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Abstract – Dyslipidemia is one of the major derangement observed in type 2 diabetes mellitus which further leads to various life threatening complications. Certain herbal disulphides/thiols are known to cater a solution towards regulation of this diabetes mellitus induced dyslipidemia. It is known that Diallyl Disulphide (DADS), a component of garlic extract possess hypolipidemic activities and may have therapeutic applications, but the metabolite of DADS, acrolein is toxic and poses certain disadvantages. Certain modified /altered disulphides, shown to possess significant hypolipidemic action with lesser toxic effects. In the present study, we tried to establish the hypolipidemic activities of Diaceto-Dipropyl-Disulphide (DADPDS), a synthetic modified disulphide in alloxan diabetic rats. The results show a significant decrease in plasma cholesterol (60%), plasma triacylglycerol (42%), liver tissue cholesterol (41%) and liver tissue triacylglycerol (23%), indicating the usefulness of this modified disulphide in controlling diabetes induced dyslipidemia.

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Hypolipidemic Effects of Diaceto-Dipropyl-Disulphide on Alloxan Diabetic Rats

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Abstract - Dyslipidemia is one of the major derangement observed in type 2 diabetes mellitus which further leads to various life threatening complications. Certain herbal disulphides/thiols are known to cater a solution towards regulation of this diabetes mellitus induced dyslipidemia. It is known that Diallyl Disulphide (DADS), a component of garlic extract possess hypolipidemic activities and may have therapeutic applications, but the metabolite of DADS, acrolein is toxic and poses certain disadvantages. Certain modified /altered disulphides, shown to possess significant hypolipidemic action with lesser toxic effects. In the present study, we tried to establish the hypolipidemic activities of Diaceto-Dipropyl-Disulphide (DADPDS), a synthetic modified disulphide in alloxan diabetic rats. The results show a significant decrease in plasma cholesterol (60%), plasma triacylglycerol (42%), liver tissue cholesterol (41%) and liver tissue triacylglycerol (23%), indicating the usefulness of this modified disulphide in controlling diabetes induced dyslipidemia.

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I. INTRODUCTION

Diabetes mellitus is a systemic metabolic disease characterized by hyperglycemia, hyperlipidemia, hyperaminoacidemia and hypoinsulinemia that results from decrease in both insulin secretion and insulin action (1). Dyslipidemia, a complication associated with diabetes mellitus leads to profound alteration in the concentration and composition of lipid profile in the body which lead to the increase in the lipid concentration in the liver cells (2-4). It is evident that during diabetes the level of total lipids, triacylglycerol and total cholesterol increases both in plasma and tissue significantly (2). There are many herbal products which are proved to have the beneficial effect in significantly lowering the lipid levels during diabetes. Garlic's sulphur compounds specifically, DADS (Diallyl Disulphide) is known to inhibit the lipogenic enzymes and reduces cholesterol and triacylglycerol synthesis (5-8). Apart from these known beneficial effects of garlic

and its products, there are reports that misuse or overuse of these may produce (9). Acrolein, the possible metabolite of DADS is thought to be responsible for its toxic effects (10,11). It was thought that unsaturation of DADS may be responsible for its toxic effects by giving rise to toxic product, acrolein and the hypolipidemic activity lies in its disulphide nature. Few attempts were made in the past to prepare and use certain synthetic disulphides which mimic the beneficial effects of DADS but devoid of any toxic effects (12). In the present study an attempt was made to assess the hypolipidemic effect of Diaceto-dipropyl-disulphide (DADPDS), a synthetic, modified disulphide in alloxan diabetic rats.

II. MATERIALS AND METHODS

All chemicals employed in the present study were of Analar grade (A.R). Alloxan was procured from Loba chemie and thiopropanol (3-mercapto-1-propanol) from Sigma- Aldrich Company USA. This thiopropanol was used for the preparation of Diaceto-dipropyl-disulphide (DADPDS).

