ISSN 2582 – 6050

Available Online at www.ijpba.info
International Journal of Pharmaceutical & Biological Archives 2022; 13(4):165-172

RESEARCH ARTICLE

Protective Effect of *Glycyrrhiza glabra* Linn in Aniline-Induced Spleen Toxicity in Rats

Laxmi Verma, Ragini Bundela, Sourabh Jain, Karunakar Shukla

Department of Pharmacology, College of Pharmacy, Dr. A. P. J. Abdul Kalam University, Indore, Madhya Pradesh, India

Received: 20 October 2022; Revised: 25 November 2022; Accepted: 04 December 2022

ABSTRACT

Glycyrrhiza glabra (GG) is an herbal plant which has lots of medicinal properties such as antimicrobial, antioxidant, anti-inflammatory, antitussive, antidiabetic, antiviral, anticancer, antimutagenic, antiulcer, and hepatoprotective. The phytochemicals present in GG have been of immense importance in phototherapeutics. The present study was designed to evaluate the protective effects of GG on hematological and biochemical changes in aniline-induced spleen toxicity in rats. Wistar rats of either sex (200–300 g) were used in the study and each group contains six rats. Splenic toxicity was induced in rats by administration of aniline hydrochloride (AH; 100 ppm) in drinking water for a period of 30 days. Treatment groups received GG (50, 100 and 200 mg/kg/day, p. o) along with AH for 30 days. At the end of treatment period, various serum and tissue parameters were evaluated. AH-treated rats showed a significant alteration in body weight, spleen weight, feed consumption and water intake, hematological parameters (Hemoglobin, red blood cells, white blood cells, and total iron content), biochemical parameters (total iron content, total protein, lipid peroxidation, reduced glutathione, and nitric oxide), and membrane bound phosphatase (ATPase). The treatment with GG (50, 100, and 200 mg/kg/day, p. o) for 30 days along with AH showed significant recovery in aniline-induced splenic toxicity. Conclusion: The present result showed that involvement of oxidative and nitrosative stress in aniline-induced splenic toxicity and GG protects the rats from the toxicity, which might be due to its antioxidant property and the presence of different phytochemicals.

Keywords: Aniline hydrochloride, antioxidant, *Glycyrrhiza glabra*, spleen toxicity

INTRODUCTION

Aniline is a toxic aromatic amine, widely used in chemical industry, particularly in the manufacture of dyes, resins, varnishes, perfumes, pigments, herbicides, fungicides, explosives, isocyanates, hydroquinone, and rubber chemicals.[1] Chronic exposure to aniline leads to the development of splenomegaly, increased erythropoietic activity, hyper pigmentation, hyperplasia, and fibrosis.[2-4] The clinical symptoms of aniline exposure such as cyanosis, weakness, dizziness, headache, stupor, loss of coordination, and coma occur rapidly (within 1–3 h) after ingestion or skin contact.[3] Earlier studies have shown that aniline exposure leads to the formation of oxidative and nitrosative stress which are due to iron overload and induction of lipid peroxidation. The excess production of free radicals could attack proteins and nucleic acid, leading to structural and functional changes in the spleen.[5] Natural products having antioxidant property are gaining importance in disease prevention, where oxidative stress has been involved. *Glycyrrhiza glabra* (GG) (Family: *Fabaceae*) is a traditional medicinal plant used in various ancient medicine systems and documented across the globe for its ethanopharmacological value to cure varieties of ailments. Glycyrrhizin is the major active constituent obtained from GG roots, one of the most widely used in herbal preparations

***Corresponding Author:**
Sourabh Jain,
E-mail: drsourabh294@gmail.com

for the treatment and management of chronic diseases. A number of studies have been reported describing the different secondary metabolites of the Glycyrrhiza species. GG contains more than 20 triterpenoids and nearly 300 flavonoids.[6] As per the previous literature *Glycyrrhiza glabra* (GG) showed antitumor, antimicrobial, antiviral, anti-inflammatory, immune regulatory, and several other activities that contribute to the recovery and protection of various systems in human body. [6] Based on its potent antioxidant activity and traditional uses, the present study was designed to assess the effect of GG on aniline exposure-induced spleen toxicity in rats by evaluating different biochemical parameters.

