on day 30 in the SDC rats.

Fig. 4 shows that the levels of total cholesterol in NC rats

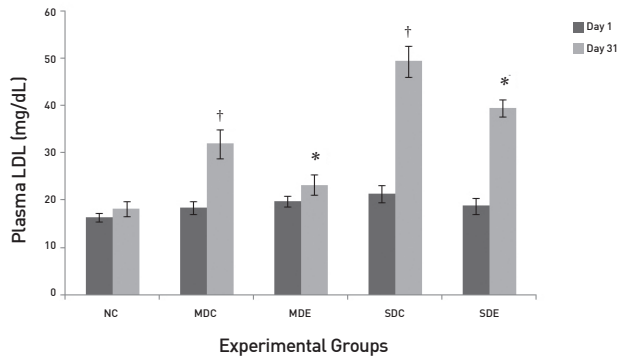


Figure 5 Plasma LDL levels, expressed as means \pm SEMs, $n = 5$ for each treatment ($P < 0.05$, $^{\dagger}P < 0.001$), in rats treated with SRM compared with their baseline values.

NC, normal control; MDC, moderately diabetic control; MDE, moderately diabetic experimental; SDC, severely diabetic control; SDE, severely diabetic experimental, LDL, low-density lipoprotein cholesterol; SEM, standard errors of the mean; SRM, *Sida rhombifolia* methanol extract.

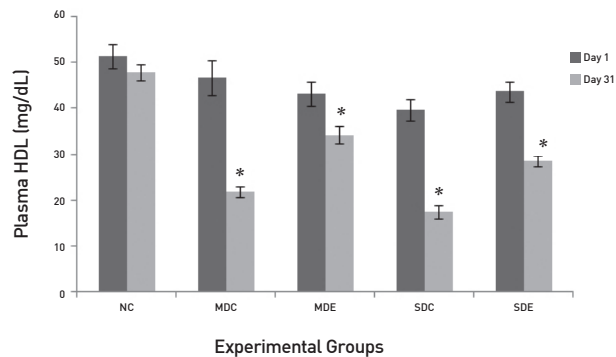


Figure 6 Plasma HDL levels, expressed as means \pm SEMs, $n = 5$ for each treatment ($P < 0.05$, $^{\dagger}P < 0.001$), in rats treated with SRM compared with their baseline values and the values in the normal control rats on the corresponding day.

NC, normal control; MDC, moderately diabetic control; MDE, moderately diabetic experimental; SDC, severely diabetic control; SDE, severely diabetic experimental, HDL, high-density lipoprotein cholesterol; SEM, standard errors of the mean; SRM, *Sida rhombifolia* methanol extract.

on day 31 differed significantly from the levels on day 1 in the MDC and the SDC rats ($P < 0.001$). The levels of cholesterol in these latter two groups were elevated significantly from their baseline values. In SDC rats, levels shot up to 272.66 ± 13.63 mg/dL from its baseline value of 114.99 ± 4.41 mg/dL. In MDC rats, the elevation was not that large, showing only a 16% increase from its baseline. The EX groups also showed significantly different results. In the MDE rats, the levels were really under control, and they did

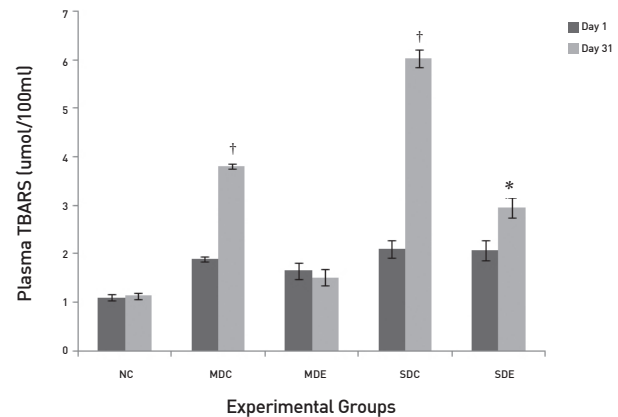


Figure 7 Plasma TBARS levels, expressed as means \pm SEMs, $n = 5$ for each treatment ($P < 0.05$, $^{\dagger}P < 0.001$), in rats treated with SRM compared with their baseline values and the values in the normal control rats on the corresponding day.

NC, normal control; MDC, moderately diabetic control; MDE, moderately diabetic experimental; SDC, severely diabetic control; SDE, severely diabetic experimental, TBARS, thiobarbituric acid reactive substances; SEM, standard errors of the mean; SRM, *Sida rhombifolia* methanol extract.

not show any spikes, but in the SDE rats, the levels were extremely high as compared to the baseline values ($P < 0.05$). Also, the levels in the MDE and the SDE rats showed no significant difference from the levels in the SDC rats, even though the levels in those two groups were slightly lower than the levels in the SDC group.