III. SYNTHESIS OF DADPDS

5 grams of thiopropanol was treated with 1N iodine in potassium iodide solution drop by drop till a light yellow colour persists and the contents were dissolved in 100 ml diethyl ether. To this 10 ml ice cold acetyl chloride was added and mixed. The above mixture was kept at 10-15° C for 3 hours. The separated ether layer was washed twice with 25 ml portions of ice cold saturated sodium chloride solution. Later washed 4 times with 0.1N sodium hydroxide solution in saturated sodium chloride then washed once with 10 ml 0.1N sodium thiosulphate in saturated sodium chloride and finally washed once with 10 ml glass distilled water. Later the ether layer was clarified with anhydrous sodium sulphate and dried at 50-55°C for 30 minutes. The residue was Diaceto-Dipropyl-Disulphide. This was employed to feed rats in the present study.

IV. ANIMALS

Adult male albino rats weighing 150-200g randomly selected from Central animal house of the Basaveshwara Medical College, Chitradurga were employed for the present study. A commercial standard pellet diet (Amruth Rat Feed supplied by Pranav Agro

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industries, Pune, India) and water was made available to animals *ad libitum*. Animals were maintained in a controlled environment ($25 \pm 5^\circ \text{C}$) with light-dark cycles in an experimental room simulating natural conditions. All the animals are cared according to the rules and regulations of the CPCSC (Committee for the purpose of Control and Experiments on Animals), New Delhi and IAEC (Institutional Animal Ethical Committee) of Basaveshwara Medical College, Chitradurga.

V. INDUCTION OF DIABETES MELLITUS

The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of freshly prepared alloxan monohydrate (150 mg/kg body weight) (13,14) in sterile normal saline. The diabetes was confirmed by examining urine sample for sugar by using standard urine glucose strips (Qualigens). The rats whose urine showed a positive test for glucose for 3 consecutive days were labelled as diabetic. The treatment with DADPDS was started on 5th day after alloxan injection and was considered as first day of treatment.

VI. GROUPING

The rats were divided into 3 groups comprising 6 rats in each group as follows.

a) Group 1

Served as normal rats maintained on normal lab diet and water *ad libitum* and were received 30ml of normal saline per kg body weight daily for 30 days.

b) Group 2

Served as diabetic control rats maintained on normal lab diet and water *ad libitum* and were received 30ml/kg body weight normal saline daily for 30 days.

c) Group 3

Served as DADPDS treated diabetic rats. The group consists of alloxan diabetic rats, maintained on normal lab diet and water *ad libitum*, and was received 100mg/kg body weight of DADPDS as 30ml suspension daily for 30days.

After the stipulated period, the rats of group 1, 2 and 3 were sacrificed by anaesthetizing. Blood samples were collected using heparin as an anticoagulant. Liver tissue was procured, blotted smoothly to remove blood stains and kept individually in clean dry glass beaker with aluminium foil cover at $0-4^\circ \text{C}$ for further usage. Blood samples were centrifuged at 3000 rpm for 5 mins, the separated plasma was employed for estimation of lipid parameters - total cholesterol (15) and triacylglycerol (16). A part of the liver tissue was homogenized with 9 parts of chloroform-methanol mixture (1:1, v/v) for 5 mins using Potter Elvehjem tissue homogenizer and the mixture was centrifuged at 3000 rpm for 5 minutes. The supernatant was employed for

the estimation of lipid parameters - total cholesterol (15) and triacylglycerol (16).

Another part of liver was homogenised with 9 parts of 5% cold TCA for 5 mins, and extract was employed for estimation of thiobarbituric acid reactive substances (TBARS) (17). A third part of liver tissue was homogenized with 9 parts of cold phosphate buffer (pH -7.4) for 5 mins and centrifuged at 3000 rpm for 5 minutes and the supernatant was used for the estimation of total thiol groups (18).

VII. STATISTICAL EVALUATION

Results obtained in the present study are expressed as their Mean $\pm$ SD. The data entry was carried out using Microsoft Office Excel and statistically analysed and probability (p value) was calculated by students't' test.

