Hypolipidemic and Antioxidant Effects of Diallyl Disulphide and Diaceto-Dipropyl Disulphide on Renal Tissue in Alloxan Diabetic Rats - A Comparative Study

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ABSTRACT

BACKGROUND

Diabetic nephropathy (DN) is one of the commonest causes for end stage renal disease. Renal accumulation of lipids is one of the pathological finding seen in diabetic nephropathy. Diallyl disulphide (DADS), a principle component of garlic oil, is known for its hypolipidemic properties. Diaceto-dipropyl disulphide (DADPDS) is a structural analogue of DADS, and is more palatable and less toxic to diabetic rats. Hence this animal experiment study was undertaken to compare the hypolipidemic and antioxidant effects of DADS and DADPDS on diabetic rat renal tissue and to evaluate the better disulphide among the two that may be used as adjuvant drug in treating or preventing diabetic nephropathy.

METHODS

This was an animal experimental comparative study. 24 male albino rats were grouped (6 rats in each group) into normal, diabetic control, DADS and DADPDS treated diabetic rats. Diabetes was induced in albino rats by intraperitoneal injection of alloxan. DADS and DADPDS were fed by gastric intubation for 90 days. After stipulated time, kidneys were dissected out and its total lipids, cholesterol and phospholipid levels were estimated along with tissue thiobarbituric acid reactive substances (TBARS) levels.

RESULTS

Renal tissue total lipids, cholesterol and phospholipids were significantly decreased in DADS and moderately decreased in DADPDS treated diabetic kidneys, when compared to diabetic control rats. But TBARS levels were significantly decreased in DADPDS rat kidneys compared to DADS treated rat kidneys.

CONCLUSIONS

In this comparative study, we note that DADS has better lipid-lowering effect on renal tissue of alloxan diabetic rats compared to DADPDS. On the other hand, DADPDS has low renal toxic effects, as indicated by low TBARS levels and improvement in blood glucose levels, when compared to DADS treated diabetic rats. Hence, DADS can be used as an adjuvant drug, only in atherogenic diabetic patients without nephropathy and DADPDS can be used as an adjuvant drug in diabetic nephropathy patients.

KEYWORDS

Diallyl-Disulphide, Diallyl-Dipropyl Disulphide, Diabetic Nephropathy, Renal Lipids

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BACKGROUND

It is estimated that India is going to lead in the number of diabetic cases when compared to other countries in the world. Among the various microvascular and macrovascular complications of diabetes, diabetic nephropathy is most common and fatal complication seen, which is a burden to the country's health care facility.

Diabetic Kidney

Diabetic nephropathy is the commonest cause of end stage renal disease, worldwide. Along with important roles played by factors such as oxidative stress, advanced glycation end-products, polyol pathways, hyperglycaemia induced alterations in renin angiotensin aldosterone and others inflammatory pathways; abnormal lipid metabolism and renal accumulation of lipids have also been proposed to play a role in the pathogenesis of diabetic nephropathy.[1] Several workers have shown the presence of lipid deposits in the kidney of diabetic human and experimental animals and they have proposed that these lipid deposits may play an important role in the pathogenesis of diabetic kidney disease.[2,3] Earlier these lipid deposits in kidney were attributed to increased levels of serum lipids. However, Sun et al.[4] showed an increased renal lipid synthesis to be responsible for this. It is shown that there is an increased lipid peroxidation in diabetic renal tissue which also contributes to the pathogenesis of diabetic nephropathy.[5-9]

Plants are most commonly studied for their anti-diabetic properties. Among various plants biochemical substances studied, disulphides have shown to mimic like insulin acting agents. The most commonly studied plant disulphides are of species belonging to Allium group. Disulphides of garlic, (Allium sativum Linn) are well known for their antidiabetic, antihyperlipidemic, antiatherogenic, antioxidant as well as anticarcinogenic properties.[10-13] Diallyl disulphide (DADS), the natural and principle disulphide compound of garlic oil, is probably responsible for the above-mentioned beneficial functions of garlic. But DADS is very pungent oil and is often difficult to feed rats. Also, few studies have shown toxic effects of DADS on rat kidneys.[14,15] But its structural analogue, diaceto- dipropyl disulphide (DADPDS) is pleasant smelling, more palatable, less toxic and possesses similar anti-diabetic properties.[16] This DADPDS was synthesised by using thiopropanol in our study.

