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Effect of DADS on alterations of liver function in alloxan induced diabetic male albino rats

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Abstract

Introduction: Diabetes Mellitus is a syndrome characterized by a loss of glucose homeostasis from defective insulin secretion and its action, both resulting in impaired metabolism of carbohydrate, lipid and protein. Liver is one of the insulin dependent tissues which are severely affected in diabetes. The hypoglycemic effect of garlic has been reported. We aimed to study the effect of Diallyl Disulphide (DADS), a principle compound of garlic oil on liver function.

Aim: To assess the effect of DADS on alteration of liver function in alloxan induced diabetic male albino rats.

Materials and Methods: Healthy adult male albino rats weighing around 100-150 grams were randomly selected from the animal house at Shri B.M.Patil Medical College & Hospital, Bijapur. Diabetes was induced using alloxan and was treated with DADS. After a stipulated time the rats were anesthetised and sacrificed to collect the blood and liver tissue. Various parameters were estimated in the blood and homogenised liver tissue using standard procedures. Liver histological section were prepared and observed under the microscope.

Results: There was significant reversal of histological and biochemical changes in the liver of DADS treated alloxan induced diabetic rats when compared to the alloxan induced diabetic rats.

Statistics: One way ANOVA followed by post hoc't' test was done.

Conclusion- From the above findings it can be concluded that the DADS a principle compound of garlic, definitely has the hepatoprotective effect in diabetic rats, with least adverse effects.

Key words : Diallyl Disulphide (DADS), Diabetes Mellitus (DM), Hepatoprotective.

Introduction

Diabetes mellitus is a syndrome resulting from variable interactions of hereditary and environmental factors and characterised by abnormal insulin secretion or insulin receptor, affecting metabolism involving carbohydrate, protein and fats in addition to damaging beta cells of pancreas, liver and kidneys (1). Diabetes Mellitus could be treated by nutritional therapy/drug therapy and others. But the drug therapy would have its own limitations and side effects. The liver is insulin dependent tissue that plays a vital role in glucose and lipid homeostasis and is severely affected in diabetes (2). Insulin deficiency in diabetic rats lead to degenerative changes in liver causing an elevation in serum Aspartate transaminase (AST) and Alanine transaminase (ALT) and disturbances in antioxidant and free radical levels. Herbal extracts specifically extracts of *Allium sativum* (garlic) have been known to possess hypolipidemic as well as hypoglycemic actions (3,4), which is attributed to its organosulphur compound. The principle organosulphur compound is Diallyl Disulphide (DADS) (3, 4).

Aim

The present study was undertaken to assess the effect of DADS on alteration of liver function in alloxan induced diabetic male albino rats.

Materials and Methods

Alloxan and Diallyl disulphide (DADS) were procured from sigma Aldrich chemicals. All the other chemicals employed were of analytical grade. Healthy Wister strain male albino rats weighing around 100-150 grams were randomly selected from

the animal house, BLDE University's, Shri B. M. Patil Medical College, Hospital and Research Centre, Bijapur, India, for the present study. The experiments were conducted in accordance with Committee for the purpose of Control and Supervision of Experimental Animals (CPCSEA), New Delhi and Institutional Animal Ethical Committee (IAEC) of Shri B. M. Patil Medical College, Hospital and Research Centre, Bijapur, India. These animals were divided into four groups with six rats in each group as follows: Group I: Normal Control, Group II: Diabetic Control, Group III: DADS treated Normal rats, Group IV: DADS treated Diabetic rats. Group I and II rats were given 3ml of normal saline per kg body weight through gastric intubation for 30 days, stock lab diet and water was provided ad libitum. Group III and IV were given 100mg/kg body weight of DADS as 3ml of suspension per kg body weight through gastric intubation for 30 days, stock lab diet and water was provided ad libitum.