VIII. RESULTS

The results of the present study are narrated in table -1 and in graphs 1-6. Table-1 shows the levels of plasma cholesterol, liver tissue cholesterol, plasma triacylglycerol, liver tissue triacylglycerol, liver TBARS and liver total thiol groups in normal group(group 1), alloxan diabetic group(group 2) and in DADPDS treated alloxan diabetic group(group 3).

It is seen from the table and from the graphs given that there is a significant raise in plasma cholesterol ($p < 0.001$), plasma triacylglycerol ($p < 0.001$), liver tissue cholesterol ($p < 0.001$), liver tissue triacylglycerol ($p < 0.001$), and liver TBARS levels ($p < 0.001$) in group 2 as compared to group 1 whereas levels of liver tissue total thiol group were significantly lowered ($p < 0.05$) in group 2 as compared to group 1. Further it is evident from the table and graphs that there is a significant decrease in plasma cholesterol ($p < 0.001$), plasma triacylglycerol ($p < 0.001$), liver tissue cholesterol ($p < 0.001$), liver tissue triacylglycerol ($p < 0.001$) and in liver tissue TBARS levels ($p < 0.001$) in group 3 as compared to group 2, whereas, a moderate raise in liver tissue total thiol groups observed in group -3 rats compared to group-2 rats (refer table -1, graphs 1-6).

IX. DISCUSSION

Dyslipidemia which is a common abnormality associated with diabetes mellitus may be resulting from insulin deficiency (19), a similar picture may be seen in alloxan diabetic rats as it is known that alloxan induce profound β -cell damage of islets of Langerhans leading to insulin deficiency (20,21). Earlier it is shown by C .S. Yadav (2) that in alloxan diabetic rat, the lipid levels both in liver and plasma rise by about 48 -55%. The results depicted in table- 1 agrees with this and there is an increase of 58% and 18% respectively in plasma total cholesterol and triacylglycerols and also an increase of 21% and 36% in liver total cholesterol and liver triacylglycerols respectively.

Many herbal extracts have been employed as lipid lowering substances since long time. Garlic (*Allium sativum*) and its extracts are the best known for their hypolipidemic actions (5). The hypolipidemic effects of garlic has been attributed to its principle organosulphur compound, Diallyl disulphide (DADS) which is known to inhibit HMG CoA reductase and possibly reduce plasma/tissue cholesterol levels (22-23). But the over use of garlic may induce many toxic effects (10) due to acrolein a possible metabolite of DADS.

In order to overcome this harmful effect of DADS certain synthetic disulphides with lesser to moderate hypolipidemic benefits been employed by earlier workers (24). DADPDS, a low molecular weight acetylated disulphide been employed for its hypolipidemic actions in alloxan diabetic rats in the present study. A 100 mg/kg body weight dosage of this disulphide shows a significant hypolipidemic effect ($p<0.001$) and hypocholesterolemic ($p<0.001$) effect (refer table -1 and graphs 1-6). DADPDS is a disulphide, similar to any other disulphide, undergoes reduction to its component thiols by utilizing NADPH (25) as shown below:



Hence decreases cellular NADPH levels thereby causing a decrease in lipid and cholesterol synthesis as it is known that HMG CoA reductase, the key enzyme of cholesterol biosynthesis as well as glycerol -@-dehydrogenase requires NADPH , thus a reduction in the cellular NADPH could decrease cholesterol as well as triacylglycerol synthesis. Further it is known that DADS is an inhibitor of HMG CoA reductase and decreases the activity of this enzyme hence induces hypocholesterolemia (26). DADPDS, a synthetic disulphide employed in the present study which is similar to DADS, a small molecular weight disulphide may induces hypocholesterolemia in alloxan diabetic rats probably by inhibiting HMG CoA reductase (refer table).Thus causing a decrease in plasma as well as liver tissue cholesterol(refer table-1, graph 1 & 3).

It is also known that many lipogenic enzymes are thiol enzymes (25)and the results of present study given in table 1 shows that in group 3 the total liver tissue thiol groups has significantly raised ($p<0.05$) as compared to group 2. Suggesting that DADPDS improve the cellular thiol group status probably by reducing the free radical levels by acting as a free-radical scavenging agent (refer table 1 , graph 5 & 6).

