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Effect of Captopril and Allylmercaptocaptopril on Antioxidant Status in Streptozotocin induced Diabetic Rats

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Abstract: Aim: To compare the effects of captopril and Allylmercaptocaptopril (CPSSA) on antioxidant status in Streptozotocin induced diabetic rats.
Method: In this study we determined the effect of captopril and CPSSA on Total antioxidant status (TAS), Total oxidative status (TOS) and Total oxidative stress index (TOSI). The body weight measurement and blood glucose levels were checked for every week until the end of the study by using the GOD/POD method. The parameters are found by using the eral method, by observing the absorbance at 560nm for TOS, 444nm for TAS. The TOSI was found by taking the percentage ratio of TOS and TAS.
Results: The TOS value of Allylmercaptocaptopril was less (15.18 ± 2.16) when compared to Captopril (18.03 ± 3.14) which indicate that the level of free radicals was low in case of Allylmercaptocaptopril treated group than Captopril. In contrast the TAS value of Allylmercaptocaptopril was more (2.57 ± 0.16) when compared to Captopril (1.63 ± 0.14). All these values predict that the Allylmercaptocaptopril has more antioxidant activity than the captopril.
Conclusion: The present study revealed the Allylmercaptocaptopril was having more potent action of reducing the TOS in the serum levels and has the higher extent of the TAS than the captopril
Key words: Allylmercaptocaptopril, captopril, Free radicals and antioxidant activity.

INTRODUCTION:

Hyperglycemia occuring in diabetes is the crucial factor that is responsible for the development of oxidative stress and reactive oxygen species (ROS) which are the main mediators of cellular damage in diabetes. Increased lipid peroxidation and reduced antioxidant enzyme activity found to be associated with progression of albuminuria in diabetes. Angiotensin-II blockage by the angiotensin converting enzyme inhibitors (ACEI's) shows to increase the activity of antioxidant enzymes during the diabetes[1,2]. There are several sources of ROS in diabetes, including defective mitochondrial metabolism[3], glucose autoxidation[4], NADPH oxidation and synthesis of advanced glycation in product.

Increased oxygen free radical activity, coupled with reduced protection against oxidative stress, could play a role in the etiology of neurovascular abnormalities in experimental diabetes mellitus[5].

High blood glucose level determines over production of ROS by the mitochondria electron transport chain. High reactivity of ROS determines chemical changes in virtually all cellular components, leading to DNA and protein modification and lipid peroxidation[6,7].

The present study was sought to investigate effectiveness of captopril and allylmercaptocaptopril

which combines the advantages of both molecules, captopril and allicin, which operate by different mechanisms. The covalently bonded reactive product of captopril and allicin leads to allylmercaptocaptopril (CPSSA), a nonsymmetric disulfide.

MATERIALS AND METHODS

Captopril was kindly provided by WOCKHARD Ltd, Aurangabad, Maharashtra, India. Streptozotocin, diallylsulfide was provided by Sigma chemical company, St. Louis, Missouri. Allicin was prepared from diallylsulfide by a modified method of Stoll and Seebeck et al [8]. Allylmercaptocaptopril was synthesized from aliicin and captopril according to the previously reported method of Miron et al [9] with some major modifications.

2.1 PHARMACOLOGICAL STUDIES:

Male albino rats of Wistar strain (weighing 145 - 170 g) are used for the study. Rats used for the study obtained from the animal house stock of the Department of Pharmacology, SRM College of Pharmacy, Kattankulathur, India and handle in accordance with the guidelines as per the "Institutional Animal Ethical Committee"(IACE) and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) rules. Animals allowed to free access of tap water and standard chow diet up to the end of the experimental period and divided in to the 4 groups; each group consists of 8 rats.

INDUCTION OF DIABETES AND DOSING

Diabetes was induced to the all the groups of animals except the normal group Streptozotocin (STZ) 60mg/kg was given through intraperitonial route by dissolving STZ in citrate buffer.