Study protocol

Induction of diabetes was done by intraperitoneal injection of freshly prepared aqueous alloxan monohydrate (150mg per kg body weight) (5) in sterile water to overnight fasted rats. Later stock lab diet and water was provided *ad libitum*. The urine of the rats, which showed positive for sugar after alloxan treatment for 3 consecutive days, was labeled as diabetic rats. On the completion of stipulated period, rats were anaesthetised and sacrificed. Blood was collected in heparinised tubes. Liver tissue was procured, then smoothly blotted it to dry, weighed and kept in clean dry beakers covered with aluminium foil. Blood samples were employed for estimation of various parameters – blood glucose by O-Toludine method (6), AST and ALT activities by Reitman and Frankel method (7). One gram of the liver tissue was homogenized with 10ml of phosphate buffer (pH-7.4) using potter Elvejham tissue homogenizer and the resultant mixture was centrifuged at 3000 rpm for 5 minutes. The clear supernatant of phosphate buffer extract was employed for the estimation of total thiols by nitroprusside method (8), AST and ALT activities by Reitman and Frankel method (7). One gram of the liver tissue was homogenized with 10ml of 5% cold TCA for 5 minutes using potter Elvejham tissue homogenizer and the resultant mixture was centrifuged at 3000 rpm for 5 minutes. The clear supernatant was employed for estimation of thio barbituric acid reactive substance (TBARS) levels (9). A part of liver tissue was fixed in 10% buffered formalin and embedded in paraffin. 5µm section were cut and stained with hematoxylin and eosin (H&E) (10, 11). The sections were examined under light microscope (10x & 40x) and photomicrographs were taken with connected camera.

Gravimetry

The body weight of all the animals of each group was recorded on the day 1 of the treatment and on the day of sacrifice. The liver weight was determined after dissecting out and blotting it dry in a single pan balance to evaluate the hepato - somatic index. Hepato - somatic index is the ratio of liver weight after dissection to body weight at the time of sacrifice.

Statistics

All the results are expressed as mean $\pm$ standard deviation. The statistical analysis was done using one way analysis of variance (ANOVA) followed by post hoc't' test to determine the significant difference between the groups. A p value less than 0.05 was selected as the point of minimal statistical significance.

Results

The results of the experiments conducted to assess the Diallyl Disulphide (100mg/kg body weight) induced changes in gravimetry, liver functioning, liver oxidative stress and liver histology are given in Table 1 to 3 and Fig. 1 to 4.

Table 1 shows a significant decrease in final body weight in group II when compared to group I (where there is increase in final body weight), suggesting loss of body weight in alloxan induced diabetic rats. No significant change in final body weight was observed in group I and III. An increase in final body

weight was observed in group IV compared to group II, indicating DADS administration improved diabetic body weight loss. Hepato - somatic index was increased significantly in group II when compared to group I while after the treatment with DADS in diabetic rats, hepato somatic index was decreased, indicating an improvement in liver recovery after treatment with DADS.

Examination of H&E stained sections of the control group (group I) showed normal architecture (Fig. 1a & 1b). In diabetic rats (group II) liver tissue section showed distortion in the arrangement of cells around central vein, periportal fatty infiltration with focal necrosis of hepatocytes were observed (Fig. 2a & 2b). In DADS treated normal rats (group III) the architecture was similar to that of normal control (Fig. 3a & 3b). In group IV, DADS treated diabetic rats, there was reduction in necrosis of hepatocytes, fatty infiltration and derangement of cells (Fig. 4a & 4b), when compared to group II.

Table 3 depicts a significant elevation in blood glucose in group II compared to group I, suggesting diabetes induced hyperglycemia. When group I was compared with group III no significant change was observed, suggesting normoglycemic effect of DADS on normal control rats. There was a significant reduction in blood glucose observed in group IV compared to group II, depicting the hypoglycemic effect of DADS on alloxan induced diabetic rats.

Table 2 and 3 showed a significant elevation in liver tissue and plasma AST and ALT in group II when compared to group I, suggesting diabetes induced liver derangement. When group I was compared with group III for the above said parameters, no significant change was observed. A significant lowering of liver tissue and plasma AST and ALT was observed in group IV when compared to group II, suggesting the liver tissue recovery with DADS treatment in alloxan induced diabetic rats.