Objectives

This animal experiment study was undertaken to assess and compare the hypolipidemic and antioxidant actions of DADS and DADPDS in renal tissue of alloxan diabetic rats and thus to evaluate the better disulphide among the two that may be used in treating or preventing diabetic nephropathy.

METHODS

This animal experimental study was conducted in Basaveshwara Medical College, Chitradurga and the duration of this animal study was 90 days from September 2017 to November 2017. All chemicals employed in the present study were of analar grade (A.R). Alloxan was procured from Loba chemie. DADS and thiopropanol were procured (3-mercapto-1-propanol) from Sigma- Aldrich Company USA. This thiopropanol was used for the preparation of diaceto-dipropyl disulphide.

Synthesis of DADPDS

DADPDS was synthesised from thiopropanol as explained by Veena GR et al.[16] 5 gms of thiopropanol was taken and was treated with 1N iodine in potassium iodide solution, slowly drop by drop, till a light-yellow colour persists and the contents were dissolved in 100 ml diethyl ether. 10 ml ice cold acetyl chloride was added to this solution and mixed well.

The above mixture was kept at 10 - 15˚C for 3 hours. Two separated liquid layers are seen. The separated ether layer was washed twice with 25 ml portions of ice-cold saturated sodium chloride solution. Thus, obtained solution was washed 4 times with 0.1 N sodium hydroxide solution in saturated sodium chloride then washed once with 10 ml 0.1 N sodium thiosulphate in saturated sodium chloride and finally was washed once with 10 ml glass distilled water. Then the ether layer was clarified with anhydrous sodium sulphate and dried at 50 - 55˚C for 30 minutes. Thus, obtained residue was diaceto-dipropyl-disulphide. This was used to feed the rats in the present study and its hypolipidemic and antioxidant effects were compared with DADS.

Calculation of Optimum Dose of DADS and DADPDS

A pilot study was conducted on different experimental rat groups by feeding them on DADS and DADPDS of 50, 100, 150 and 200 mg/kg body weight. After 2 months of treatment, the rats were sacrificed; blood and renal tissue were dissected out and were used for estimation of transaminases, TBARS and lipid levels. The TBARS and transaminases levels were raised in 150 and 200 mg/kg body weight, DADS and DADPDS treated rat groups.

Hence, it was found that the optimum dose of both DADS and DADPDS was 100 mg/kg body weight, as there was maximum hypolipidemic effect and minimum TBARS levels rise, in both blood and renal tissue (refer Table 2).

Maintenance of Animals

24 male albino rats, weighing 200 - 250 gms were randomly selected from Central Animal House of Basaveshwara Medical College, Chitradurga. The animals were maintained on a standard rat feed, room temperature of 28^0C and water available ad libitum.

Induction of Diabetes

The albino rats were fasted overnight and a single intraperitoneal injection of freshly prepared alloxan (150 mg/kg body wt.) in sterile normal saline, was given. The

animals which showed blood glucose levels more than 250 mg/dl and urine showed consistent glucosuria, were considered diabetic animals. The feeding of DADS and DADPDS was started on 5th day after alloxan injection and was considered as first day of treatment.

Grouping of Rats

Male albino rats were divided into four groups consisting of six rats in each group, as follows:

I. *Normal Rats* – The rats were given 30 ml of normal saline per kg body weight, through gastric intubation daily for 90 days.
II. *Diabetic Control Rats* - The rats were given normal saline 30 ml/kg body weight, through gastric intubation daily for 90 days.
III. *DADS treated Diabetic Rats* – The rats were given DADS (100 mg/kg body weight) prepared in normal saline, given as 30 ml/kg body weight suspension, through gastric intubation daily for 90 days.
IV. *DADPDS Treated Diabetic Rats* – The rats were given DADPDS (100 mg/kg body weight) prepared in normal saline, given as 30 ml/kg body weight suspension, through gastric intubation daily for 90 days.