Thus it may be concluded that DADPDS at the dosage employed in the present study has a definite hypolipidemic and hypocholesterolemic action in alloxan diabetes rats.

Table 1: Showing the levels of total cholesterol, triacylglycerol in plasma and liver tissue, as well as TBARS and –SH group content in liver tissue of normal group, Alloxan diabetic group and DADPDS treated alloxan diabetic group

Groups	TOTAL CHOLESTEROL		TRIACYLGLYCEROL		Liver TBARS μmol/MDA/g	Liver total - SH GROUP mg/g T
	Plasma mg/dl	Tissue mg/g T	Plasma mg/dl	Tissue mg/g T		
NORMAL RATS GROUP 1 (n=6)	39.68 ± 3.40	4.50 ± 0.39	128.2 ± 2.83	16.64 ± 2.15	4.26 ± 1.26	0.94 ± 0.11
ALLOXAN DIABETIC RATS GROUP 2 (n=6)	95.0*** ± 18.10	5.7*** ± 0.48	176.4*** ± 2.0	26.8*** ± 1.6	13.84*** ± 1.40	0.81* ± 0.06
DADPDS TREATED ALLOXAN DIABETIC RATS GROUP 3 (n=6)	37.5*** ± 4.18	3.37*** ± 0.58	102.2*** ± 2.0	20.48*** ± 1.9	4.33*** ± 1.22	0.94* ± 0.10

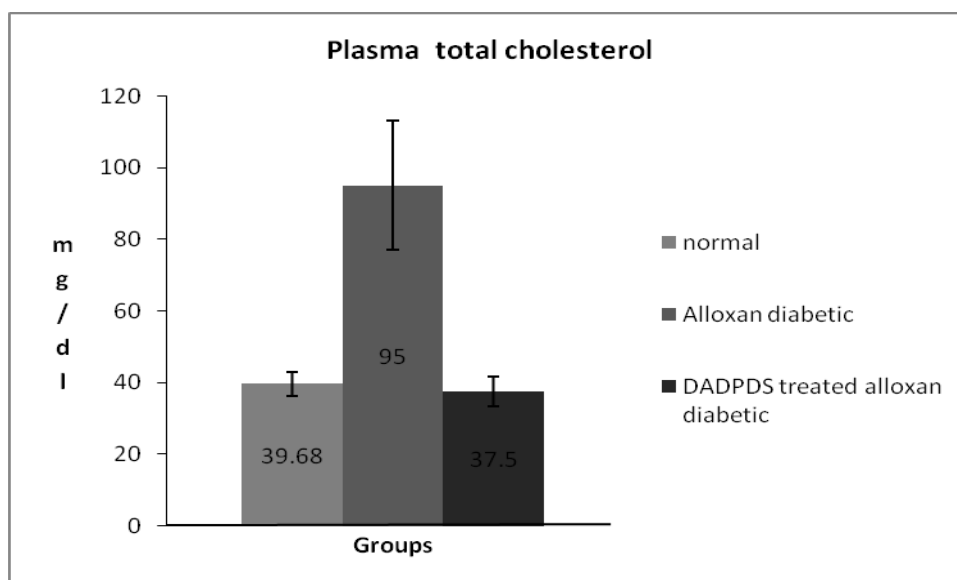
Note :