Table 2 shows a reduction in liver tissue total thiols and increase in TBARS in group II compared to group I, depicting diabetes induced reduction in antioxidants and increase in free radicals. No significant change is observed in between group I and III. An increase in liver tissue total thiols and decrease in TBARS levels was observed in group IV compared to group II suggesting improvement in antioxidant levels upon treatment with DADS in alloxan induced diabetic rats.

Table 1 Gravimetry

Changes in body weight and Hepato Somatic index in Normal and Alloxan induced Diabetic rats before and after treatment with DADS (100mg/kg body weight) for 30 days.

S.No	Parameter	Group I (n=6)	Group II (n=6)	Group III (n=6)	Group IV (n=6)	F Value	P Value
1	Initial Body weight (g)	260+9 ^{a}	257+16.8^{a}	251.5+6.9^{a}	253.5+9.3^{a}	0.7444	0.5388
2	Final Body weight (g)	276.6+11^{a}	238+18.1^{b}	266.6+8.1^{a}	242+7^{b}	15.38	0.0000
3	% Body weight Change	6+1.34^{a}	-8.23+2^{b}	5.6+0.9^{a}	-4.7+1.8^{c}	120.3	0.0000
4	Hepato-Somatic Index (g/Kg)	29+0.49^{a}	36+ 1.2^{b}	30.5+1.8^{a}	33.2+0.7^{c}	33.27	0.0000

Note: Each value is mean $\pm$ SD of 6 observations in each group. In each row values with different superscripts (a, b, c) are significantly different from each other ($p<0.05$).

Table 2 Tissue Biochemistry
Changes in liver tissue AST, ALT, Sulphydryl groups and TBARS in Normal and Alloxan induced Diabetic rats before and after treatment with DADS (100mg/kg body weight) for 30 days.

S.No	Parameter	Group I (n=6)	Group II (n=6)	Group III (n=6)	Group IV (n=6)	F Value	P Value
1	Sulphydryl Groups (μm/g)	$1.09+0.13^{a}$	$0.75+0.18^{b}$	$1.06+0.15^{a}$	$0.86+0.11^{b}$	6.448	0.0034
2	TBARS (μmMD/g)	$4.97+1.65^{a}$	$6.14+1.8^{a}$	$5.04+1.58^{a}$	$5.55+1.93^{a}$	0.5140	0.6776
3	AST(U/L)	$27+3.5^{a}$	$42.5+7.07^{b}$	$30.8+2.99^{a}$	$33.5+5.74^{c}$	9.268	0.0005
4	ALT(U/L)	$41+5.1^{a}$	$54.6+5.0^{b}$	$39.4+7.8^{a}$	$47.4+3.28^{c}$	8.013	0.0012

Note: Each value is mean ± SD of 6 observations in each group.
In each row values with different superscripts (a, b, c) are significantly different from each other ($p<0.05$).

Table 3 Blood Biochemistry
Changes in blood glucose, AST and ALT in Normal and Alloxan induced Diabetic rats before and after treatment with DADS (100mg/kg body weight) for 30 days.

S.No	Parameter	Group I (n=6)	Group II (n=6)	Group III (n=6)	Group IV (n=6)	F Value	P Value
1	Blood Glucose (mg/dl)	$99.6+7.2^{a}$	$473+24.1^{b}$	$93.5+6.05^{a}$	$323+25.8^{c}$	580.1	0.0000
2	AST(U/L)	$28.5+6.8^{a}$	$58+4.7^{b}$	$31.5+6.9^{a}$	$38+9.2^{c}$	17.79	0.0000
3	ALT(U/L)	$35.5+7.5^{a}$	$69+5.9^{b}$	$37.5+8.1^{a}$	$50.8+6.1^{c}$	25.72	0.0000

Note: Each value is mean ± SD of 6 observations in each group.
In each row values with different superscripts (a, b, c) are significantly different from each other ($p<0.05$).