Fig. 5 shows the effects of SRM on plasma LDL levels. The results are slightly different from those for total cholesterol, but the trends are similar. The levels in both control groups, MDC and SDC, were significantly different from their baseline values. The maximum increase in the level of LDL in the MDC group was 43% higher than the baseline value of 18.4 ± 1.29 mg/dL. In the SDC group, a very large increase was noted. The plasma LDL level increased to 49.47 ± 3.27 mg/dL ($P < 0.001$) from a baseline value of 21.34 ± 1.87 mg/dL, which was more than a 100% increase. Contrary to these increments, treated rats in the MDE group on day 31 showed a non-significant increase from the baseline value; however, the LDL level in the MDE group differed significantly from the level in the MDC group on day 31 ($P < 0.001$). In the SDE group, the results were not that significant. The SDE group showed a decreased level, and it differed significantly from the value in the SDC group, but the rats in the SDE group could not achieve LDL levels in the normal range.

The effects of SRM on the HDL levels in MD and SD rats are presented in Fig. 6. The fig shows a significant difference between the plasma HDL levels in the NC group and those in EX control groups, SDC and MDC, on day 31 ($P < 0.05$) Significant differences also existed between the baseline levels on day 1 and the final levels on day 31 in both

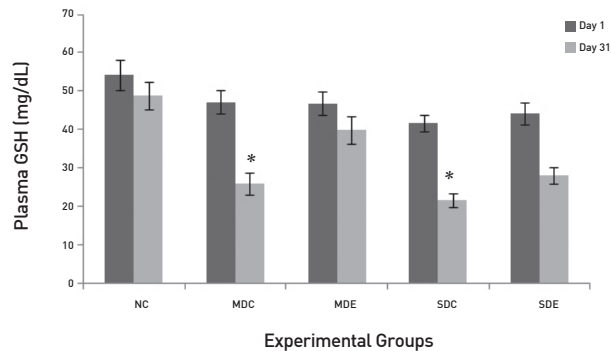


Figure 8 Plasma GSH levels, expressed as means \pm SEMs, $n = 5$ for each treatment ($P < 0.05$, $^{\dagger}P < 0.001$), in rats treated with SRM compared with their baseline values and the values in the normal control rats on the corresponding day.

NC, normal control; MDC, moderately diabetic control; MDE, moderately diabetic experimental; SDC, severely diabetic control; SDE, severely diabetic experimental, GSH, glutathione; SEM, standard errors of the mean; SRM, *Sida rhombifolia* methanol extract.

the MDC and the SDC groups. The levels of HDL on day 31 in the MDC and the SDC groups showed 54% and 56% reductions, respectively, compared to their baseline levels. The results in the EX group, however, paint a different picture. The levels in the MDE group showed only an 8% reduction from its baseline value of 43.23 ± 2.72 mg/dL while those in the SDE group showed a 35% reduction. Nevertheless, the results are still significant when compared to the decreased HDL level in the SDC group ($P < 0.05$).

The effects of SRM on TBARS in diabetic rats are demonstrated in Fig. 7. The fig shows that the levels of TBARS in the MDC and the SDC rats differed significantly on day 31 ($P < 0.001$ and $P < 0.001$, respectively) from their baseline values on day 1 and from the levels in the NC group. In the case of the MDE group, the levels of TBARS were low compared to the baseline value, but still differed significantly from the levels in the NC group. In the SDE group, a 45% increase in the level of TBARS, as compared to the baseline value was noted, but this increase was much lower than the 200% increase in the SDC group ($P < 0.05$).

The effects of SRM on GSH in diabetic rats are demonstrated in Fig. 8. The fig shows that the levels of GSH were maintained in the NC group up to day 31. Levels in the EX controls, MDC and SDC, on day 31 were significantly decreased compared to their baseline levels on day 1 ($P < 0.05$). On the other hand, in the MDE group, a reduction was noted, but not to the extent observed in the MDC and the SDC groups. SRM treatment failed to maintain the reduced GSH levels in the SDE group on day 31, and the reduction was smaller than it was in the SDC and the MDC groups.

At the beginning of the treatment, all the diabetic rats weighed between 230 and 280 g. By the end of the experi-

ment, EX rats in the MDE group had maintained their BW when compared to the SDC and the SDE groups, in which weight loss was noticed.

4. Discussion

The present study indicates that SRM has a significant effect on OGTTs conducted in normal rats. The most effective doses were 200 and 300 mg/kg BW. The OGTT curves peaked after 1 hour in the EX groups, just as in the NC. At the 3 hours after SRM treatment, a significant reduction was noted in the EX2 and the EX3 groups as compared to the levels in the NC group. This shows that SRM does not interfere with the absorption of glucose from the intestine. Glucose absorption has a normal time of 1 hour. The non-significant differences among the levels of glucose in the control and the EX groups also support this fact. The significantly low levels of glucose in the EX2 and the EX3 groups indicate the possible involvement of SRM with either insulin release or enhancement of its sensitivity, which might have caused the rapid clearance of glucose from the circulatory system as compared to the control and the EX1 groups. The present study also notes that SRM reduced the glucose levels at 2 hours after its administration in MD rats at a dose of 200 mg/kg and in SD rats at a dose of 300 mg/kg. The effect was more pronounced in the MD rats. Moreover, long-term administration of SRM to diabetic rats resulted in the normalization of glucose levels in MD rats, but not in SD rats. These results are consistent with previous finding [6, 8]. These results indicate that some compounds that enhance glucose clearance from the circulation either by increasing its transport and/or by enhancing its usage at the cellular level may be present in the extract. The non-significant results in the SD rats might be due to the amount of SRM administered being insufficient to clear the high glucose load in the circulatory system.