Procedure

On completion of 90 days, the rats were anaesthetised using ether and were sacrificed. Venous blood was collected in heparinised tubes. Kidneys were dissected carefully and their total weight was noted. Immediately the kidneys were processed as follows. One part of kidney was homogenised with 9 parts of chloroform methanol (1 : 1 v / v) and the extract was used for total lipids,[17] total cholesterol[18] and phospholipid levels.[19] A second part of kidney was homogenised with 9 parts of trichloro acetic acid (10 %) and extract was used for the estimation of thiobarbituric acid reactive substances (TBARS) levels.[20] Whole blood was employed for glycated haemoglobin estimation.[21] A part of whole blood was centrifuged at 3500 rpm for 6 - 8 minutes and the plasma was used for glucose.[22]

Statistical Analysis

The results obtained were statistically analysed by student 't' test.

Ethics

This animal experiment was conducted according to the norms approved by ministry of social justice and empowerment, Government of India, and Institutional Animal Ethics Committee (IAEC) guidelines of Basaveshwara Medical College, Chitradurga, India.

RESULTS

From the results of our study, we note that there was significant decrease (P < 0.001) in body weight of diabetic-control group rats along with diallyl disulphide (DADS) and diaceto-dipropyl disulphide (DADPDS) treated diabetic rats when compared with normal group rats. Our study showed no significant changes in kidney weights of rats belonging to all the groups. The most probable reason for this may be there was decrease protein content due to increased protein catabolism, but this was compensated by increase in the lipid synthesis seen in diabetes mellitus (Refer table 3). Kidney-body weight ratio in diabetic control groups, DADS and DADPDS treated diabetic rats was significantly decreased (P < 0,001) when compared to normal rats. And when compared between DADS and DADPDS treated rats, DADS treated diabetic rats showed no significant loss of body weight and kidney weight compared to DADPDS treated diabetic rats (Refer table 1).

	Rat Weight (gms)	Kidneys Weight (gms)	Kidney Weight/Body Weight Ratio
Normal (n = 6)	323.81 ± 55.65	2.21 ± 0.18	0.0071 ± 0.0009
Diabetic control (n = 6)	217.85 ± 31.40***	1.97 ± 0.24	0.0091 ± 0.0008***
DADS (n = 6)	197.16 ± 50.32	1.87 ± 0.45	0.0094 ± 0.0007
DADPDS (n = 6)	234.5 ± 17.67	2.01 ± 0.13	0.0085 ± 0.0007

Table 1. Rats Weights, Kidneys Weight and Relative Kidney Weights in All the 3 Groups

Note: 1. Number of parenthesis indicate the number of animals in each group.
2. Significance level: - * p < 0.05; ** p < 0.01; *** p < 0.001 [when compared with the above group in the table. #p < 0.05; ## p < 0.01; ### p < 0.001 [when compared with the diabetic control group]

	Plasma Glucose (mg/dl)	Glycated Haemoglobin (%)	Renal Tissue TBARS (µ mol/g)
Normal (n = 6)	112.26 ± 19.6	0.39 ± 0.12	8.11 ± 1.76
Diabetic control (n = 6)	632.66 ± 102.08***	1.62 ± 0.15***	20.51 ± 4.62***
DADS (n = 6)	272.0 ± 5.94***	1.56 ± 0.12***	14.28 ± 2.31***
DADPDS (n = 6)	264.23 ± 87.41***	1.25 ± 0.19**	10.68 ± 2.29***/###

Table 2. Plasma Glucose, Glycated-Hb and Renal Tissue TBARS Levels in All the 3 Groups

Note: 1. Number of parenthesis indicate the number of animals in each group.
2. Significance level: - * p < 0.05; ** p < 0.01; *** p < 0.001 [when compared with the above group in the table] #p < 0.05; ## p < 0.01; ### p < 0.001 [when compared with the diabetic control group]