MATERIALS AND METHODS

Drugs and Chemicals

Standardized extracts of GG were obtained as gift sample from Green ChemPvt Ltd, Bangalore, India, along with certificate of analysis. Aniline hydrochloride (AH); 2,2'-dipyridy l, 5,5'-dithiobis-(2-nitrobenzoic acid); and N-(1-Napthyl) ethylene diaminedihydrochloride were purchased from HiMedia Lab. Pvt Ltd, Mumbai. All the other chemicals used in the study were of analytical grade and procured from standard supplier.

Preparation of Drug Solution

GG was dissolved in distilled water and 100 ppm (100 mg/l) of Aniline HCL (100 ppm) was prepared in distilled water. All the drug solutions were freshly prepared before starting experiment.

Experimental Animals

Wistar rats of either sex (200–300 g) were used in the study. The animals were procured from Lachmi biotech, Pvt. Ltd, Pune. Rats were placed separately in polypropylene cages (4–6 per cage) with paddy husk as bedding. The animals were maintained under standard laboratory conditions at temperature 23 ± 20°C, relative humidity 45–55 ± 10%, and 12 h light and 12 h dark cycles throughout the experiments. Animals had free access of water and standard laboratory feed (Prashant Enterprises, Pune). The animals were shifted to the laboratory 1 h before the experiment. The experimental protocol was approved by the Institutional Animal Ethic Committee.

Aniline-Induced Splenic Toxicity in Rats

Firoze khan reported that aniline metabolized to variety of oxidized products. Oxidation of amino group in aniline results in the formation of phenylhydroxylamine (PHA), which can be further oxidized to nitrosobenzene (NB). PHA enters the erythrocytes within the liver and reacts with oxy hemoglobin to yield methemoglobin (MetHb) and NB. Since one of the major functions of the spleen is to remove damage red cells, aniline-damage erythrocytes are expected to be scavenged by splenic phagocytes resulting in splenic toxicity.[7]

Experimental Protocol

The rats were divided into five different groups (n = 6)

- Group I: Normal control
- Group II: Rats received Aniline HCl (100 ppm, p. o) in drinking water for 30 days
- Group III: Rats received Aniline HCl (100 ppm) through drinking water and GG (50 mg/kg/day, p. o), for 30 days
- Group IV: Rats received Aniline HCl (100 ppm) through drinking water and GG (100 mg/kg/day, p. o), for 30 days
- Group V: Rats received Aniline HCl (100 ppm) through drinking water and GG (200 mg/kg/day, p. o), for 30 days.

Biochemical Evaluation

General parameter such as body weight, spleen weight, water intake, and feed consumption were studied in between and at the end of experiment. At the end of treatment period, blood was withdrawn from retro-orbital plexus using glass capillary and serum was separated and used for the estimation of hemoglobin (Sahli'shaemometer method), red

blood cells (RBCs), and white blood cells (WBCs) count using hemocytometer.[8] Serum was used for the estimation of iron content,[9] protein content was estimated using standard diagnostic kits (Span Diagnostic kit).

Assessment of Markers of Oxidative Stress

Tissue homogenization

The animals were euthanized using human procedure; spleen was quickly transferred to ice-cold tris-hydrochloride buffered saline (pH7.4). Spleen was cross-chopped with surgical scalpel into fine slices, suspended in chilled 0.25 M sucrose solution, and quickly blotted on a filter paper. The tissue was, then, minced and homogenized in chilled tris-hydrochloride buffer (10 Mm, pH 7.4) to a concentration of 10% w/v. The homogenate was centrifuged at 10,000 rpm at 00 C for 15 min using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the determination of lipid peroxidation,[10] reduced glutathione (GSH),[11] and nitric oxide (NO)[12] level, whereas the sediment was used for the estimation of membrane bound phosphatase such as Na^+/K^+ ATPase,[13] Ca^{++}ATPase,[14] and Mg^{++}ATPase.[15]

Statistical Analysis

All the values are presented as mean ± S.E.M. Statistical significance between more than two groups was tested using one-way analysis of variance followed by the Dunnett "t" test comparison test as appropriate using computer based fitting program (Prism 5). Differences were considered to be statistical significant when $P < 0.05$.

