

Antioxidant Activity of Polyherbal Formulation on Streptozotocin Induced Diabetes in Experimental Animals

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ABSTRACT

ESF/AY/250 and ESF/AY/500 polyherbal formulation intended to use for diabetic patients has been screened for antioxidant activity. For antioxidant studies, both were administered orally for 45 days at a dose of 250mg and 500mg/kg body weight to Streptozotocin induced diabetic male Wistar rats. All the animals were sacrificed on the 45th day and the levels of LPO, SOD, CAT, GP_x & GSH in kidney and liver of control and experimental rats were studied. The poly herbal 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/250 and ESF/AY/500 lowers Streptozotocin induced Lipid per oxidation and alters SOD, CAT, GP_x and GSH enzymes to reduce oxidative stress.

Keywords: Polyherbal; Streptozotocin; 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 effects and thus the search for new drugs/compounds is essential to overcome the diabetic problems [2]. Diabetes mellitus is one of the most common endocrine metabolic disorder. 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/250 and ESF/AY/500 is a polyherbal formulation composed eight medicinal plants namely *Aerva lanata*, *Aegle marmelos*, *Ficus benghalensis*, *Catharanthus roseus*, *Bambusa arundinaceae*, *Salacia reticulata*, *Syzygium cumini* and *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/250 and ESF/AY/500 on liver and kidney LPO, SOD, CAT, GP_x and GSH in Streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Animals

Wistar male rats (100- 200 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 [IAEC, Registration No. 160/1999/CPCSEA] of Rajah Muthiah Medical College, Annamalai University, Chidambaram, Tamil Nadu, South India.

Test drug and chemicals

An ayurvedic proprietary poly herbal formulation, ESF/AY/250 and ESF/AY/500 capsules. 250mg and 500mg of the poly herbal formulation powder was weighed and dissolved in 0.5% 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.

Preparation of the polyherbal formulation ESF/AY/250 and ESF/AY/500

The selected parts of respective plants were subjected for Soxhlation. Ethanol (90% v/v) was used as menstrum. The individual extracts were lyophilized. The poly herbal formulations (ESF/AY/250 and ESF/AY/500) of all the eight lyophilized extracts were prepared in fixed ratios as per [Table 1]

Table - 1 Composition of ingredient(s) present in polyherbal formulation

Botanical name	Common name	Part used	Ingredients of polyherbal formulation (stock sample) *(mg)	
			ESF/AY 250mg	ESF/AY500mg
Aegle marmelos	Kuvalam	Leaves	25	50
Bambusa arundinaceae	Bamboo	Leaves	50	100
Eruca sativa	Yellow julienne	Leaves	50	100
Aerva lanata	Astmabayata	Aerial	25	50
Catharanthus roseus	Vinca	Aerial	25	50
Ficus benghalensis	Peraal	Bark	25	50
Salacia reticulata	Saptarangi	Bark	25	50
Syzygium cumini	Njaval	Bark	25	50

* This stock sample was used at the dose mentioned in the experiment

Experimental design

The rats (n=30) were divided into five groups of six animals each. The rats (n=24) were injected with Streptozotocin dissolved in physiological saline at a dose of 120 mg/kg body weight and it was randomly distributed into Groups II, III, IV & V.

Group I: served as control and it received 0.5% CMC.

Group II: served as the toxic control and it received Streptozotocin (60 mg/kg).

Group III: Streptozotocin (60 mg/kg) induced diabetic rats and treated with Glibenclamide

Group IV: Streptozotocin (60 mg/kg) induced diabetic rats and treated with ESF/AY/250 (250 mg/kg body weight).

Group V: Streptozotocin (60 mg/kg) induced diabetic rats and treated with ESF/AY/500 (500 mg/kg body weight).

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. The tissue were weighed and 10% tissue homogenate was prepared with 0.025M Tris-HCl buffer, P^H 7.5. After centrifugation at 10,000 ×g for 10 min, the resulting supernatant was used for enzyme assays for the estimation of non-enzymatic and enzymatic antioxidants.

Estimation of lipid peroxidation of rat liver and kidneys

Lipid peroxidation in liver and kidney was estimated calorimetrically by measuring thiobarbituric acid reactive substances (TBARS) using the method of Fraga *et al.* [1988], 0.1 ml of tissue homogenate was treated with 2 ml of TBA-trichloroacetic acid-HCl reagent (0.37% TBA, 0.25 M HCl and 15% TCA, 1:1:1 ratio), placed for 15 min in a water bath and then cooled and centrifuged at 3500 ×g for 10 min at room temperature, the absorbance of clear supernatant was measured at 535 nm against a reference blank. Values were expressed as Mm/100 g tissue [10].

