AMELIORATION OF ALLOXAN-INDUCED HYPERGLYCAEMIA BY *ALOE ARBORESCENS* MILLER. AND ITS POSSIBLE MECHANISM

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Summary

Oxidative stress induced by alloxan has been shown to damage pancreatic β-cell and produce hyperglycaemia in rats. *Aloe arborescens* leaves are being used by tribals as a medicine for diabetes. The present study examined the antidiabetes and antioxidant potential of fresh *Aloe arborescens* juice and its role in reducing hepatic and renal toxicity induced by alloxan. Antidiabetic activity of the juice was carried out in the alloxan diabetic rats. Reduction in blood sugar could be seen from 6th day after continuous administration of the extract. *In vitro* free radicals scavenging activity was estimated by inhibition of lipid peroxidation (LP), hydroxyl radical and superoxide radicals. Oxidative stress produced by alloxan was found to be significantly lowered by the administration of the juice. This was evident from a significant decrease in lipid peroxidation level in serum as well as in liver induced by alloxan. Superoxide dismutase, catalase and glutathione levels were found to be increased. The changes in hepatic and renal functions during alloxan induction were also reversed after the continuous administration of *A. arborescens* juice. These results indicated that *A. arborescens* juice effectively reduced the oxidative stress, levels of kidney and renal functions induced by alloxan and produce a reduction in blood sugar.

Key words: Anti-oxidant Enzymes, Anti-diabetes, *Aloe arborescens,* Alloxan toxicity

Introduction

Role of the oxygen radicals in the causation of diabetes is under intense research. Type I diabetes is produced mainly by the autoimmune mechanism. β-cell destruction is produced among other things by the free radicals of oxygen generated by cytotoxic T lymphocytes and macrophage [1]. Oxygen radicals have also play a role in the insulin resistance during type II diabetes [2].

Many indigenous Indian medicinal plants have been found to be useful in the management of diabetes [3]. However, the mechanism of action of most of these drugs is not understood at present. Antioxidant drugs have significant role in the management of diabetes and as such many of the herbal drugs used in diabetes have been reported to have antioxidant activity [4]. *Aloe* species have also been used for centuries for their laxative, anti-inflammatory, immunostimulant, antiseptic [5], wound and burn healing [6], antiulcer [7] and antitumour [8] activities. *Aloe arborescens* is a large, multi-stemmed shrub, and its juice is used by tribals against adult onset diabetes. It was also found to inhibit pancreatic cancer induced by carcinogens [9]. In the present study, we have determined the antidiabetes and antioxidant activity of *Aloe arborescens* and its role to reduce hepatic and renal toxicity induced by pro-oxidants.

Methods

Preparation of *Aloe arborescens* juice

The leaves of *Aloe arborescens* Miller. (Family-Liliaceae) were collected from Wyanad and it was identified by Dr. Ansari, Botanist, Malabar Botanical Garden, Kozhikkode, Kerala. Fresh gel was taken out with blunted knife. Twenty grams of fresh gel was crushed in the mortar and suspended in 40 ml of distilled water. This juice was passed through a muslin cloth, and used for the experiments. *Zingiber officinale* (Ginger) was collected from Thrissur local market. The 100 g of fresh ginger was crushed and macerated in 500ml of distilled water (24 hours at 5°C) and filtered. Extraction was repeated and the pooled extract was evaporated to dryness in vacuum. The percentage yield of aqueous extract was 10.6%.

Animals and Chemicals

Male Wistar albino rats (250-300g) were obtained from the Veterinary College, Mannuthy, Kerala. They were housed in ventilated cages and

fed with a pelleted diet (Lipton, India Ltd) and water *ad libitam*. All experiments were carried out as per the Institutional Ethics Committee. Alloxan monohydrate was obtained from Sigma, St. Louis, M.O. 1-Chloro-2,4-dinitrobenzene, glutathione and 5-5-dithiobis (2-nitrobenzoic acid) were purchased from Sisco Research Laboratory, Mumbay, India. Thiobarbituric acid was obtained from E-Merck, Germany. All other chemicals used were of analytical reagent grade.

Determination of *in vitro* antioxidant activity

Aloe arborescens juice was diluted (1000 mg/ml) and various concentrations were used for the *in vitro* experiments. The antioxidant levels of *A. arborescens* juice was compared with water extract of ginger (*Zingiber officinale*) which is a known antioxidant [10].