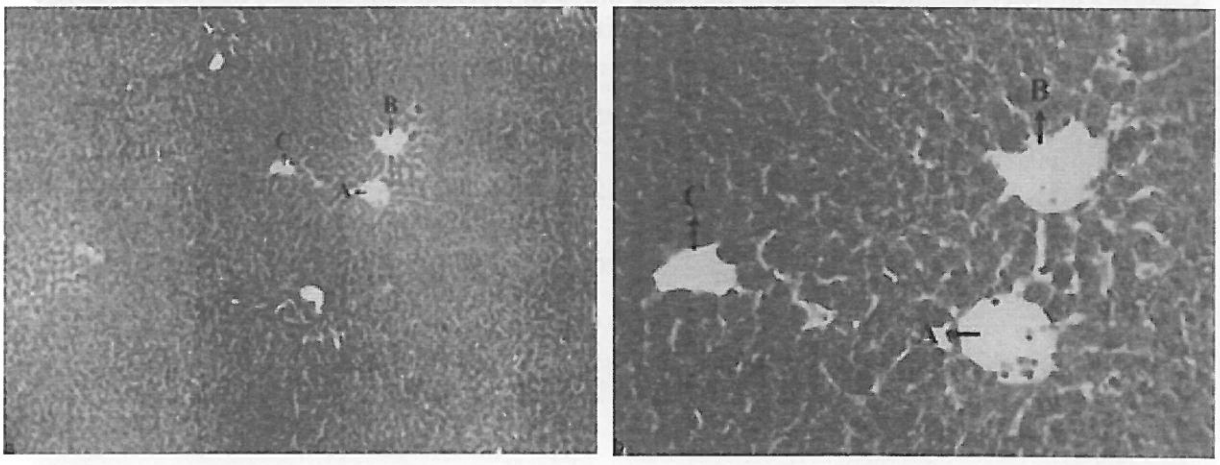

Fig.1 (a) Liver section of normal control group I (10x), (b) Liver section of normal control group I (40x) showing hepatocytes and portal triad comprising (A) Hepatic artery, (B) Hepatic vein, (C) Bile duct.

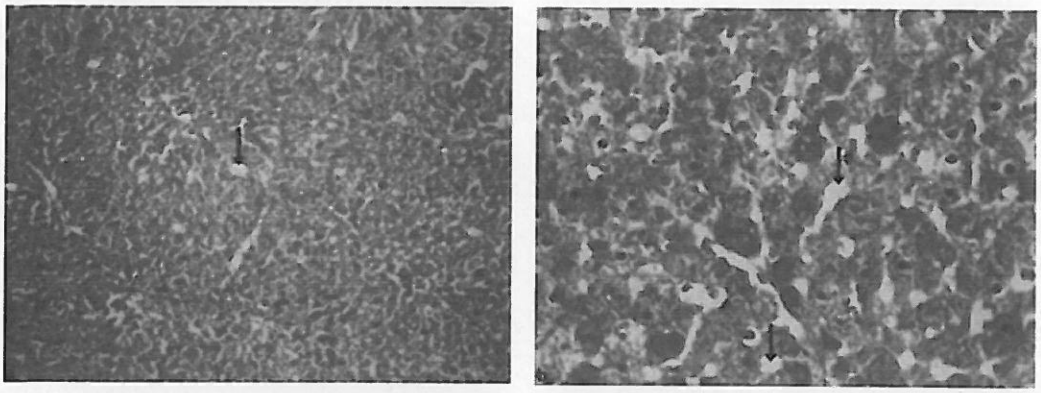

Fig.2 (a) Liver section of diabetic rat group II (10x), (b) Liver section of diabetic rat group II (40x) showing features of fatty liver (arrow marks indicate deposition of fat)