	Total Lipids mg/g	Total Cholesterol mg/g	Phospholipids mg/g
Normal (n = 6)	24.85 ± 4.48	0.810 ± 0.07	7.62 ± 1.86
Diabetic control (n = 6)	40.60 ± 4.80***	0.985 ± 0.10***	12.45 ± 2.15***
DADS (n = 6)	27.84 ± 6.51***	0.848 ± 0.10***	9.12 ± 3.16***
DADPDS (n = 6)	33.72 ± 5.67#	0.945 ± 0.06	7.76 ± 0.82###

Table 3. Renal Lipid levels (TL, TC and PL) in All the 3 Groups

Note: 1. Number of parenthesis indicate the number of animals in each group.
2. Significance level: - * p < 0.05; ** p < 0.01; *** p < 0.001 [when compared with the above group in the table] #p < 0.05; ## p < 0.01; ### p < 0.001 [when compared with the diabetic control group]

Blood glucose along with glycated haemoglobin and renal tissue TBARS levels were significantly increased (P < 0.001) in alloxan diabetic-control rats, DADS treated and DADPDS treated diabetic rats, when compared with normal group rats. And when compared in between DADS and DADPDS treated rats, DADPDS treated diabetic rats showed significant lower levels of plasma glucose (P < 0.001), lower levels of glycated haemoglobin (P < 0.01) and lower levels (P < 0.001) of renal tissue TBARS when compared to DADS treated diabetic rats (Refer table 2).