The normal group (group-1), STZ control group (group-2) diabetic rats receiving no treatment which were given tap water, Captopril receiving group which were given captopril at libitum in drinking water at a concentration of 50 mg/kg i.p (group-3), Allylmercaptocaptopril was given at a dose of 50mg/kg i.p (group- 4). After animals are considered to be diabetic if they had serum glucose level >130mg/dl, and decrease in their body weight. Upto the seventh week i.e, end of the experiment blood samples from all the rats in the study were collected once in a week, collected by retro-orbital puncture. The photometric estimation of glucose in plasma was done based on GOD/POD method done as described in the manufacturers (Merck Specilist Pvt Ltd) instruction manual.

At the end of the experiment the blood samples were collected and were measured for the Total plasma peroxide concentration, Total Antioxidant status, and for total oxidative stress index.

BIOCHEMICAL ANALYSIS

At the end of the experimental period, all the animals are sacrificed. Blood sample is collected in heparinised tubes and centrifuged at 1600 x g for 15 min. Plasma used for the estimation of total antioxidant status (TAS), total oxidative status (TOS) and the ratio percentage of the TOS to the TAS potential gives the oxidative stress index, an indicator of the degree of oxidative stress[10].

Both TOS and TAS are measured by the using a novel automated colorimetric measurement method by Erel et al[11].

STATISTICAL ANALYSIS

Results of all parameters were expressed as mean ± standard deviation for each group. One way ANOVA followed by Dunnett test.

RESULTS:

Estimation of body weight:

Table 1 shows the change in the body weight of the diabetic induced animals and the diabetic + drug treated animals. It has been observed that there was a decrease in the diabetic control group, Due to the potent antioxidant activity of Allylmercaptocaptopril than the captopril the decrease in the body weight in allylmercaptocaptopril treated groups was less when compared to captopril treated rats.

Estimation of blood glucose levels

From table 2 the allylmercaptocaptopril and captopril treated groups were having the decrease in the blood glucose levels due to their high antioxidant activity ,but when compared to captopril allylmercaptocaptopril has more effect on blood glucose level than captopril.

BIOCHEMICAL PARAMETERS

From the table 3 the TOS value of Allylmercaptocaptopril was less (15.18 ± 2.16) when compared to Captopril (18.03 ± 3.14) which indicate that the level of free radicals was low in case of Allylmercaptocaptopril treated group. In contrast the TAS value of Allylmercaptocaptopril was more (2.57 ± 0.16) when compared to captopril (1.63 ± 0.14) and the TOSI index of Allylmercaptocaptopril (0.587) was less when compared to Captopril (1.106). All these values predict that the Allylmercaptocaptopril has more antioxidant activity and more effective in maintaining the homeostasis of redox balance between oxidation and antioxidation than captopril.

Table:1 Effect of Allylmercaptocaptopril and Captopril on body weight of Streptozotocin induced diabetic rats.

S. No	Groups	Initial Body Weight(g)	1st week (g)	2nd week(g)	3rd week (g)	4th week (g)	5th week (g)	6th week (g)	7th Week (g)
1	Normal	146.27± 1.87*	150.36 ±1.30**	152.87 ±2.69**	156.81 ±1.62**	165.01 ±1.41**	172.45 ±2.12**	174.77 ±1.92**	177.49 ±3.59**
2	Diabetic control	155.2 ± 2.34	140.11 ± 1.16	132.71 ± 2.61	126.13 ± 2.30	119 ± 1.06	112.37 ± 2.41	107 ± 1.12	102.91 ± 0.19
3	Diabetic + Captopril	153.47 ± 2.12ns	147.17 ± 1.52**	136.33 ± 2.71ns	132.47 ± 0.38*	130.07 ± 3.45**	131.65 ± 1.96**	132.76 ± 0.08**	134.81 ± 2.26**
4	Diabetes+ allylmer-captocapt-opril	167 ± 2.76**	158.33 ± 2.01**	152.16 ± 0.32**	148.06 ± 1.18**	149.96 ± 2.12**	148 ± 1.40**	149.56 ± 2.39**	153.04 ± 2.16**

Each value is represented as Mean ± SEM, Number of animals (n) = 8, *P< 0.05, **P< 0.01, nsP> 0.05 vs Diabetic control group. One way ANOVA followed by Dunnett test.

Table: 2 Effect of Allylmercaptocaptopril and Captopril on Blood Glucose Levels BGL (mg/dl) in Streptozotocin induced diabetic rats.