The superoxide scavenging activity of was determined by the method of McCord and Fridovich [11]. Hydroxyl radical scavenging activity was determined by measuring inhibition of hydroxyl radical generated by Fenton reaction [12]. Lipid peroxidation was induced in rat liver homogenate by incubating with Fe^{2+-}ascorbate for 1h and lipid peroxide formation was determined by the estimation of thiobarbituric acid reacting substances (TBARS) formation [13].

Determination of Glucose tolerance

Male Wistar rats (150 – 200 g) were divided into four groups. Control rats (Group 1) were given 1 ml distilled water. Fresh juice of *Aloe arborescence* (200 and 1000 mg/ kg b.wt.) was given orally to second and third groups. Insulin (2 IU/animals) was administered to group 4 by intraperitoneal injection. Glucose (2 g/kg b. wt.) was administered orally to all groups immediately after the drug administration. Blood samples were collected from the tail vein just prior to drug administration and 30 min, 1, 2 and 4 hour after the glucose loading and blood glucose levels were measured by GOD/POD enzymatic method [14].

Determination of antidiabetes activity of Aloe arborescence in alloxan induced diabetic animals

Diabetes was induced in male rats by injecting (ip.) a single dose of alloxan monohydrate (120 mg/kg b.wt). Serum glucose level was checked after 72 hour. Animals with serum glucose level greater than 250 mg/dl were considered diabetic and used for the study. The rats were divided into five groups of 6 rats each. Group I was normal rats and Group II (diabetic control) animals were given distilled water.

Group III and IV were given *A. arborescens* juice orally at a dose of 200 and 1000 mg/kg b. wt. respectively on third day after alloxan treatment. Group V was given insulin (2 IU/ animal) once daily as intraperitoneal injection. Fasting blood samples were collected from the tail vein third day after alloxan treatment prior to the extract administration and at 1, 2, 4 and 6 hour intervals after the juice administration. In the multidose study, animals were continued with the same dose of the extract once daily for 15 days. Blood was collected one hour after drug administration and serum glucose levels were measured on 3, 6, 9, 12, 15 and 18th day. The body weight of the animals was monitored at every week.

Determination of *in vivo* antioxidant activity

The diabetic and drug treated animals were sacrificed on 18th day after alloxan administration. Blood was collected by heart puncture and serum was separated. The liver was separated, washed with normal saline and kept in the freezer (-20°C) till the experiment carried out. The liver samples were homogenized with Tris-HCl buffer.

Erythrocytes were prepared by the method of Minami and Yoshikawa [15] and superoxide dismutase was estimated by the modified method of McCord and Fridovich [11]. The assay is based on the ability of the enzyme to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide (O_2), which is generated by the photo reduction of riboflavin. One unit of enzyme activity is defined as amount of enzyme giving 50% inhibition of the reduction of NBT and expressed as Units/mg protein.

Catalase was estimated in the erythrocytes and liver tissue by the method of Aebi [16] by measuring the rate of decomposition of hydrogen peroxide (H_2O_2) at 240 nm. Lipid peroxidation (LPO) levels in liver was estimated using thiobarbituric acid (TBA) method of Ohkawa [13] by using 1,1,3,3 tetramethoxy propane as standard and the serum lipid peroxidation was estimated by the method of Satoh [17]. Glutathione was estimated both in blood and liver tissue by the method of Moron [18].

Determination of liver and kidney function

Normal and diabetic animals were treated with *Aloe* juice for 15 days at dose levels of 200 and 1000 mg/kg body weight. They were sacrificed on 18th day after alloxan treatment. Blood was collected and serum was separated and alkaline phosphatase was measured by the chemical reaction of phenol with 4-aminoantipyrine.

Glutamate pyruvate transaminase in serum was done according to DNPH method. Blood urea nitrogen (diacetyl monoxime) and creatinine (picrate Jaffe method) were estimated. Protein was determined by the method of Lowry et al., [19]. Total leukocyte counts [20] were made on 0, 7th and 14th day after alloxan administration. Haemoglobin was estimated with Drabkin's reagent [21]. The liver glycogen was estimated by the method of Hassid and Abraham [22].