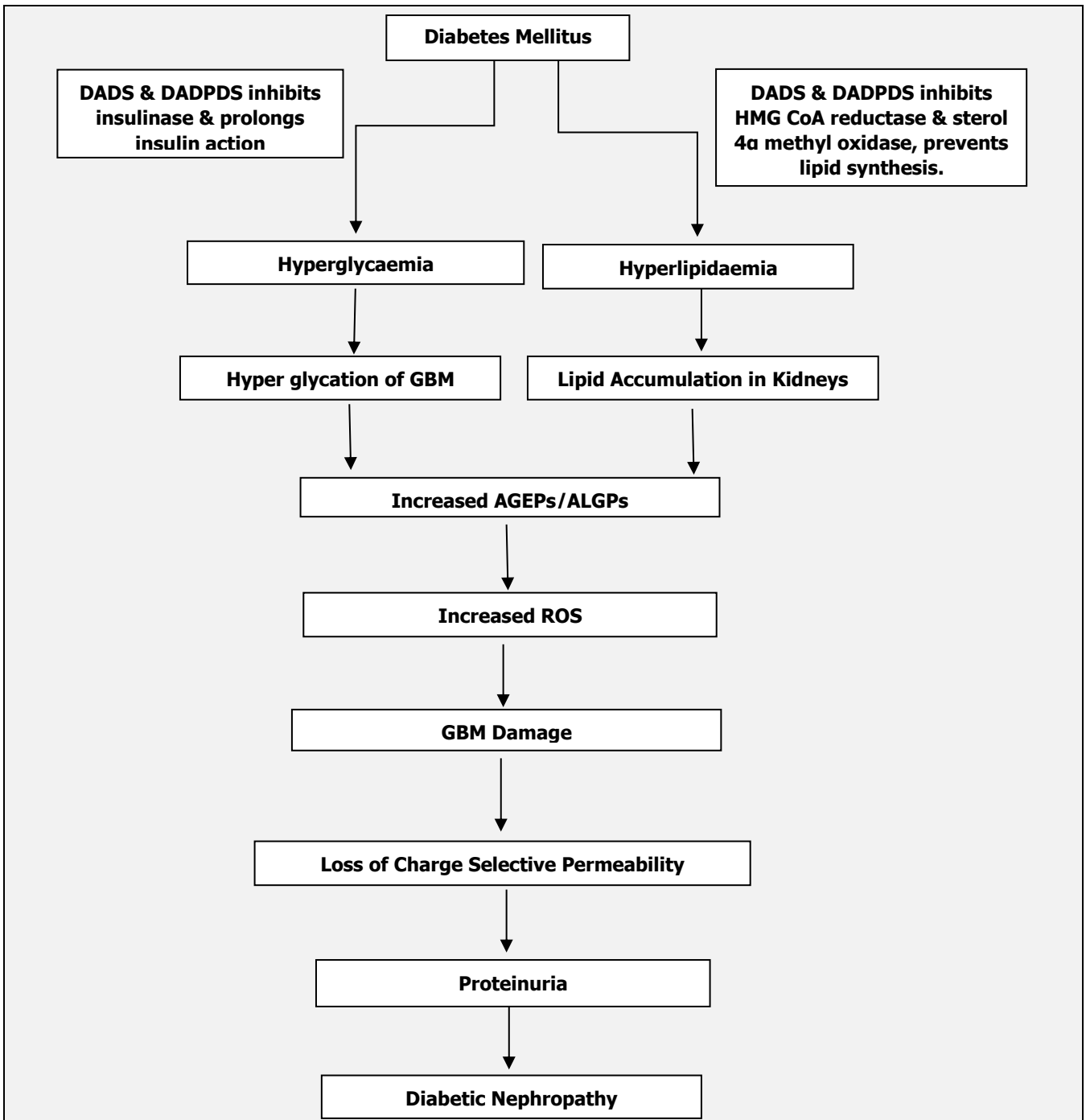


Diagram 1. Probable Mechanism of Action of DADS and DADPDS in Prevention of Hyperlipidaemia and Renal Accumulation of Lipids, in Alloxan Diabetic Rats and thus Preventing DN. (* Advanced Glycated End Products/Advanced Lipid Glycation Products)

The renal tissue total lipids, cholesterol and phospholipids had increased significantly ($P < 0.001$) in diabetic control rats when compared to normal group rats. On treatment with DADS and DADPDS for 90 days in alloxan diabetic rats, there was significant decrease ($P < 0.001$) in renal tissue total lipids, cholesterol and phospholipids when compared to diabetic control rats. Further when compared in between DADS and DADPDS treated diabetic rats, there was no statistically significant difference found (Refer table 3). On comparison between renal hypolipidemic action of DADS and DADPDS treated diabetic rats, with diabetic-control rats, DADS treated diabetic rats shows better ($P < 0.001$) hypolipidemic actions when compared DADPDS treated rats ($P < 0.05$). To highlight the results, DADS treated diabetic rats showed better renal-tissue lipid lowering effect than DADPDS treated rats and DADPDS treated diabetic rats showed better hypoglycaemic effects than DADS treated rats, when compared to diabetic-control rats.

DISCUSSION

In our study, administration of alloxan (150 mg/kg body weight) has induced diabetes mellitus in the albino rats which is evidenced by increased plasma glucose levels and

glycated haemoglobin levels in group II, III and group IV rats (refer table 2). Alloxan specifically damages the beta cells of Langerhans of pancreas and causes severe lack of insulin, thus causing diabetes mellitus in rats. Here in this study the rats were studies around 90 days of induction of diabetes mellitus by injecting alloxan. The levels of glycated haemoglobin have been shown to be an important parameter of chronic glycaemic control in diabetes. The decrease in body weight of diabetic rats is due to increase in the protein catabolism mainly in skeletal muscles that helps to channel amino acids for gluconeogenesis, decrease in protein uptake and increased lipolysis due to insulin deficiency.[23] Insulin is an important regulator of many enzymes involved in lipolysis and lipogenesis, and its deficiency causes major changes in the activity of these enzymes thereby affecting overall lipid metabolism and lipid profiles of various tissues.[24]

Our study showed no significant changes in kidney weights of rats belonging to all the groups. The most probable reason for this may be there was decrease protein content due to increased protein catabolism but this was compensated by increase in the lipid synthesis seen in diabetes mellitus. Increase in the relative weight of kidneys (ratios between weights of kidneys and that of body weight) in diabetic rats (refer table 1) is due to increase in lipogenesis and lipid deposition in kidneys,[4,25] as evident in the table 2. There is growing evidence that abnormal lipid metabolism and renal accumulation of lipids play a role in the pathogenesis of diabetic nephropathy.[26]

