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Research Article

Antioxidant potential of a polyherbal formulation (Diabet) on alloxan induced oxidative stress in rats

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ABSTRACT

Diabet, a polyherbal formulation intended to use for diabetic patients has been screened for antioxidant activity. For antioxidant studies, Diabet was administered orally for 30 days at a dose of 500 mg/kg body weight to alloxan induced diabetic male Wistar rats. All the animals were sacrificed on the 31st day and the levels of LPO, SOD, CAT, GPx and GSH in kidney and liver of control and experimental rats were studied. The formulation exhibited significant antioxidant activity showing increased levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and decreased level of lipid peroxidation. These results showed that treatment with Diabet lowers Alloxan induced LPO and alters SOD, CAT, GPx and GSH enzymes to reduce oxidative stress.

Keywords: Diabet; Alloxan; Marker enzymes in liver and kidney; Antioxidant activity

INTRODUCTION

Diabetes mellitus is one of the most common endocrine metabolic disorders. It is one of the most prevalent chronic diseases in the world affecting nearly 25% of the population. Oxidative stress has been shown to have a role in the causation of diabetes and as such antioxidants may have a role in the reduction of diabetes and related problems [1]. Herbal medicines are frequently considered to be less toxic and more free from side-effects than synthetic ones [2]. After the introduction of insulin therapy, the use of traditional treatments for diabetes generally declined in occidental societies, although some traditional practices are continued for prophylactic purposes and as adjuncts to conventional therapy. In the traditional system of Indian medicinal plant formulation and several cases, combined extracts of plants are used as drug of choice rather than individual. Many of these have shown promising effect [3].

Diabet is an herbal drug compound for diabetes mellitus produced by herbal galenicals, Chennai, Tamil Nadu, India. It is a combination of six medicinal plants namely *Curcuma longa, Coscinium fenestratum, Strychnos potatorum, Tamarindus indica, Tribulus terrestris and Phyllanthus reticulatus.* Some of these are known to possess antidiabetic effect and have been used in the indigenous system of medicine to treat diabetes mellitus [4-7]. The present investigations was undertaken to study the effects of Diabet on liver and kidney LPO, SOD, CAT, GPx and GSH in alloxan-diabetic rats.

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MATERIALS AND METHODS

Animals

Wistar rats of either sex (150-180 g body weight) were used for this study. The animals were kept under a standard condition maintained at 23°C-25°C and given a standard pellet diet (Hindustan lever, Bangalore, India). The experimental protocol was approved by Animal Ethics Committee of Sri Ramachandra Medical College and Research Institute, Porur, Chennai.

Test drug and chemicals

An ayurvedic proprietary formulation, Diabet capsules were obtained from M/S Herbal galenicals, Chennai. 500mg of the powder was weighed and dissolved in 5% CMC and used for animal studies. Alloxan monohydrate was purchased from Sigma Aldrich Chemicals, U.S.A. All other biochemical and chemicals used for the experiments were of analytical grade obtained from Sd Fine Chemicals Mumbai, India.

Experimental design

The rats (n=24) were divided into four groups of six animals each. The rats (n=18) were injected with alloxan monohydrate dissolved in physiological saline at a dose of 120 mg/kg body weight

and it is randomly distributed in to Groups 2,3 & 4. The level of blood glucose of 200-250 mg/dl was taken as diabetic in this study. The Group 1(n=6) rats serve as the control ant it received 1 ml of 5% CMC. The Group 2 (n=6) rats serve as the diabetic control. The Group 3 diabetic rats administered with Diabet (500 mg/kg body weight) daily for 30 days (n=6). The Group 4 Control rats administered with Diabet (500 mg/kg body weight) daily for 30 days (n=6).

Preparation of homogenate

After the experimental regimen, the animals were sacrificed under mild chloroform anesthesia. Liver and kidney were excised, immediately washed with cold saline and 10% homogenate of the liver and kidney tissues were prepared with 0.1M Tris-HCl buffer (pH 7.4). The homogenate was used for assaying the enzyme activities.

Biochemical parameters

Estimation of lipid peroxidation products (Malondialdehyde):

The lipid peroxidation (LPO) products present in the tissue sample were estimated by the thiobarbuturic acid (TBA) method [8].Which measured malondialdehyde (MDA) reactive product at 548nm.

Estimation if superoxide dismutase (SOD):

SOD activity was measured by correlation between total antioxidant status and lipid peroxidation. Maximum auto oxidation occurred at pH 7.4 and that has been used as the basis for the assay of this enzyme [9].

Estimation of catalase (CAT):

Catalase activity was measured according to the method described by Sinha [10].

Estimation of reduced glutathione (GSH):

The protein free filtrate obtained after precipitation with meta-phosphoric acid is made to react with 5-5' dithiobis (2- nitrobenzoic acid) (CDNB). The CDNB and sulphydryl groups form a relatively stable yellow colour whose absorbance is measured at 420nm against blank [11].