Statistical analysis

The quantitative measurements were made on six animals in each group and the values were expressed as mean ±SD. Data obtained were subjected to oneway analysis of variance (ANOVA) followed by Bartlett's test or student 't' test and values with $P<0.05$ were considered significant.

Results

A. arborescens juice was found to scavenge the superoxide generated by photoreduction of riboflavin in a concentration dependent manner. The concentration needed for 50% scavenging of superoxides was 54 µg/ml. The concentration needed for 50 % inhibition of hydroxyl radical generation was 70.5 µg/ml. Lipid peroxidation induced by Fe^{3+}/ascorbate in rat liver homogenate was found to be inhibited and the concentration needed for 50 % inhibition was 52.2 µg/ml. Concentrations needed for 50% scavenging of superoxides, hydroxyl radical and lipid peroxidation by ginger were 20.0, 150.0 and 30.0 µg/ml respectively (Table 1) indicating that *A. arborescens* has significant antioxidant activity which was comparable to ginger extract.

Table 1: Effect of *Aloe arborescens* juice on *in vitro* antioxidant activity

	Concentration needed for 50% inhibition of oxygen radicals (µg/ml)		
Treatment	**Superoxide**	**Hydroxyl radical**	**Lipid peroxidation**
A. arborescens (Juice)	54.0±1.5	70.5±1.8	52.2±1.9
Z. officinale (Water extract)	22.0±1.2	150.0±2.5	30.0±1.1

Values are mean ±SD, n=3

Administration of 2 g glucose/kg b.wt. to the normal rats increased the serum glucose level from 77.8± 5.1 to 147.5 ±6.4 at 60 minutes and then reduced to normal at 240 minutes. Administration of the juice (200 and 1000 mg/kg b.wt.) suppressed the elevation of serum glucose level in a dose dependent manner (Figure 1).

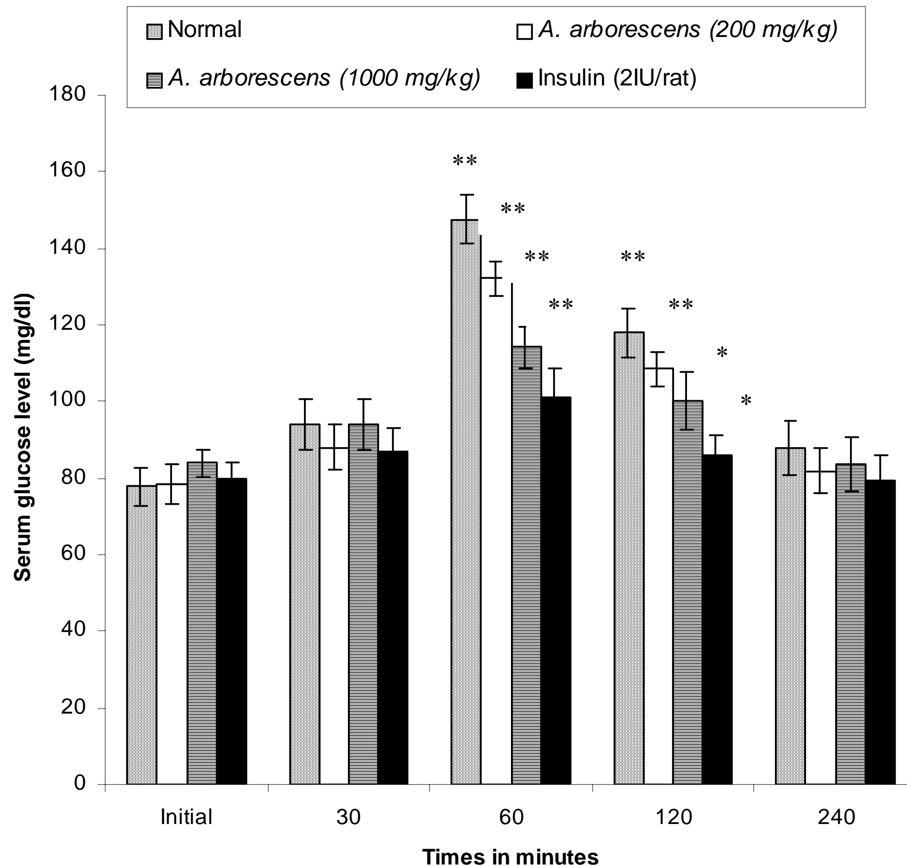

Figure 1. Effect of *Aloe arborescens* on glucose tolerance in normal rats.

