Antioxidant Potential Of Byesukar, A Polyherbal Formulation On Alloxan Induced Oxidative Stress In Rats

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Abstract
Byesukar, a polyherbal formulation intended to use for diabetic patients has been screened for antioxidant activity. For antioxidant studies, byesukar 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, GST and GR in kidney and liver of control and experimental rats were studied. The extracts exhibited significant antioxidant activity showing increased levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione s-transferase (GST) and glutathione reductase (GR) and decreased level of lipid peroxidation. These results showed that treatment with byesukar lowers alloxan induced LPO and alters SOD, CAT, GPx, GST and GR enzymes to reduce oxidative stress.

Keywords: Byesukar; 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 medicine 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].

Byesukar is a herbal drug compound for diabetes mellitus produced by A.Z. Siddha Research Foundation, Chennai, India. It is a combination of three medicinal plants namely Cassia auriculata (bark), Eugenia jambolana (seeds) and Thespesia populnea (leaf). Some of these are known to possess antidiabetic effect and have been used in the indigenous system of medicine to treat diabetes mellitus [4, 5]. The present investigations was undertaken to study the effects of byesukar on liver and kidney SOD, CAT, GSH, GPx, GST and GR in alloxan-diabetic rats.

Materials and Methods
Animals
Twenty four male wistar rats of body weights ranging from 150-200g were obtained from PSG Institute of Medical Sciences, Coimbatore. The animals were fed on a pellet diet (Sai Durga Feeds, Bangalore) and water ad libitum. All animal experiments conducted during the present study got prior permission and followed the guidelines of Institutional Animal Ethics Committee (IAEC).

Test drug and chemicals
Byesukar, a herbal medicinal drug was a gift from A.Z. Siddha Research Foundation, Chennai. 250 g of herbal drug powder were soaked overnight in 750 ml of 95% ethanol. This suspension was filtered and the residue was resuspended in an equal volume of 95% ethanol for 48 hours and filtered again. The two filtrates were pooled and the solvents were evaporated in a rotary evaporator at 40-50°C under reduced pressure. The yield of the extract was around 10-15 g. It was stored at 4°C. This extract was dissolved in distilled water and used in this study.

Alloxan monohydrate was purchased from BDH Chemicals, Poole, England. All other biochemicals and chemicals used for the experiments were of analytical grade.

Induction of diabetes
The rats (n=18) were injected with alloxan monohydrate dissolved in physiological saline at a dose of 120 mg/kg body weight. The level of blood glucose of 200-250 mg/dl was taken as diabetic in this study.

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Experimental design
The rats (n=24) were divided into four groups of six animals each.

Group I: Control rats (n=6).
Group II: Rats administered with alloxan monohydrate (120 mg/kg body weight) by intraperitoneal injection (n=6).
Group III: Diabetic rats administered with Byesukar (500 mg/kg body weight) daily for 30 days (n=6).
Group IV: Control rats administered with Byesukar (500 mg/kg body weight) daily for 30 days (n=6).

After the experimental regimen, the animals were sacrificed under mild chloroform anaesthesia.

Preparation of homogenate
A 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
The following biochemical parameters were analysed. Lipid Peroxidation (LPO) was estimated according to the method of Uchiyama et al. [6]. Superoxide dismutase (SOD) activity of kidney and liver tissue was determined by nitrite method of Das et al. [7]. Catalase (CAT) activity was estimated by the method of Sinha [8] by measuring the rate of decomposition of hydrogen peroxide at 240 nm. Reduced glutathione (GSH) activity in kidney and liver tissue were measured by the method of Moron et al. [9] based on the reaction with 5,5’ dithiobis (2-nitrobenzoic acid). Glutathione peroxidase (GPx) activity of kidney and liver tissue was determined by the method of Rotruck et al. [10] based on the degradation of hydrogen peroxide in the presence of reduced glutathione. Glutathione S-transferase (GST) activity of kidney and liver sample was estimated by the method of Habig et al. [11] based on rate of increase in conjugate formation between reduced glutathione and 1-chloro-2,4-dinitrobenzene. Glutathione reductase (GR) activity was measured by Beutler’s method [12] based on the amount of reduced form of nicotinamide adenine dinucleotide phosphate consumed during the conversion of oxidised glutathione to reduced glutathione.

Statistical analysis
Data was expressed as mean ± standard deviation (SD). The biochemical parameters were analysed using one way Analysis of Variance (ANOVA) and the group means were compared by Duncans Multiple Range Test [13].

