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Phytochemical Investigation and Antioxidant Activity of a Polyherbal Formulation (ESF/AY/500) on Streptozotocin Induced Oxidative Stress in Rats

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ABSTRACT

ESF/AY/500, a polyherbal formulation intended to use for diabetic patients has been screened for antioxidant activity. For antioxidant studies, ESF/AY/500 was administered orally for 21 days at a dose of 500 mg/kg body weight to Streptozotocin induced diabetic male Wistar rats. All the animals were sacrificed on the 22nd 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 (GP_x), and reduced glutathione (GSH) and decreased level of lipid peroxidation. These results showed that treatment with ESF/AY/500 lowers Streptozotocin induced LPO and alters SOD, CAT, GP_x and GSH enzymes to reduce oxidative stress.

Keywords: Anti diabetic; polyherbal; *Eruca sativa*; streptatozocin; Antioxidant.

INTRODUCTION

Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fats and proteins. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin. Several drugs such as biguanides and sulfonylureas are currently available to reduce hyperglycemia in diabetes mellitus [1]. These drugs have side effect and thus search for new drug/compound is essential to overcome the diabetic problems [2].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 [3]. Herbal medicines are frequently considered to be less toxic and more free from side

effects than synthetic ones [4]. 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 [5].ESF/AY/500 is a polyherbal formulation composed of eight medicinal plants, It is a combination of six medicinal plants namely *Aerva lanata, Aegle marmelos, Ficus benghalensis, Catharanthus roseus, Bambusa arundinaceae, Salacia reticulata and Szygium cumini* with that of '*Eruca sativa'* Some of these are known to possess anti diabetic effect and have been used in the indigenous system of medicine to treat diabetes mellitus [6-9].the present investigations was undertaken to study the effects of ESF/AY/500 on liver and kidney LPO, SOD, CAT, GP_x and GSH in Streptozotocin diabetic rats.

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^{o}C-25^{o}C and given a standard pellet diet (Hindustan lever, Bangalore, India). The experimental protocol was approved by Animal Ethics Committee [IAEC, Registration No. 160/1999/CPCSEA] of Rajah Muthiah Medical College, Annamalai University, Chidambaram**,** Tamil Nadu, and South India

Test drug and chemicals

An ayurvedic proprietary formulation, ESF/AY/500 capsules 500mg of the powder was weighed and dissolved in 5% CMC and used for animal studies. Streptozotocin 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 Streptozotocin dissolved in physiological saline at a dose of 120 mg/kg body weight and it is randomly distributed in to Groups II, III & IV. The level of blood glucose of 200-250 mg/dl was taken as diabetic in this study. The Group I (n=6) rats serve as the control ant it received 1 ml of 5% CMC. The Group II (n=6) rats serve as the diabetic control. The Group III diabetic rats administered with ESF/AY/500 (500 mg/kg body weight) daily for 21 days (n=6). The Group IV Control rats administered with ESF/AY/500 (500 mg/kg body weight) daily for 21 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 [10].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 [11].

Estimation of catalase (CAT):
Catalase activity was measured according to the method described by Sinha [12].

Estimation of reduced glutathione (GSH):
The protein free filtrate obtained after precipitation with metaphosphoric 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 [13].

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 using trichloroacetic acid [14] (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.

RESULT

Table 1 and 2 shows the effect of ESF/AY/500 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 (0.274 ± 0.02) which were significantly lowered to 0.161 ± 0.02 by the administration of ESF/AY/500. Similarly higher level of liver lipid peroxide in diabetic control animals (0.287 ± 0.02) was significantly reduced to 0.169 ± 0.06 by the administration of ESF/AY/500.