1. The values are expressed as Mean ± SD.
2. Number in parentheses indicates the number of animals in that group.

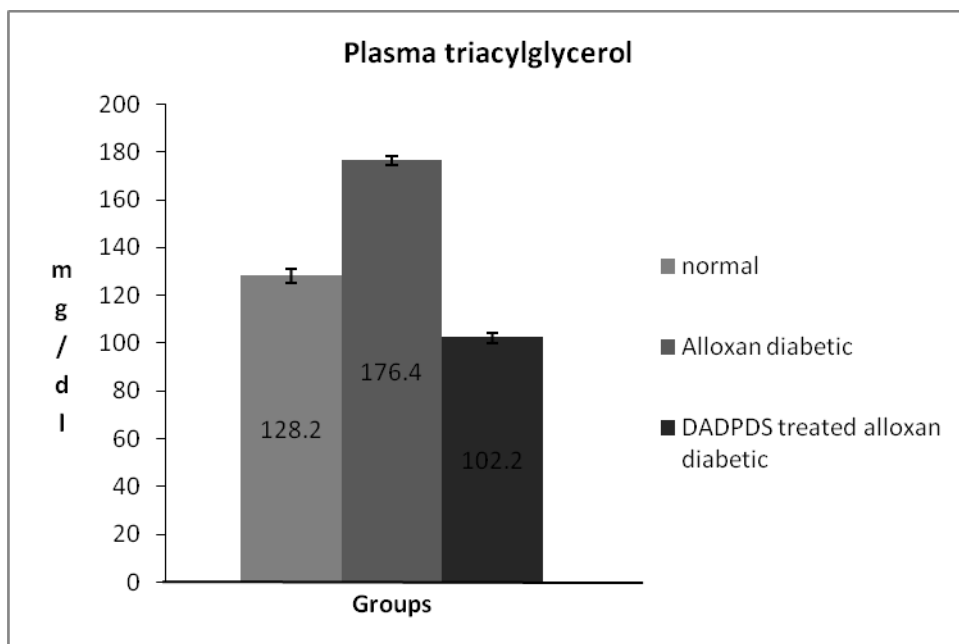
3. Statistical evaluation – probability level *p<0.05, **p<0.01, ***p<0.001

X. GRAPHS

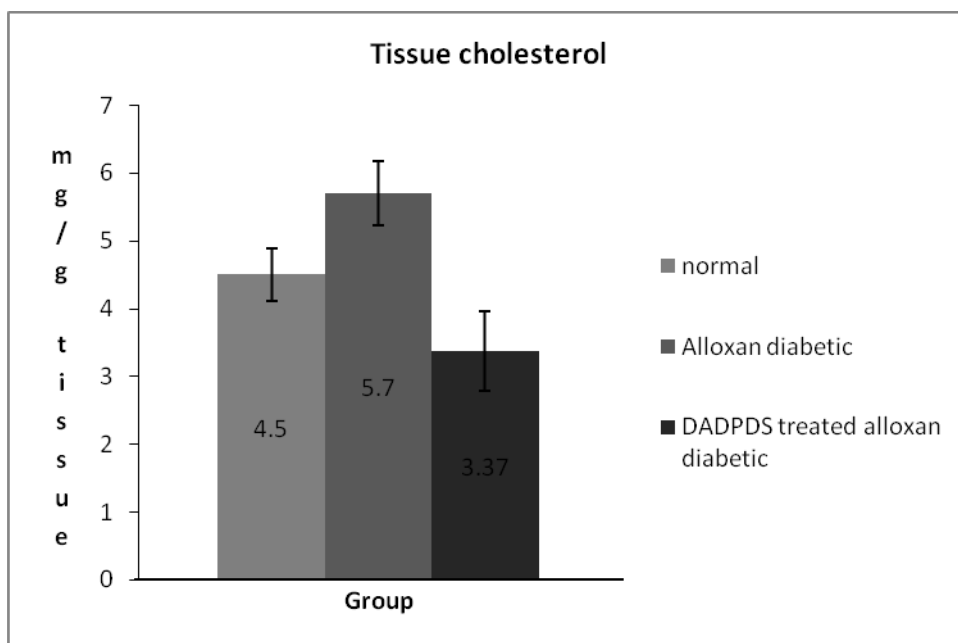
Graph -1-1 showing plasma total cholesterol level in normal, alloxan diabetic and DADPDS treated alloxan diabetic rats