Results
Table 1 and 2 shows the effect of byesukar on LPO levels in kidney and liver of control and experimental animals. The kidney lipid peroxide levels were high in the case of control animals (2.79 ± 0.16) which was significantly lowered to 1.61 ± 0.16 by the administration of byesukar. Similarly higher level of liver lipid peroxide in control animals (2.87 ± 0.16) was significantly reduced to 1.69 ± 0.16 by the administration of byesukar.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Basal</th>
<th>FeSO₄</th>
<th>Ascorbate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.49 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.71 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.46 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic</td>
<td>1.25 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.99 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.79 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + Byesukar</td>
<td>0.75 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.92 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.61 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control + Byesukar</td>
<td>0.45 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.61 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.38 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=6). Values not sharing a common letter differ significantly at P<0.05 by DMRT
Units: LPO = mmoles of MDA formed/100 g

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Basal</th>
<th>FeSO₄</th>
<th>Ascorbate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.61 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.14 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.51 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic</td>
<td>1.84 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.05 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.87 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + Byesukar</td>
<td>0.82 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.41 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.69 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control + Byesukar</td>
<td>0.56 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.04 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.43 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=6). Values not sharing a common letter differ significantly at P<0.05 by DMRT
Units: LPO = mmoles of MDA formed/100 g
Table 3 represents the effect of byesukar on tissue SOD and CAT activity of normal and experimental group. The low level of kidney SOD in diabetic control animals (3.43 ± 0.35) was found to be elevated on byesukar treatment (6.66 ± 0.54). Similarly lower level of liver SOD in diabetic control animals (8.21 ± 0.57) was also found to be increased (11.23 ± 0.56) on byesukar treatment. Kidney Catalase level was found to be elevated from 5.44 ± 0.36 to 7.19 ± 0.39 in byesukar treated diabetic rats. Similarly decreased Catalase levels in liver (4.14 ± 0.46) during alloxan induced diabetes was found to be significantly increased (7.28 ± 0.54) by the extract.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Kidney SOD</th>
<th>Kidney catalase</th>
<th>Liver SOD</th>
<th>Liver catalase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.67 ± 0.45c</td>
<td>8.69 ± 0.83c</td>
<td>12.77 ± 1.06c</td>
<td>8.25 ± 0.25c</td>
</tr>
<tr>
<td>Diabetic</td>
<td>3.43 ± 0.35c</td>
<td>5.44 ± 0.36c</td>
<td>8.21 ± 0.57c</td>
<td>4.14 ± 0.46c</td>
</tr>
<tr>
<td>Diabetic + Byesukar</td>
<td>6.66 ± 0.54b</td>
<td>7.19 ± 0.39b</td>
<td>11.23 ± 0.56b</td>
<td>7.28 ± 0.54b</td>
</tr>
<tr>
<td>Control + Byesukar</td>
<td>7.50 ± 0.40b</td>
<td>8.10 ± 0.49b</td>
<td>11.01 ± 0.90b</td>
<td>8.03 ± 0.53b</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=6). Values not sharing a common letter differ significantly at P<0.05 by DMRT.

Units: SOD = 50% inhibition of nitrite/min/mg protein
CAT = nmole of H₂O₂ decomposed/min/mg protein

Tables 4 and 5 represent the effect of byesukar on tissue GST and GR activity of normal and experimental group. The low level of kidney GST in diabetic control animals (16.57 ± 0.47) was found to be elevated on byesukar treatment (21.07 ± 0.51). Similarly lower level of liver GST in diabetic control (19.96 ± 0.78) were found to be increased (30.70 ± 0.57) on byesukar treatment. Diabetic animals treated with byesukar showed a significant increase in kidney and liver GR levels from 8.00 ± 0.53 to 11.00 ± 0.41 and 9.72 ± 0.59 to 13.16 ± 0.82 respectively.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Kidney GSH</th>
<th>Liver GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.47 ± 0.35c</td>
<td>6.59 ± 0.29c</td>
</tr>
<tr>
<td>Diabetic</td>
<td>2.57 ± 0.22d</td>
<td>3.61 ± 0.24d</td>
</tr>
<tr>
<td>Diabetic + Byesukar</td>
<td>3.98 ± 0.25e</td>
<td>5.90 ± 0.45e</td>
</tr>
<tr>
<td>Control + Byesukar</td>
<td>4.30 ± 0.25e</td>
<td>6.48 ± 0.33e</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=6). Values not sharing a common letter differ significantly at P<0.05 by DMRT.