Table 1: Effect of ESF/AY/500 on the levels of lipid peroxides in kidney of control and experimental rats

Groups	Basal	$FeSO_4$	Ascorbate
Diabetic control	0.069 ± 0.02	0.191 ± 0.02	0.166 ± 0.08
Non diabetic control	0.135 ± 0.16^{a*}	0.289 ± 0.06^{a*}	0.274 ± 0.02^{a**}
Diabetic + ESF/AY/500	0.075 ± 0.04^{b*}	0.206 ± 0.02^{b*}	0.161 ± 0.02^{b**}
Non diabetic +ESF/AY/500	0.058 ± 0.04$^{c\ ns}$	0.189 ± 0.04^{cns}	0.142 ± 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 ESF/AY/500 on the levels of lipid peroxides in liver of control and experimental rats

Groups	Basal	$FeSO_4$	Ascorbate
Diabetic control Groups I	0.061 ± 0.02	0.214 ± 0.02	0.151 ± 0.02
Non diabetic control Groups II	$0.184 \pm 0.06^{a*}$	$0.305 \pm 0.02^{a*}$	$0.287 \pm 0.02^{a**}$
Diabetic + ESF/AY/500Groups III	$0.094 \pm 0.02^{b*}$	$0.241 \pm 0.02^{b*}$	$0.169 \pm 0.06^{b**}$
Non diabetic + ESF/AY/500Groups IV	0.066 ± 0.01^{cns}	0.216 ± 0.02^{cns}	0.143 ± 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 ESF/AY/500 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 ESF/AY/500 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 ESF/AY/500 treatment. Kidney Catalase level was found to be elevated from 0.644±0.02 to 0.819 ± 0.02 in ESF/AY/500 treated diabetic rats. Similarly decreased Catalase levels in liver (0.514± 0.04) during Streptozotocin induced diabetes were found to be significantly increased (0.828 ± 0.04) by ESF/AY/500.

Table 3: Effect of ESF/AY/500 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
Diabetic control Groups I	0.767 ± 0.04	0.969 ± 0.06	1.377 ±0.01	0.925 ± 0.02
Non diabetic control Groups II	$0.443 \pm 0.02^{a*}$	$0.644 \pm 0.02^{a*}$	$0.921 \pm 0.06^{a*}$	$0.514 \pm 0.04^{a*}$
Diabetic + ESF/AY/500 Groups III	$0.758 \pm 0.02b^{*}$	$0.819 \pm 0.02b^{*}$	$1.223 \pm 0.06b^{*}$	$0.828 \pm 0.04^{b*}$
Non diabetic +ESF/AY/500 Groups 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 H2O2 decomposed/min/mg protein

Table 4 shows the Effect of ESF/AY/500 on glutathione and GP_x levels in the tissues of normal and treated animals. ESF/AY/500 treated diabetic rats showed a significant increase in kidney and liver GSH levels from 0.347 ± 0.02 to 0.488 ± 0.05 and 0.451 ± 0.04 to 0.670 ± 0.05 respectively similarly ESF/AY/500 treated diabetic animals showed a significant increase in kidney and liver GP_x levels from 0.354 ± 0.02 to 0.467 ± 0.04 and 0.586 ± 0.04 to 0.859 ± 0.08 respectively.

Table 4

Groups	Kidney GSH	Kidney GPx	Liver GSH	Liver GP_x
Diabetic control Groups I	0.537 ± 0.02	0.769 ± 0.09	0.575 ± 0.02	0.936 ± 0.06
Non diabetic control Groups II	$0.347 \pm 0.02^{a*}$	$0.451 \pm 0.04^{a**}$	$0.354 \pm 0.02^{a*}$	$0.586 \pm 0.04^{a**}$
Diabetic + ESF/AY/500 Groups III	$0.488 \pm 0.05^{b*}$	$0.670 \pm 0.05^{b**}$	$0.467 \pm 0.04^{b*}$	$0.859 \pm 0.08^{b**}$
Non diabetic +ESF/AY/500 Groups IV	0.520 ± 0.05^{cns}	0.758 ± 0.03^{cns}	0.535 ± 0.02^{cns}	0.960 ± 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.
GP_x = 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 Streptozotocin 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 GP_x in rats treated with the herbal formulation since GP_x has been shown to inactivate lipid peroxidation [15].