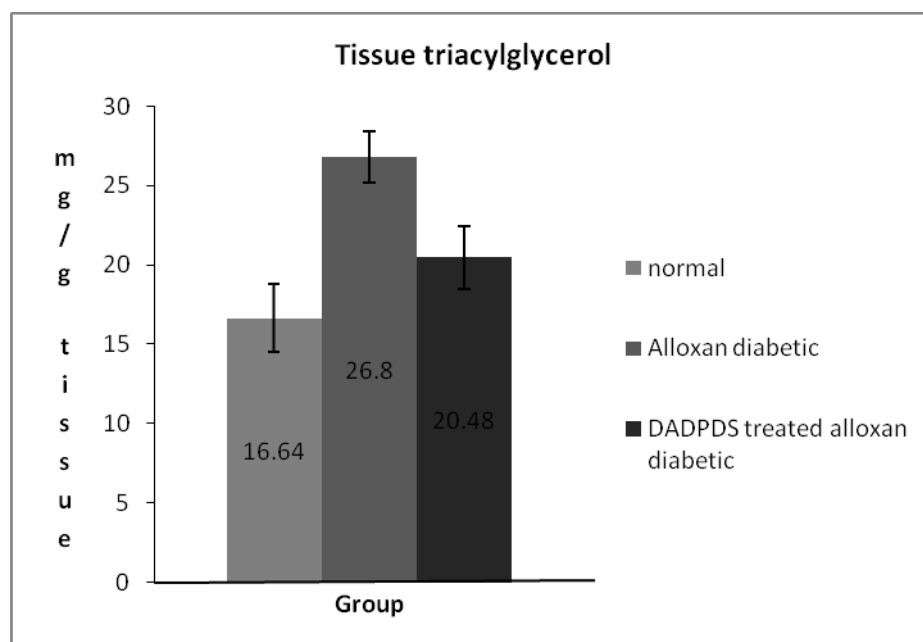
Graph - 2 showing plasma triacylglycerol levels in normal, alloxan diabetic and DADPDS treated alloxan diabetic rats



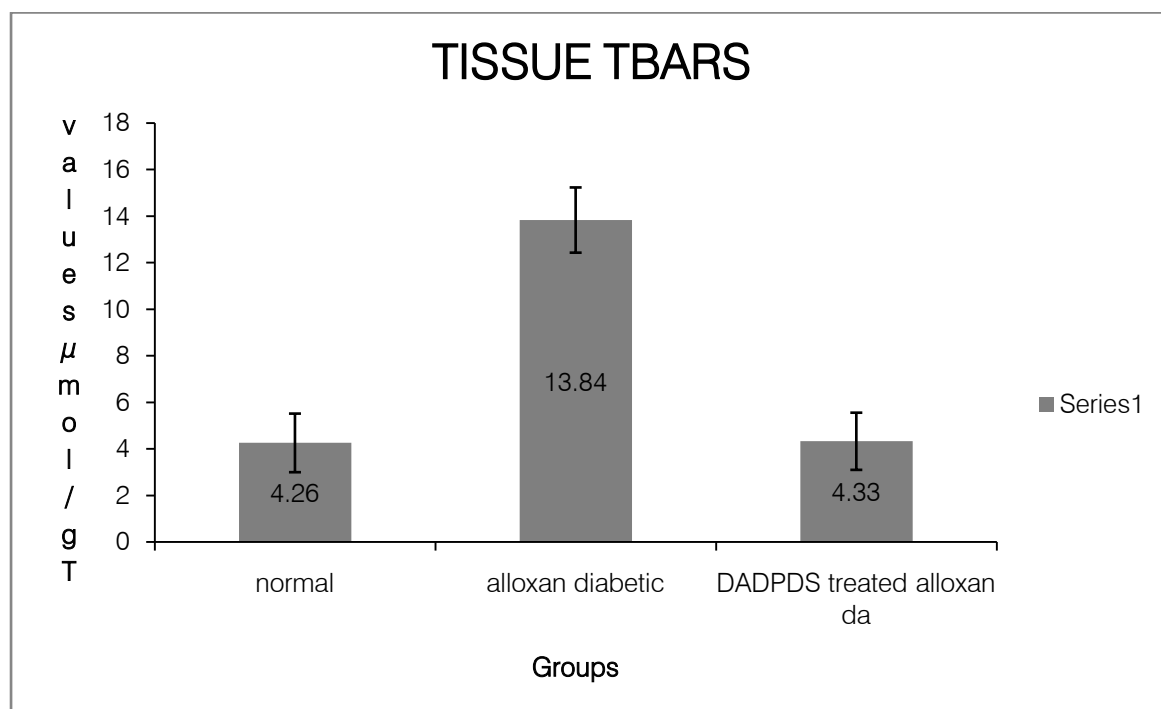
Graph -3 showing liver tissue total cholesterol level in normal, alloxan diabetic and DADPDS treated alloxan diabetic rats