The lipid profiles showed similar patterns. The levels of plasma TGs, LDL and cholesterol were very high in the MDC and the SDC groups, but low in the MDE group. In the SDE group, a decrease was noticed, but it was not significant. High levels of postprandial lipids and lipoproteins in the circulatory system are features of diabetes [12] and it happens because of the enhanced lipid absorption and increased production of chylomicron particles in TGs and decreased clearance from the circulatory system [13]. Persistent hyperlipidaemia occurs because of the reduced lipoprotein lipase (LPL) and hepatic lipase (HL)-mediated lipolysis of chylomicrons and very low-density lipoprotein (VLDL) and because of the reduced clearance of LDL in cases of severe diabetes [14]. Non-enzymatic glycosylation of apoprotein B of LDL interferes with specific LDL receptor binding and its transport, hence reducing the clearance of LDL cholesterol [15]. The overall reduction in the LDL level in the MDE group indicates the possibility of active transport of LDL, which could be due to the reduced glycosylation of the LDL molecule caused by glucose levels being normal in these groups. Hence, the clearance of glucose from the circulatory system due to the administration of SRM in MD rats has alleviated the diabetic condition

and, hence, improved the dyslipidemic conditions.

Plasma HDL levels were very low in the MDC, SDC and SDE groups, but the levels of HDL were maintained in the MDE group. During diabetic conditions, the levels of TGs and LDL are usually very high with an associated decrease in the HDL level [16]. An increased transfer of cholesterol from HDL to TG-rich lipoproteins and the subsequent transfer of TGs to HDL have been reported in patients with diabetes [17]. Because of the hydrolysis of TG-rich HDL particles by HL, HDL particles are rapidly catabolized and cleared from the plasma. The normal glucose levels in the SRM-treated MDE rats might have reduced the rapid transport of TGs. The normal levels of TGs in the MDE rats also support this fact and could be the reason behind the rising levels of HDL in this group. In the case of SDE rats the opposite must have taken place due to the high glucose levels.

Persistent hyperglycaemia induces glycosylation of macromolecules, and especially of LDL, by a superoxide-dependent pathway and results in oxidative stress [18]. Prolonged oxidative stress can cause diabetic complications because of lipid peroxidation chain reactions [19]. MDA is the end product of lipid peroxidation; hence, the level of MDA is an indication of the degree of lipid peroxidation. In the present study, significant elevations in the levels of MDA were noticed in both the MDC and the SDC rats. The levels of MDA showed marked reduction in the MDE group where rats received SRM treatment. Here, the results showed non-significant differences from the baseline value. Thus, SRM appears not only to reduce glucose levels but also to suppress the oxidative stress that commences because of persistent hyperglycaemia. In the SDE group, the MDA levels were also reduced, but the reduction was not as significant as it was in the MDE group. GSH is a tri-peptide present in all mammalian cells and participates in many metabolic processes for the protection of cells against free radicals and toxic metabolites [20, 21]. The decrease in the levels of reduced GSH represents its increased utilization because of oxidative stress. A significant reduction was noticed in the control groups SDC and MDC. Treatments with SRM in the MDE group resulted in the level of GSH being maintained, but that was not seen in the SDE group; the failure of SRM to do so indicates a high degree of oxidative stress.

Our previous findings and the findings of other reports show that SR extracts, including methanol extracts, have strong free-radical scavenging activity and that they are rich in phenol content. Phenolic compounds are also reported to have anti-diabetic activities [22, 23]. The hypoglycaemic effect of phenols is linked with reduced intestinal absorption of dietary carbohydrates, modulation of the enzymes involved in glucose metabolism, improvement of beta-cell function and insulin action, and their anti-oxidative and anti-inflammatory properties [24]. SRM is also rich in phenols, but the results in the MD rats and the SD rats were not consistent. Possibly, the phenols that are present in SRM only have mild anti-diabetic effects. In the present study, diabetes was induced by using alloxan monohydrate. This compound is reported to damage B cells by generating oxidative stress, and SRM alleviated diabetic conditions. Possibly in the MD rats, the extent

of damage might not have been that severe, so SRM with its strong free-radical scavenging properties managed to scavenge the free radicals generated by alloxan and helped the anti-oxidant system to restore its pre-diabetic status. The improvement in the oxidative stress in the MDE rats could be due to the phenol content of the SR extract and its free-radical scavenging activities.

5. Conclusion

SRM ameliorates alloxan induced diabetes in moderately diabetic rats and is unable to produce similar effects in severely diabetic rats.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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