Estimation of Superoxide Dismutase (SOD)

The activity of superoxide dismutase (SOD) was assayed by the method of Kakkar, *et al.*, [1984] based on the oxidation of epinephrine adrenochrome transition by enzyme. The post-mitochondrial rat suspension of rat liver 0.5ml was diluted with distilled water 0.5ml. To this chilled ethanol 0.25ml and chloroform 0.15ml was added. The mixture was shaken for 1 min and centrifuged at 2000 ×g for 10 min. The PMS 0.5ml was added with 1.5ml PBS buffer (P^H7.2). The reaction initiated by the addition of 0.4ml epinephrine and change in optical density OD (min⁻¹) was measured at 470 nm. SOD activity was expressed as U/mg of protein. Change in OD (min⁻¹) at 50% inhibition to adrenochrome transition by the enzyme was taken as one enzyme unit [11].

Estimation of Catalase (CAT)

Catalase (CAT) was estimated by the method of Sinha *et al.*, [1972]. The reaction mixture 1.5 ml vol contained 1.0 ml of 0.01 M phosphate buffer (P^H7.0) 0.1 ml of tissue homogenate and 0.4 ml of 2 M H₂O₂. The reaction was stopped by the addition of 2.0 ml dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). Then the absorbance was measured at 530 nm; CAT activity was expressed as μM of H₂O₂ consumed/min/mg protein [12].

Estimation of Glutathione peroxidase (GPx)

Glutathione peroxidase (GPx) was measured by the method described by Rotruck *et al.* [1973] Briefly, the reaction mixture contained 0.2 ml 0.4 M phosphate buffer (P^H7.0), 0.1ml 10 mM

sodium azide, 0.2 ml tissue homogenized in 0.4M phosphate buffer $P^H 7.0$. 0.2 ml tissue homogenized in 0.4 M, phosphate buffer, $P^H 7.0$, 0.2 ml reduced glutathione, 0.1 ml 0.2 mM hydrogen peroxide. The contents were incubated for 10 min at $37^\circ C$, 0.4 ml 10% TCA was added to stop the reaction and centrifuged at $3200 \times g$ for 20 min. The supernatant was assayed for glutathione content using Ellman's reagent (19.8 mg 5,5'-dithiobisnitrobenzoic acid [DTNB] in 100 ml 0.1% sodium nitrate). The activities were expressed as μg of GSH consumed/ min/mg protein [13].

Estimation of reduced glutathione(GSH)

Reduced glutathione (GSH) was measured by the method of Ellman et al., [1959]. The PMS of rat liver (720 μl) and 5% TCA were mixed to precipitate the protein content of the supernatant. After centrifugation at $10,000 \times g$ for 5 min, the supernatant was taken. DTNB (5,5'-dithio-bis(2-nitrobenzoic acid) Ellman's reagent was added to it and the absorbance was measured at 412 nm. A standard graph was drawn using different concentration of standard GSH solution. GSH contents were calculated in the PMS of rat liver [14].

Statistical analysis

Statistical analysis was carried out using Graph Pad PRISM software (version 4.03). One way ANOVA was used, followed by Dunnett's t-tests (2005). The data represent mean \pm SEM. The minimum level of significance was set at $p < 0.05$. All assays were conducted in triplicate and statistical analysis was done.

Table -2 Effect of ESF/AY/250 and ESF/AY/500 on the activities of LPO, CAT, GSH, GP_x, and SOD in kidney of diabetic rats

Groups	Kidney				
	LPO	CAT	GSH	GP _x	SOD
Control	0.232 \pm 0.01	0.969 \pm 0.06	0.537 \pm 0.02	0.769 \pm 0.09	0.767 \pm 0.04
Toxic Control	0.455 \pm 0.02	0.644 \pm 0.02	0.347 \pm 0.02*	0.451 \pm 0.04	0.443 \pm 0.02
Standard (Gilbenclimide)	0.241 \pm 0.02*	0.916 \pm 0.04	0.520 \pm 0.05**	0.758 \pm 0.03	0.760 \pm 0.04
ESF/AY/250	0.298 \pm 0.01	0.724 \pm 0.05**	0.424 \pm 0.01	0.568 \pm 0.02*	0.535 \pm 0.01
ESF/AY/500	0.256 \pm 0.02	0.819 \pm 0.02**	0.488 \pm 0.05	0.670 \pm 0.05***	0.758 \pm 0.02

Each value represents mean \pm SEM, $n = 6$. P value $< 0.001 = ***$, P value $< 0.01 = **$, P value $< 0.05 = *$ Units: SOD = expressed as U/mg of protein. GSH = μg of GSH consumed/min/mg protein. GP_x = μg of GSH utilized/min/mg protein. CAT = expressed as μM of H_2O_2 consumed/min/mg protein.