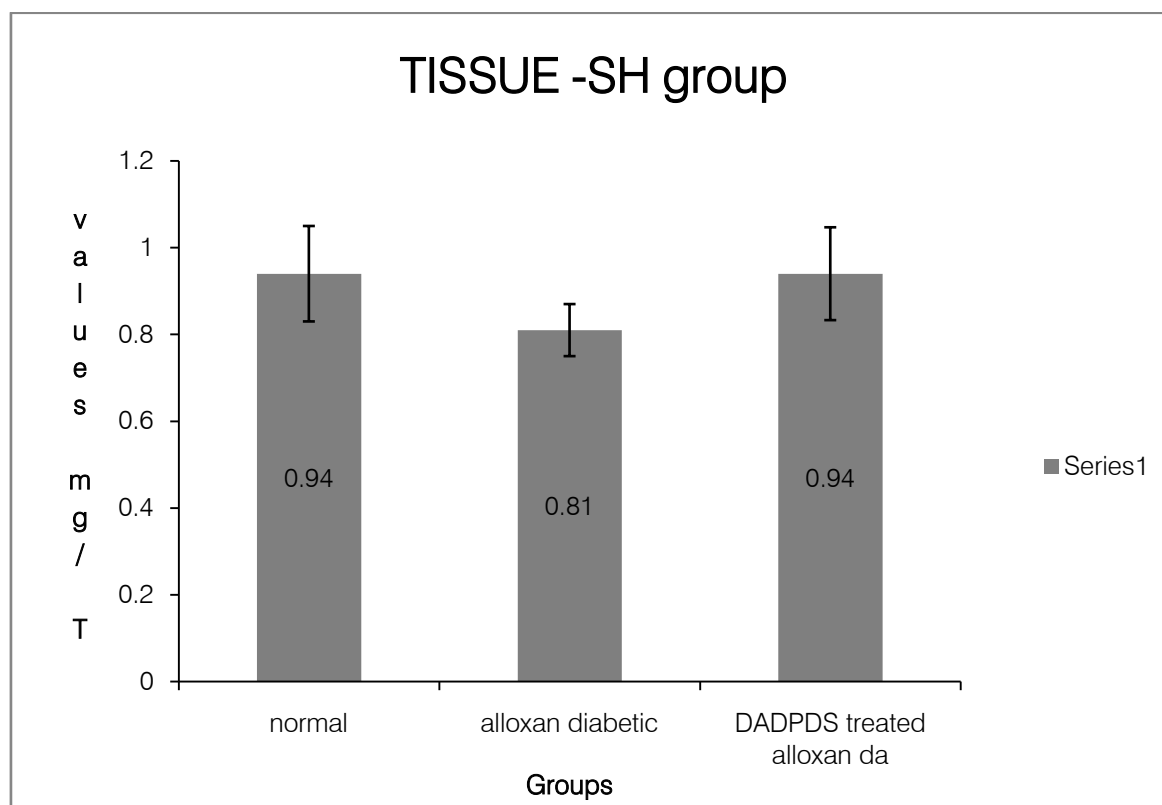
Graph -4 showing liver tissue triacylglycerol levels in normal; alloxan diabetic and DADPDS treated alloxan diabetic rats



Graph - 5 showing liver tissue TBARS content in normal, alloxan diabetic and DADPDS treated alloxan diabetic rats



Graph -6 showing liver tissue total – SH group content in normal, alloxan diabetic and DADPDS treated alloxan diabetic rats



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