Numbers of animals used per group were six. Glucose levels were estimated initially, 30,60,120 and 240 minutes intervals.
*p<0.05, **p<0.001 when compared with initial value of the same group.

Single administration of the juice (200 mg/kg, b.wt.) on 3rd day after alloxan administration did not produce a significant reduction in serum glucose level. At a dose level of 1000 mg/kg, b.wt. there was 8.4% reduction in glucose level at 6th hour (data not included).

Continuous administration (200 mg/kg, b.wt.) produced 26.9 % reduction in the elevated glucose level on 18th day after alloxan administration. At a dose of 1000 mg/kg b.wt. there was 42.3% reduction in blood glucose level on 18th day ($P<0.001$). Administration of insulin (2 IU/animal) produced 53.2% reduction in the elevated serum glucose level on 18th day (Table 2).

Table 2. Effect of *Aloe arborescens* juice on the blood sugar levels in alloxan induced diabetic rats (Multidose study)

Treatment	Serum glucose level (mg/dl)					
Group	3rd Day	6th Day	9th Day	12th Day	15th Day	18th Day
					4	
1	342.5 ± 22.4	410.2 ± 10.4	428.8 ± 13.5*	459.6 ± 22.1*	367.1 ± 22.8*	381.3 ± 23.6
2	361.1 ± 23.2	326.2 ± 19.3	307.4 ± 13.5	294.5 ± 16.6	272.9 ± 8.3*	263.9 ± 5.0*
3	415.1 ± 30.14	379.0 ± 32.6	345.7 ± 28.6	312.1 ± 20.2	70.0 ± 23.5*	239.6 ± 24.3**
4	444.9 ± 26.2	430.6 ± 20.3	360.3 ± 13.9	305.0 ± 15.4**	51.7 ± 12.7**	208.3 ± 8.7**

Values are mean ± SD, n=6, * $P < 0.05$, ** $P < 0.001$ when compared to value on 3rd day after alloxan treatment of the same group

The body weights of normal and diabetic animals were recorded before treatment, and 1st, 7th and 14th day after drug treatment. In normal animals there was a slight increase in the body weight. Alloxan diabetic animals showed significant decrease in the body weight from 7th day. Administration of *A. arborescens* juice reduced the weight loss induced by alloxan administration in a dose dependent manner and in the animal treated with 1000 mg/kg drug, the body weights of the animals were higher than the initial weight of the same group (Figure 2).

SOD levels in blood were significantly ($p<0.005$) increased after the juice treatment (Table 3). There was no significant increase in the liver weight. There was only marginal increase in the values of blood and liver catalase, which were reduced by alloxan injection.

Glutathione levels were significantly decreased in alloxan-induced diabetic rats when compared with normal animals. The GSH levels were increased ($p<0.005$) in the liver tissue after treatment with the drug and no significant increase was shown in blood GSH after the

juice or insulin treatment. Tissue lipid peroxidation was decreased by the administration (1000 mg/kg b.wt.) of the juice (P<0.005) and there was no significant decrease in the serum lipid peroxidation by the juice administration.

There was elevation of serum ALP and GPT in alloxan-induced diabetes when compared with normal animals. Animals treated with 1000 mg/kg of the juice showed significant (P<0.001) reduction in the elevated level of ALP and GPT. The liver glycogen was significantly (P<0.001) reduced in diabetic group. Administration of the juice (1000 mg/kg, b.wt.) or insulin treatment significantly (P<0.001) prevented the reduction in the glycogen level.