Estimation of glutathione peroxidases (GPx):

Glutathione peroxidases play a major role in the scavenging the hydroxyl radical produced by free radicals and its activity was measured by the termination reaction [12] using trichloroacetic acid (TCA).

Statistical analysis

Statistical analysis was carried out using GraphPad PRISM software (version 4.03). One way ANOVA was used, followed by Dunnet's multiple comparison tests (2005). The data represent mean ± SEM. The minimum level of significance was set at $p < 0.05$. All assays were conducted in triplicate and statistical analysis was done.

RESULTS

Table 1 and 2 shows the effect of Diabet on LPO levels in kidney and liver of control and experimental animals. The kidney lipid peroxide levels were high in the case of diabetic control animals (0.289 ± 0.02) which were significantly lowered to 0.171 ± 0.02 by the administration of Diabet. Similarly higher level of liver lipid peroxide in diabetic control animals (0.297 ± 0.02) was significantly reduced to 0.179 ± 0.06 by the administration of Diabet.

Table 1: Effect of Diabet on the levels of lipid peroxides in kidney of control and experimental rats

Groups	Basal	FeSO4	Ascorbate
Control	0.059 ± 0.02	0.181 ± 0.02	0.156 ± 0.08
Diabetic	$0.145 \pm 0.16^{a*}$	$0.299 \pm 0.06^{a*}$	$0.289 \pm 0.02^{a**}$
Diabetic + Diabet	$0.085 \pm 0.04^{b*}$	$0.201 \pm 0.02^{b*}$	$0.171 \pm 0.02^{b**}$
Control + Diabet	$0.055 \pm 0.04^{c\ ns}$	0.179 ± 0.04^{cns}	$0.159 \pm 0.06^{c\ ns}$

Each value represents mean ± SEM, n = 6.
a Group I vs group II. b Group II vs groups III .c Group IV vs Group I
** P < 0.01, ** P < 0.05, ns non-significant (one-way ANOVA followed by Dunnet's t-test). Units: LPO = n moles of MDA formed/min/mg protein*

Table 2: Effect of Diabet on the levels of lipid peroxides in liver of control and experimental rats

Groups	Basal	FeSO4	Ascorbate
Control (Group I)	0.071 ± 0.02	0.224 ± 0.02	0.161 ± 0.02
Diabetic (Group II)	$0.194 \pm 0.06^{a*}$	$0.315 \pm 0.02^{a*}$	$0.297 \pm 0.02^{a**}$
Diabetic + Diabet (GroupIII)	$0.092 \pm 0.02^{b*}$	$0.251 \pm 0.02^{b*}$	$0.179 \pm 0.06^{b**}$
Control + Diabet (Group IV)	0.066 ± 0.01^{cns}	0.214 ± 0.02^{cns}	0.153 ± 0.06^{cns}

Each value represents mean ± SEM, n = 6.
a Group I vs group II. b Group II vs groups III .c Group IV vs Group I
** P < 0.01, ** P < 0.05, ns non-significant (one-way ANOVA followed by Dunnet's t-test). Units: LPO = n moles of MDA formed/min/mg protein*

Table 3 represents the effect of Diabet on tissue SOD and CAT activity of normal and experimental group. The low level of kidney SOD in diabetic control animals (0.443± 0.02) was found to be elevated on Diabet treatment (0.758 ± 0.02). Similarly lower level of liver SOD in diabetic control animals (0.921±0.06) was also found to be increased (1.223 ± 0.06) on Diabet treatment. Kidney Catalase level was found to be elevated from 0.644±0.02 to 0.819 ± 0.02 in Diabet treated diabetic rats. Similarly decreased Catalase levels in liver (0.514± 0.04) during alloxan induced diabetes were found to be significantly increased (0.828 ± 0.04) by diabet.

Table 3: Effect of Diabet on the activities of SOD and CAT in kidney and liver of control and experimental rats

Groups	Kidney SOD	Kidney catalase	Liver SOD	Liver catalase
Control (Group I)	0.767 ± 0.04	0.969 ± 0.06	1.377 ±0.01	0.925 ± 0.02
Diabetic (Group II)	0.443± 0.02^{a*}	0.644±0.02^{a*}	0.921±0.06^{a*}	0.514± 0.04^{a*}
Diabetic+Diabet (GroupIII)	0.758 ± 0.02^{b*}	0.819 ± 0.02^{b*}	1.223 ± 0.06^{b*}	0.828 ± 0.04^{b*}
Control+Diabet (Group IV)	0.760 ± 0.04^{cns}	0.910 ± 0.04^{cns}	1.301 ± 0.08^{cns}	0.913 ± 0.06^{cns}

Each value represents mean ± SEM, n = 6.
a Group I vs group II. b Group II vs groups III .c Group IV vs Group I
** P < 0.01, ** P < 0.05, ^{ns} non-significant (one-way ANOVA followed by Dunnet's t-test). Units: SOD = 50% inhibition of nitrate/min/mg protein*
CAT = µ moles of H_2O_2 decomposed/min/mg protein