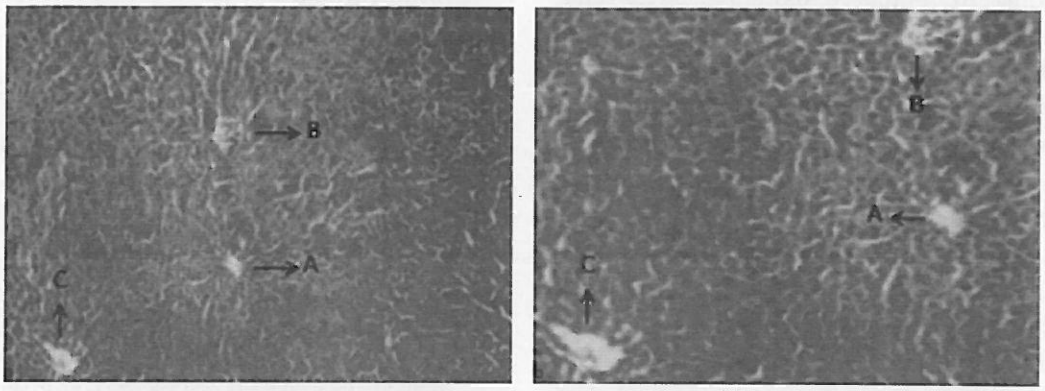

Fig. 3 (a) Liver section of DADS treated normal rats group III (10x), (b) Liver section of DADS treated normal rats group III (40x) showing normal hepatocytes and portal triad comprising (A) Hepatic artery, (B) Hepatic vein, (C) Bile duct.

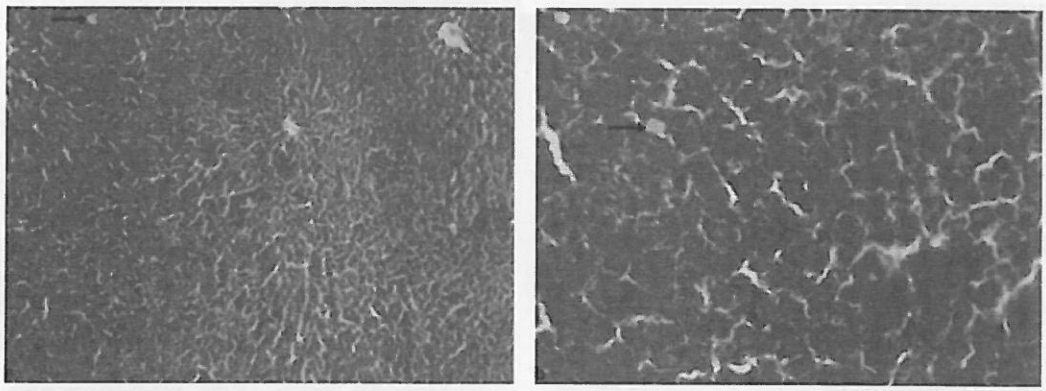

Fig. 4 (a) Liver section of DADS treated diabetic rats group IV (10x), (b) Liver section of DADS treated diabetic rats group IV (40x) showing hypolipidemic changes indicated by arrow marks.

Discussion

In this study a significant decrease in serum glucose was observed in DADS treated alloxan induced diabetic rats. In alloxan induced diabetic rats, i) the degenerative changes of liver histology were similar to the earlier observations (1, 12, 13, 14). ii) the absence of insulin showed a marked structural alteration in the liver histological sections. iii) the major alteration was periportal fatty infiltration and necrosis of hepatocytes. This damage is partially reversed by DADS treatment and is similar to that observed by *Vinca rosea* extract in alloxan induced diabetic rats by Ghosh *et al* (1). Prolonged hyperglycemia will produce reactive oxygen species (ROS), leading to increased oxidative stress and decreased antioxidant levels (15, 16). In our study in untreated diabetic rats, an increase in liver tissue TBARS was observed, which is considered as marker of oxidative stress and decrease in total thiol groups was observed, which is considered as marker for antioxidant levels. Upon treatment with DADS an improvement in TBARS and total thiol groups was observed. Liver cell destruction led impairment in permeability of liver cell membrane resulted in elevation of serum and liver tissue AST and ALT (17) in untreated diabetic rats. Our study showed that diabetic rats treated with DADS showed a reversal of histological and biochemical changes in the liver functioning.

Conclusion

In the present study, consumption of 100 mg/kg body weight of DADS reversed most of the histological and biochemical changes in liver of the diabetic rats. This effect was due to the hypoglycemic nature of the DADS. In addition, an associated increase in oxidative stress in diabetes was significantly reduced by DADS consumption. So we can conclude that DADS had a significant hepato-protective role in diabetic rats and offers promising perspectives deserve further investigations.

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