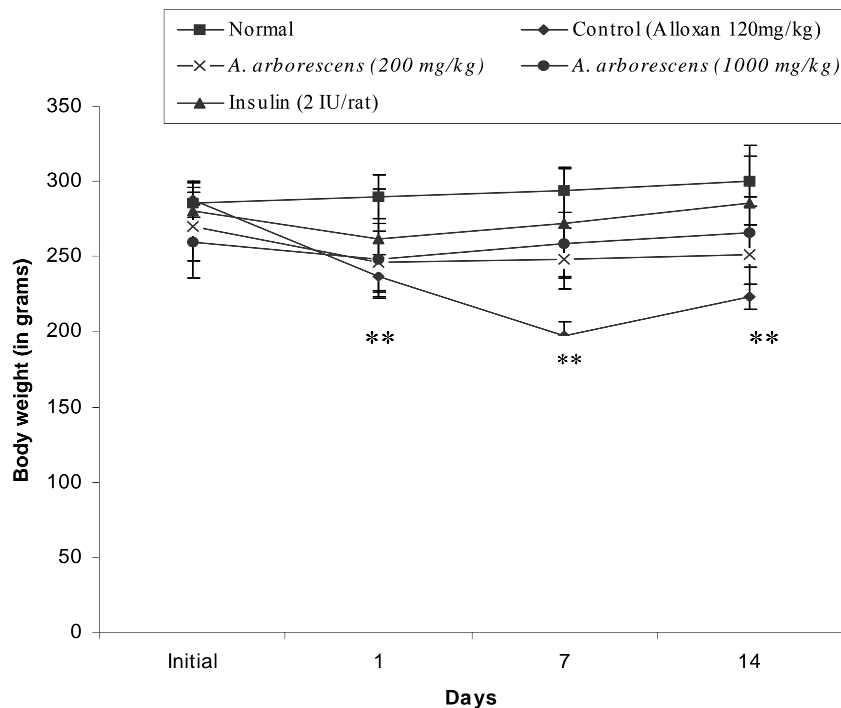

Figure 2: Effect of *Aloe arborescens* on body weight changes in alloxan-diabetic rats
Numbers of animals used per group were six. Body weights were measured initially, day 1, 7 and 14 after the animals were made diabetic. **p<0.001 when compared with initial value of the same group.

Renal function parameters like creatinine and blood urea nitrogen (BUN) were also elevated significantly (P<0.001) in the alloxan diabetic rats when compared with normal rats. The elevated creatinine level by alloxan administration was found to be reduced by the administration of 1000 mg/kg b.wt. of the juice. Elevated levels of BUN were also found to be reduced significantly in animals treated with 1000mg/kg dose of the juice or insulin (Table 3).

SOD-Superoxide dismutase; LPO-Lipid peroxidation; GSH-Glutathion; ALP-Alkaline Phosphatase; GPT-Glutamate pyruvate transaminase; BUN- Blood urea nitrogen

Units: A-U/g Hb, B- U/mg protein, C- U/mg Protein/min, D-n moles/ml, E-n moles/mg protein, F-n moles/g Hb, G-n moles/mg protein, H- KA/dl, I- U/ml, J-mg/g tissue, K-mg/dl, L- mg/dl.
Diabetic group is compared with normal (* P<0.05); alphabets letters indicate comparison between diabetic and treated groups. Means having different alphabets are significant at P<0.05, but the same letters are not significant.

The effect of *A. arborescens* juice on total WBC in alloxan diabetic animals is shown in Table 4. The juice treatment significantly prevented the reduction in WBC count observed in diabetic control rats.

Table 3: Effect of *Aloe arborescens* in the alteration of antioxidant levels and hepatic and renal function induced by alloxan

Parameters		Normal	Diabetic	*Aloe arborescens*		Insulin (2 IU/rat)
				200mg/kg	1000mg/kg	
SOD	Blood^{A}	675.2 ±10.6	422.5±18.9$^{\alpha}$*	455.7±16.7^{a}	520.2±20.1^{b}	525.1±20.7^{b}
	Liver^{B}	12.7±0.95	7.5±0.18^{a}*	8.0±0.71^{ab}	9.0±0.62^{bc}	9.1±0.95^{c}
Catalase	Blood^{A}	8.7±1.21	5.5±0.82^{a}*	6.2±1.01^{a}	6.9±0.99^{b}	6.8±0.91^{b}
	Liver^{C}	7.5±0.79	5.2±0.82^{a}*	6.1±0.92^{a}	6.8±0.74^{b}	6.9±0.65^{b}
LPO	Serum^{D}	2.1±0.14	2.6±0.08^{ab}*	2.1±0.17^{acd}	2.3±0.06^{bce}	2.3±0.03^{de}
	Liver^{E}	3.0±0.32	4.2±0.30^{a}*	3.5±0.15^{bc}	3.6±0.17^{b}	3.2±0.29^{c}
GSH	Blood^{F}	26.7±1.53	20.3±1.53^{abc}*	22.0±1.0^{ade}	25.7±1.53^{bd}	26.5±1.32^{cde}
	Liver^{G}	38.0±2.0	29.3±1.15^{a}*	33.2±1.26^{abc}	34.8±1.04^{bd}	36.2±1.44^{cd}
ALP	Serum^{H}	18.6±1.1	36.6±3.2^{a}*	32.1±2.1^{a}	25.6±1.4^{b}	23.3±2.1^{b}
GPT	Serum^{I}	75.1±6.6	215.2±8.6^{a}*	180.1±9.8^{a}	138.6±12.6^{b}	145.1±13.7^{c}
Glycogen	Liver^{J}	25.4±0.66	17.2±0.59^{a}*	18.4±0.86^{ab}	19.8±0.43^{bc}	20.5±0.47^{c}
BUN	Blood^{K}	15.3±0.52	25.5±0.82^{abc}*	24.7±0.90^{ade}	21.7±1.61^{bd}	20.7±1.23^{cde}
Creatinine	Serum^{L}	2.7±0.11	4.2±0.15^{A}*	3.9±0.16^{AB}	3.4±0.18^{BC}	3.2±0.22^{C}