Table -3 Effect of ESF/AY/250 and ESF/AY/500 on the activities of LPO, CAT, GSH, GP_x, and SOD in liver of diabetic rats.

Groups	Liver				
	LPO	CAT	GSH	GP _x	SOD
Control	0.266 \pm 0.02	0.925 \pm 0.02	0.575 \pm 0.02	0.936 \pm 0.06	1.377 \pm 0.01
Toxic Control	0.568 \pm 0.01	0.514 \pm 0.04	0.354 \pm 0.02	0.586 \pm 0.04*	0.921 \pm 0.06
Standard Gilbenclimide	0.252 \pm 0.01	0.913 \pm 0.06**	0.535 \pm 0.02	0.960 \pm 0.02**	1.301 \pm 0.08
ESF/AY/250	0.304 \pm 0.02*	0.747 \pm 0.04	0.418 \pm 0.03**	0.627 \pm 0.01	1.172 \pm 0.02
ESF/AY/500	0.296 \pm 0.10	0.828 \pm 0.04**	0.467 \pm 0.04	0.859 \pm 0.08	1.223 \pm 0.06*

Each value represents mean \pm SEM, $n = 6$. P value $< 0.001 = ***$, P value $< 0.01 = **$, P value $< 0.05 = *$ Units: SOD = expressed as U/mg of protein. GSH = μg of GSH consumed/min/mg protein. GP_x = μg of GSH utilized/min/mg protein. CAT = expressed as μM of H_2O_2 consumed/min/mg protein.