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 [16]. 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 [17]. The decrease in SOD activity could result from inactivation by hydrogen peroxide or glycation of the enzyme, which is known to occur during diabetes [18]. 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 GP_x. GSH also functions as free radical scavenger and in the repair of radical caused biological damage [19]. It also inhibits free radical mediated lipid peroxidation [20]. Decreased glutathione levels in diabetes have been considered to be an indicator of increased oxidative stress [21]. Lowered levels of GSH may also be due to the utilization of GSH by the GP_x and GST as their substrate. ESF/AY/500 administration resulted in significant elevation of GSH in the experimental rats Table 4.

GP_x plays a pivotal role in H_2O_2 catabolism and in the detoxification of endogenous metabolic peroxides and hydroperoxides which catalyses GSH [20]. Decreased activity of GP_x may result from radical induced inactivation and glycation of the enzymes [23]. In diabetic rats treated with the formulation, significant increase in GP_x was observed Table 4. This might reflect the antioxidant potency of ESF/AY/500, which by reducing glucose levels, prevented glycation and inactivation of GP_x.

The over expression of these antioxidant enzymes in diabetic rats treated with ESF/AY/500 implies that this potential oxidant defense is reactivated by the active principles of ESF/AY/500 with a resulting increase in the capacity of detoxification through enhanced scavenging of oxy radicals.

In conclusion, the ethanolic extract of ESF/AY/500 was shown to possess antioxidant activity by increasing the levels of SOD, CAT, GP_x and GSH activities and by decreasing the levels of LPO. Further studies will be needed to purify the bioactive compounds in the ethanolic extract, and use the purified compounds for bioassay directed experiments.

REFERENCES

[1] Mutalik S, Sulochana B, Chetana M, Udupa N, Devi VP. *Indian J Exp Biol*., **2003**, 41,316-320.
[2] Noor A, Gunasekaran S, Manickam AS, Vijayalakshmi MA.*Curr. Sci*., **2008**, 94, 1070-1076.
[3] John WB. Diabetes., **1991**, 40,405.
[4] Pari L, Umamaheswari J. *Phytother Res*., **2000**, 14:1-3.
[5] Santhosh Kumari KS, Devi KS. *Ind J Exp Biol*., **1993**, 31,595- 599.
[6] Tank R, Sharma N and Dixit VP. *Indian Drugs*., **1990**, 27, 11, 587-589.
[7] Annie Shirwaikar,K. Rajendran and Punitha ISR. *J. Ethnopharmacol*., **2005**, 97, 2,369-374.
[8] Mandal TK, Biswas TK and Tripathi PC. *Aryavaidyan*., **2002**, 15,178- 186.
[9] Maitia R, Janaa D, Dasa UK and Ghosh D. *J. Ethnopharmacol*., **2004**, 92, 1, 85-91.
[10] Uchiyama M, Mahara M. *Anal Biochem*, **1978**, 86,271-278
[11] Das S, Vasisht S, Snehlata R, Das N, Srivastava LM. *Curr Sci*, **2000**, 78,486-487
[12] Sinha AK. *Anal Biochem*., **1972**, 47,389
[13] Moron MS, Defierre JW, Mannervik B. *Biochem Biophys Acta*, **1979**, 582,67-68
[14] Rotruck JT, Pope AL, Ganther HL, Swanson AB, *Science*, **1984**, 179,588.
[15] Levy U, Zaltzber H, Ben Amotz A, Kanter Y, Aviram M, ß*Pathophysiology*, **1999**, 6,157- 161
[16] Chance B, Grenstein DS, Roughton RJW, *Arch Biochem Biophy*., **1952**, 301-339
[17] Searle AJ, Wilson RL, *Int J Radiant. Biol*.,**1980**, 37,213-217
[18] Sozmen EY, Sozmen B, Delen Y, Onat T.. *Arch Med Res*., **2001**, 32,283-287
[19] Meister A. *Nutr Rev* 1984; 42:397- 410
[20] Meister A, Anderson ME. Glutathione, *Annual Rev Biochem*, **1983**, 52,711- 760
[21] Wolff SP, *Br Med Bull*, **1993**, 49,642- 652
[22] Eaton JW, *J Lab Clin Med*, **1991**, 118,3- 4
[23] Hodgson EK, Fridovich I, *Biochemistry*, **1975**, 14,5294-5299