Table 4 shows the Effect of Diabet on glutathione and GPx levels in the tissues of normal and treated animals is shown in table 4.Diabet treated diabetic rats showed a significant increase in kidney and liver GSH levels from 0.357 ± 0.02 to 0.498 ± 0.05 and 0.461 ± 0.04 to 0.690 ± 0.05 respectively similarly Diabet treated diabetic animals showed a significant increase in kidney and liver GPx levels from 0.364 ± 0.02 to 0.467 ± 0.04 and 0.576 ± 0.04 to 0.849 ± 0.08 respectively.

Table 4: Effect of Diabet on the levels of GSH and GPx in kidney and liver of control and experimental rats

Groups	Kidney GSH	Liver GSH	Kidney GPx	Liver GPx
Control (Group I)	0.547 ± 0.02	0.759 ± 0.09	0.585 ± 0.02	0.926 ± 0.06
Diabetic (Group II)	0.357 ± 0.02^{a*}	0.461 ± 0.04^{a**}	0.364 ± 0.02^{a*}	0.576 ± 0.04^{a**}
Diabetic + Diabet (GroupIII)	0.498 ± 0.05^{b*}	0.690 ± 0.05^{b**}	0.467 ± 0.04^{b*}	0.849 ± 0.08^{b**}
Control + Diabet (Group IV)	0.530 ± 0.05^{cns}	0.748 ± 0.03^{cns}	0.525± 0.02^{cns}	0.950 ± 0.02^{cns}

Each value represents mean ± SEM, n = 6.
a Group I vs group II. b Group II vs groups III .c Group IV vs Group I
** P < 0.01, ** P < 0.05, ^{ns} non-significant (one-way ANOVA followed by Dunnet's t-test). Units: GSH = µg of GSH consumed/min/mg protein.*
GPx = µg of GSH utilized/min/mg protein.

There is no significant difference in all enzyme activities between the normal (Group I) and drug treated control animals (Group IV). This indicates that the drug does not have any deleterious effect on the normal rats.

DISCUSSION

Lipid peroxidation is one of the characteristic features of chronic diabetes. Oxidative damage induced by alloxan resulted in the formation of highly reactive hydroxyl radical, which stimulates the LPO that causes destruction and damage to the cell membrane. Treatment with the herbal formulation reduced the level of lipid peroxides indicating the effective antioxidant property of the herbal drug in the moderation of tissue damage (Table 1 and Table 2). This decrease could be attributed to the increase in GPx in rats treated with the herbal formulation since GPx has been shown to inactivate lipid peroxidation [13].

SOD is an important defense enzyme which catalyses the dismutation of superoxide radicals [14]. CAT is a heme protein which catalyses the reduction of hydrogen peroxides and protects the tissues from hydroxyl radicals [15]. Therefore reduction in the activity of these enzymes (SOD, CAT) may result in a number of deleterious effects due to the accumulation of superoxide anion and hydrogen peroxide [16]. The decrease in SOD activity could result from inactivation by hydrogen peroxide or glycation of the enzyme, which is known to occur during diabetes [17]. Administration of herbal formulation increased the activities of SOD and CAT in diabetic rats (Table 3).

Glutathione is an important biomolecule against chemically induced toxicity and can participate in the elimination of reactive intermediates by reduction of hydroperoxides in the presence of GPx. GSH also functions as free radical scavenger and in the repair of radical caused biological damage [18]. It also inhibits free radical mediated lipid peroxidation [19]. Decreased glutathione levels in diabetes have been considered to be an indicator of increased oxidative stress [20]. Lowered levels of GSH may also be due to the utilization of GSH by the GPx and GST as their substrate. Diabet administration resulted in significant elevation of GSH in the experimental rats (Table 4).

GPx plays a pivotal role in H_2O_2 catabolism and in the detoxification of endogenous metabolic peroxides and hydroperoxides which catalyses GSH [21]. Decreased activity of GPx may result from radical induced inactivation and glycation of the enzymes [22]. In diabetic rats treated with the formulation, significant increase in GPx was observed (Table 4). This might reflect the antioxidant potency of Diabet, which by reducing glucose levels, prevented glycation and inactivation of GPx.

The over expression of these antioxidant enzymes in diabetic rats treated with Diabet implies that this potential oxidant defense is reactivated by the active principles of Diabet with a resulting increase in the capacity of detoxification through enhanced scavenging of oxy radicals.

In conclusion, the ethanolic extract of Diabet was shown to possess

antioxidant activity by increasing the levels of SOD, CAT, GPx and GSH activities and by decreasing the levels of LPO. Further studies will be needed to purify the bioactive compound(s) in the ethanolic extract, and use the purified compound(s) for bioassay directed experiments.

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