Table 4: Effect of *Aloe arborescens* on total white blood cells in normal and alloxan diabetic animals

Group	Before treatment	1st day	7th day	14th day
Normal	9340 ± 495.2	9305 ± 570.3	9309 ± 477.1	9257 ± 564.5
Control	9072 ± 275.0	6247 ± 281.0**	5830 ± 385.1**	7143 ± 155.7**
A. arboescens (200mg/kg b.wt.)	9384 ± 442.0	7047 ± 115.7**	7115 ± 92.6**	7534 ± 125.1**
A. arborescens (1000 mg/kg b.wt.)	9841 ± 231.9	7249 ± 695.3**	7843 ± 376.2**	8108 ± 188.7**
Insulin (2 IU/animal)	9560 ± 210.4	8217 ± 128.3**	8650 ± 203.5**	9031 ± 90.1**

Values are mean ± SE, n=6 Unit: Cells/mm^3
** P<0.001 compared to value of before treatment of the same group

Discussion

The fresh juice of the *Aloe* plant (aloes) is one of the several traditional remedies, which has several pharmacological actions. Antidiabetic activity of *Aloe vera* has been reported and is being used to manage blood sugar in clinical diabetes [23]. Less known species, *A. arborescens* is being used by tribals to manage diabetes. However there are no literatures on its activity in human or in experimental diabetes. The present study indicates that the juice of *A. arborescens* could produce significant protection against diabetes as seen by increased glucose tolerance in normal rats as well as the glucose lowering effect in alloxan diabetic rats.

The compounds responsible for the antidiabetic activity of *A. arborescens* juice are not known at present. Several anthraquinones and polyphenols are shown to be present in *Aloe* species of which barbaloin, aloeresin and aloe emodine are most important [24]. Chemopreventive activity of *Aloe* species against experimental pancreatic cancer as well as colon cancer are mainly due to the presence of aloe emodine which has shown to produce apoptosis of tumour cells [25]. *Aloe* species are also found have significant quantity of complex polysaccharide which is shown to be immunomodulatory and tumor reducing properties [26].

Our results also indicate that the juice has significant *in vitro* and *in vivo* antioxidant activity. Glutathione (GSH) acts as an antioxidant and its decrease was reported in diabetes mellitus [27]. The increased GSH content in the liver of the rats treated with *A. arborescens* may be one of the factors responsible for the inhibition of lipid peroxidation.

Alloxan produces free oxygen radicals in the body, which cause pancreatic injury and could be responsible for increased blood sugar seen in the animals. However, its action is not specific to pancreas as other organs such as liver, kidney and haemopoietic system also affected by alloxan administration as seen from the elevation of marker enzymes in liver and kidney and reduction in hematological parameters. These toxic manifestations of alloxan were reversed by the continued administration of *A. arborescens* juice.

In conclusion, oral administration of the fresh juice of *A. arborescens* was found to increase the serum glucose tolerance in normal and alloxan diabetic rats. It could also increase the antioxidant potential and reduce the liver and kidney toxicity and normalize the hematological changes induced by pro-oxidants.

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