Units: GSH = µg/mg protein

Table 5: Effect of byesukar on the activity of GPx in kidney and liver of control and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Kidney GPx</th>
<th>Liver GPx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.85 ± 0.38c</td>
<td>8.26 ± 0.60c</td>
</tr>
<tr>
<td>Diabetic</td>
<td>2.64 ± 0.24d</td>
<td>4.76 ± 0.34d</td>
</tr>
<tr>
<td>Diabetic + Byesukar</td>
<td>3.67 ± 0.32e</td>
<td>7.49 ± 0.29e</td>
</tr>
<tr>
<td>Control + Byesukar</td>
<td>4.25 ± 0.20e</td>
<td>8.50 ± 0.41e</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=6). Values not sharing a common letter differ significantly at P<0.05 by DMRT.

Units: GPx = µg of GSH consumed/min/mg protein

Effect of byesukar on glutathione levels in the tissues of normal and treated animals is shown in table 4. Byesukar treated diabetic rats showed a significant increase in kidney and liver GSH levels from 2.57 ± 0.22 to 3.98 ± 0.25 and 3.61 ± 0.24 to 5.90 ± 0.45 respectively.

Effect of byesukar on glutathione levels in the tissues of normal and treated animals is shown in table 4. Byesukar treated diabetic rats showed a significant increase in kidney and liver GSH levels from 2.57 ± 0.22 to 3.98 ± 0.25 and 3.61 ± 0.24 to 5.90 ± 0.45 respectively.

Table 5 depicts the level of GPx in the tissues of normal and treated animals. Byesukar treated diabetic animals showed a significant increase in kidney and liver GPx levels from 2.64 ± 0.24 to 3.67 ± 0.32 and 4.76 ± 0.34 to 7.49 ± 0.29 respectively.
Table 6: Effect of byesukar on the activities of GST and GR in kidney and liver of control and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Kidney GST</th>
<th>Kidney GR</th>
<th>Liver GST</th>
<th>Liver GR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.65 ± 0.53c</td>
<td>13.77 ± 0.62c</td>
<td>34.15 ± 1.03c</td>
<td>14.40 ± 0.81c</td>
</tr>
<tr>
<td>Diabetic</td>
<td>16.57 ± 0.47a</td>
<td>8.00 ± 0.53a</td>
<td>19.96 ± 0.78a</td>
<td>9.72 ± 0.59a</td>
</tr>
<tr>
<td>Diabetic + Byesukar</td>
<td>21.07 ± 0.51b</td>
<td>11.00 ± 0.41b</td>
<td>30.70 ± 0.57b</td>
<td>13.16 ± 0.82b</td>
</tr>
<tr>
<td>Control + Byesukar</td>
<td>23.98 ± 0.80c</td>
<td>13.04 ± 0.54c</td>
<td>33.65 ± 1.00c</td>
<td>14.03 ± 0.57c</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=6). Values not sharing a common letter differ significantly at P<0.05 by DMRT.

Units: GST = µ moles of CDNB-GSH conjugate formed/min/mg protein  
CAT = µ moles of GSH utilized/min/mg protein

Units: SOD = 50% inhibition of nitrite/min/mg protein  
CAT = nmoles of H₂O₂ decomposed/min/mg protein

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

Discussion

Lipid peroxidation is one of the characteristic feature of chronic diabetes. Oxidative damage induced by alloxan resulted in the formation of highly reactive hydroxy 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 [14].

SOD is an important defense enzyme which catalyses the dismutation of superoxide radicals [15]. 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 GPx. 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 GPx and GST as their substrate. Byesukar administration resulted in significant elevation of GSH in the experimental rats (Table 4).

GPx plays a pivotal role in H₂O₂ catabolism and in the detoxification of endogenous metabolic peroxides and hydroperoxides which catalyses GSH [22]. Decreased activity of GPx may result from radical induced inactivation and glycation of the enzymes [23]. In diabetic rats treated with the formulation, significant increase in GPx was observed (Table 5). This might reflect the antioxidant potency of Byesukar, which by reducing glucose levels, prevented glycation and inactivation of GPx.

GST is a family of isoenzymes which participate in the conjugation of toxic electrophiles with GSH [24]. The decreased activity of GST in the present study may have been due to the decreased availability of GSH. The increased activity of GST in extract treated diabetic rats hasten the detoxification of the lipid peroxides (Table 6).

GR is a family of homologous proteins whose members are dimeric, NADPH dependent and FAD containing enzymes. GR maintains the cellular levels of GSH (by the reduction of oxidized glutathione), which protects the cellular membranes from peroxides [25]. The increase in GR activity implies that herbal formulation protects the tissues from oxidative damage by GSH generated from its oxidized form (Table 6).

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

Antioxidant Potential of byesukar
In conclusion, the ethanolic extract of byesukar was shown to possess antioxidant activity by increasing GSH levels, SOD, CAT, GST and GR 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.

References

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