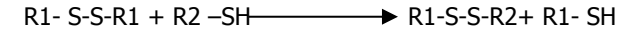
Also, studies have proved that hyperglycaemia induced increased lipid peroxidation contributes to the pathogenesis of diabetic nephropathy.[8,9] Diabetes mellitus induces dyslipidaemia and gross alterations in serum and tissue lipid levels. The antihyperlipidemic effects of garlic and its organo sulphur compounds have been repeatedly established in hyperlipidaemic rats. Our study addresses (refer table 2 and 3) that there is significant increase in total lipids, cholesterol phospholipids as well as thiobarbituric acid reactive substances (TBARS) levels in renal tissue of diabetic control rats (group II) when compared with normal rats (group I).

Thiobarbituric acid reactive substances are widely used to measure lipid peroxidation and anti-oxidant activity in experimental diabetic rats. Diabetes mellitus induced increase in free radicals may probably reduce tissue total thiols including glutathione, as a first line of defence of free radical scavenging process. The renal tissue TBARS levels shown in table 2, indicates that free radical generation as well as oxidative damage was significantly higher in diabetic control group rats along with DADS and DADPDS treated diabetic rats when compared with normal group rats. But when compared between DADS and DADPDS treated diabetic rats, DADPDS treated rats showed significant lower levels of TBARS levels in the renal tissue, suggesting that DADPDS possess higher antioxidant activity than DADS.

Disulphides from genus Allium group (like onion and garlic), have shown to possess anti-diabetic and anti-atherogenic properties. The proposed biochemical mechanism of actions of these disulphides, are as follows:

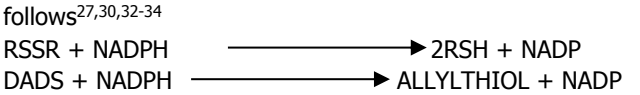
Thiol-Disulphide Exchange Reactions of Disulphides

Sulphydryl enzymes like insulinase,[27] HMG CoA reductase[28] and sterol 4α methyl oxidase[29] when treated with DADS and DADPDS may be inhibited by thiol-disulphide exchange reactions as shown below:[30,31]



Reduction to Thiols Using NADPH/NADH

DADS and DADPDS are disulphides that may undergo reduction to its thiols, using NADPH / NADH as follows[27,30,32-34]



Such reaction of DADS and DADPDS with NADPH may reduce cellular NADPH levels, hence lowers fatty acids and cholesterol synthesis, as their synthesis requires sufficient supply of NADPH.[32] Similarly, an NADPH dependent enzyme, insulinase or insulin transhydrogenase is involved in insulin degradation.[35] A decrease in NADPH levels caused by DADS and DADPDS,[30] may limit insulinase action causing increase in half-life of insulin. This leads to prolonged insulin action and hypoglycaemia.[36]

Hence on treating the alloxan diabetic rats with disulphides, both DADS and DADPDS, showed significant decrease in plasma glucose and glycated haemoglobin levels along with total lipids, cholesterol, phospholipids and TBARS levels in the renal tissue.

CONCLUSIONS

In this comparative study, we noted that diallyl disulphide (DADS) has better lipid-lowering effects on renal tissue of alloxan diabetic rats compared to diaceto-dipropyl disulphide (DADPDS). On the other hand, DADPDS has low renal toxic effects, as indicated by low thiobarbituric acid reactive substances (TBARS) levels and improvement in plasma glucose levels, when compared to DADS treated diabetic rats. DADS can be used as an adjuvant drug, only in atherogenic diabetic patients without nephropathy and DADPDS can be used as an adjuvant drug in diabetic nephropathy patients.

Further Scope of the Study

Study in larger population can be done and the effects of DADS and DADPDS can be compared with present standard anti-hyperglycaemic and anti-hyperlipidaemic drugs, which was not done in this present study.

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