S. No	Groups	0 week (mg/dl)	1st week (mg/dl)	2nd week (mg/dl)	3rd week (mg/dl)	4th week (mg/dl)	5th week (mg/dl)	6rt week (mg/dl)	7th week (mg/dl)
1	Normal	126 ± 2.98**	127.57± 1.35**	128.37 ±1.70**	127.85 ±0.04**	129 ± 1.88**	128.02 ±1.67**	127.29± 0.90**	128.11 ±1.44**
2	Diabetic control	346.01 ± 1.60	378.60 ± 3.01	381.9 ±2.43	387.62 ± 0.14	390.45 ± 0.09	392.02 ± 2.76	394.89 ± 1.66	393.11 ± 3.89
3	Diabetic + Captopril	352.4 ± 3.69ns	349.48 ±0.40**	320.11 ±3.01**	301.32 ±2.18**	292.91 ±1.86**	281.52 ±1.14**	279.92 ±4.19**	270.12 ±0.62**
4	Diabetes+ Allylmer-Captocapt-opril	369.74 ±5.98**	320.95 ±1.12**	301.11 ±0.66**	290.02 ±4.34**	275.80 ±2.07**	261.91 ±0.83**	257 ± 1.02**	245.30 ±1.14**

Each value is represented as Mean ± SEM, Number of animals (n) = 8, *P< 0.05, **P< 0.01, nsP> 0.05 vs Diabetic control group. One way ANOVA followed by Dunnett test.

Table:3 Effect of Allylmercaptocaptopril and Captopril on TOS, TAS and TOSI in Streptozotocin induced diabetic rats.

Sl no	Groups	TOS (μmol H_20_2 equiv/L)	TAS (mmol Trolox equiv/L)	TOSI (AU)
1	Normal	12.06 ± 3.26**	2.53 ± 0.15**	0.476
2	Diabetic control	27.17± 3.21	1.14 ± 0.21	2.383
3	Diabetes + Captopril	18.03 ± 3.14ns	1.63 ± 0.14ns	1.106
4	Diabetes + Allylmercaptocaptopril	15.18 ± 2.16*	2.57 ± 0.16**	0.587

Each value is represented as Mean ± SEM, Number of animals (n) = 8, *P< 0.05, **P< 0.01, nsP> 0.05 vs Diabetic control group. One way ANOVA followed by Dunnett test.

DISCUSSION

Oxidative stress plays an important role in chronic complications of diabetes and is postulated to be associated with increased lipid peroxidation. The present study was to examine the involvement of free radicals in diabetes and the role of these toxic species in lipid peroxidation and the antioxidant defence system can be estimated by the changes in the level of extracellular total antioxidants, total oxidant status, and oxidative stress index in diabetic patients.

Captopril and allicin have been reported to possess free radical scavenging activity so these drugs were used in the present study in estimation of the oxidative stress, antioxidant status and the degree of oxidative stress was assessed by measuring the blood serum levels by spectrophotometrically by eral method.

It has been proved that sulfhydryl-containing angiotensin converting enzyme inhibitors protects against free radical injury in endothelial cells and demonstrated that the SH-containing ACE agents are capable of protecting the endothelial cells against free radical induced lipid peroxidation and cell injury; the mechanism may be due to direct hydroxyl radical scavenging [(12)].

It also appears that the protective effects of sulfhydryl agents correlate better with their direct hydroxyl radical scavenging abilities than with their antiperoxidative potency[(13,14)].

In addition, it has previously been reported that captopril is very effective in scavenging free radicals, in a manner similar to glutathione, N-2-mercaptoproppionylglycine, and N-acetylcysteine, but this effect was not mimicked by Enalapril[(15)].

The mode of action of allicin in trapping of radicals and interaction with thiol containing proteins has already been summarized. This study demonstrated that in addition to its antioxidant activity, the major biological effect of allicin should be attributed to its rapid reaction with thiol containing proteins[(16)].

CONCLUSION:

It is well known that, the effects of various antioxidants in plasma are additive and the cooperation of antioxidants in human serum provides protection of the organism against attacks by free radicals. Therefore, the measurement of TOSI may reflect accurately the antioxidant status of the organism. It has a definite beneficial role against free radical mediated disorders and further study was required to study the molecular mechanisms involved in the free radical scavenging activity of Allylmercaptocaptopril.

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