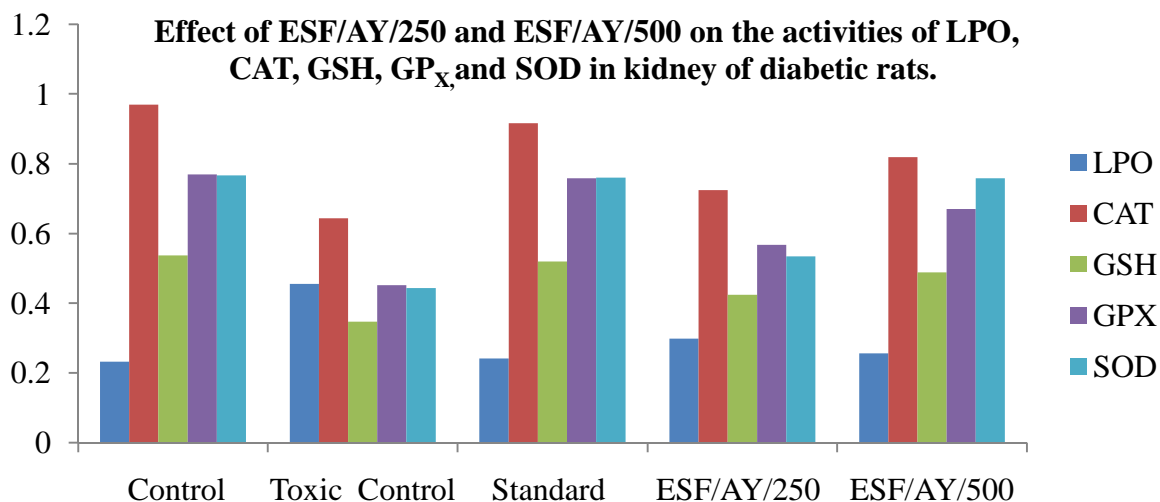


Fig.1

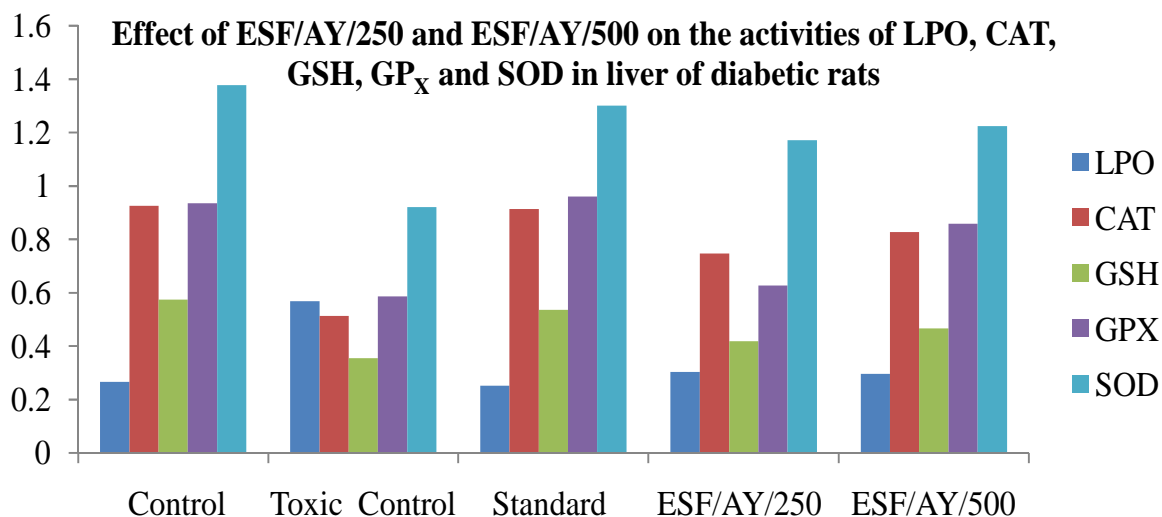


Fig.2

RESULT

Table 2 and 3 shows the effect of ESF/AY/250 and ESF/AY/500 on LPO levels in kidney and liver of diabetic rats compared with control. The kidney lipid peroxide levels were high in the case of diabetic control animals 0.455 ± 0.02 which were significantly lowered to 0.298 ± 0.01 and 0.256 ± 0.02 by the administration of ESF/AY/250 and ESF/AY/500. Similarly higher level of liver lipid peroxide in diabetic control animals 0.568 ± 0.01 was significantly reduced to 0.304 ± 0.02 and 0.296 ± 0.10 by the administration of ESF/AY/250 and ESF/AY/500.

Table 2 and 3 represents the effect of ESF/AY/250 and ESF/AY/500 on tissue SOD and CAT activity of diabetic rats compared with control. The low level of kidney SOD in diabetic control (toxic control) animals (0.443 ± 0.02) was found to be elevated on ESF/AY/250 and ESF/AY/500 treatment 0.535 ± 0.01 and 0.758 ± 0.02 . Similarly lower level of liver SOD in diabetic control animals 0.921 ± 0.06 was also found to be increased 1.172 ± 0.02 and 1.223 ± 0.06 on ESF/AY/250 and ESF/AY/500 treatment. Kidney Catalase level was found to be elevated from 0.644 ± 0.02 to 0.724 ± 0.05 and 0.819 ± 0.02 on ESF/AY/250 and

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 0.747 ± 0.04 and 0.828 ± 0.04 by ESF/AY/250 and ESF/AY/500.

Table 2 and 3 represents the effect of ESF/AY/250 and ESF/AY/500 on GP_x and GSH levels in the tissues of diabetic rats compared with control. ESF/AY/250 and ESF/AY/500 treated diabetic rats showed a significant increase in kidney and liver GP_x levels from 0.451 ± 0.04 to 0.568 ± 0.02 and 0.670 ± 0.05 and 0.586 ± 0.04 to 0.627 ± 0.01 and 0.859 ± 0.08 respectively. similarly ESF/AY/250 and ESF/AY/500 treated diabetic animals showed a significant increase in kidney and liver GSH levels from 0.347 ± 0.02 to 0.424 ± 0.01 and 0.488 ± 0.05 and 0.354 ± 0.02 to 0.418 ± 0.03 and 0.467 ± 0.04 respectively.

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 (fig 1 and fig 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 [16]. CAT is a heme protein which catalyses the reduction of hydrogen peroxides and protects the tissues from hydroxyl radicals [17]. 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 [18]. The decrease in SOD activity could result from inactivation by hydrogen peroxide or glycation of the enzyme, which is known to occur during diabetes [19]. Administration of herbal formulation increased the activities of SOD and CAT in diabetic rats.

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 [20]. It also inhibits free radical mediated lipid peroxidation [21]. Decreased glutathione levels in diabetes have been considered to be an indicator of increased oxidative stress [22]. 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.

GP_x plays a pivotal role in H₂O₂ catabolism and in the detoxification of endogenous metabolic peroxides and hydroperoxides which catalyses GSH [23]. Decreased activity of GP_x may result from radical induced inactivation and glycation of the enzymes [24]. In diabetic rats treated with the formulation, significant increase in GP_x was observed. 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 formulation ESF/AY/500 was shown to possess antioxidant activity by increasing the levels of SOD, CAT, GP_x activities, reducing 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.

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