RESULTS

At the end of 30 days body weight, water intake and food consumption was monitored and it was found to be moderately changed in AH-treated group as compared to control group. The treatment with GG (50/100/200 mg/kg/day, p. o) for 30 days showed a significant alteration in body weight, water intake, and feed consumption as compared with AH-treated group, as shown in Table 1. Spleen weight was monitored at the end of study. A significant ($P < 0.001$) increased in spleen weight was observed as compared to AH-treated group. Treatment with GG (50/100/200 mg/kg/day, p. o) for 30 days showed a significant ($P < 0.001$) improved in the weight of spleen as compared to AH-treated group [Table 1]. The RBCs and hemoglobin count were significantly ($P < 0.001$) decreased and WBCs count was significantly ($P < 0.001$) increased in AH-treated rats as compared to control animals. Treatment with GG (50/100/200 mg/kg/day, p. o) for 30 days then reduces the intensity of the blood sample as compare to AH-treated group, as shown in Table 2. The total iron content was significantly ($P < 0.001$) increased in AH-treated rats as compared to control animals. The treatment with GG (50/100/200 mg/kg/day, p. o) for 30 days showed significantly ($P < 0.001$) decrease in total iron content as compare to AH-treated group, as shown in Table 3. The level of total protein content was also monitored. It was found that rat treated with AH showed a significant ($P < 0.001$) reduction in level of protein content as compared to control rats. GG (50/100/200 mg/kg/day, p. o) treatment causes significant ($P < 0.001$) improvement in protein content compared to AH-treated rats. GG (200 mg/kg/day p. o) antioxidant showed better recovery in protein content in AH-treated group, as shown in Table 3. Lipid peroxidation in spleen homogenate was significantly ($P < 0.001$) increased in AH-treated rats as compared to control animals. Treatment with GG (50/100/200 mg/kg/day, p. o) and for 30 days showed significantly ($P < 0.001$) decrease in LPO level in spleen as compare to AH-treated group. GG (200 mg/kg/day p. o) was found to be more effective in maintaining lipid peroxidation level as compare to GG (50 mg/kg/day, p. o) and GG (100 mg/kg/day, p. o) after extensive administration of hemolytic compounds depleted erythrocyte-reduced GSH. Our study with AH confirmed GSH depletion in rat erythrocytes. GSH level was significantly ($P < 0.001$) decreased in AH-treated rats as compared to control animals. The treatment with GG (50 mg/kg/day, p. o) and GG (100 mg/kg/day, p. o) for 30 days along

Table 1: Effect GG on body weight, water intake, feed consumption, and spleen weight

Parameters	Days	Control	AH (100 ppm)	AH+GG (50 mg)	AH+GG (100 mg)	AH+GG (200 mg)
Body weight	1	287.5±0.763	283.3±0.760	283.3±0.600	278.2±0.600	277.5±0.763
	30	296.7±0.918	219.7±0.592***	252.8±0.792###	261.5±0.991###	275.5±0.341###
Feed consumption	1	21.00±0.364	19.41±0.459	20.34±0.454	20.86±0.625	19.94±0.386
	30	22.48±0.559	10.89±0.544***	16.57±0.473###	17.38±0.676###	19.37±0.543###
Water intake (mL)	1	36.61±0.137	37.44±0.199	34.70±0.135	33.45±0.105	35.52±0.101
	30	40.45±0.109	16.74±0.111***	26.47±0.191###	31.42±0.114###	34.32±0.184###
Weight of (g) spleen	30	0.589±0.024	1.284±0.014***	1.121±0.146##	0.847±0.026###	0.664±0.014###

Values are expressed as mean±SEM, (n=6). One way Analysis of variance (ANOVA) and Dunnett's test, Level of significance is considered as *P<0.05. **P<0.01, ***P<0.001 compared to control group #P<0.05, ##P<0.01, ###P<0.001 compared to AH treated group, GG: *Glycyrrhiza glabra*, AH: Aniline hydrochloride

Table 2: Effect of GG on hemoglobin level, RBCs, and WBCs counts

Blood content	Days	Control	AH (100 ppm)	AH+GG (50 mg)	AH+GG (100 mg)	AH+GG (200 mg)
HB (g/dL)	1	15.9 2±0.079	14.60±0.258	15.48±0.414	15.80±0.340	16.33±0.294
	30	16.0 8±0.343	4.45±0.201***	8.78±0.336###	12.42±0.162###	14.01±0.147###
RBCs ($\times 10^6$/cells)	1	10.08 8±0.261	9.771±0.280	9.993±0.333	10.244±0.166	10.110±0.255
	30	9.70 0±0.115	4.745±0.086***	6.245±0.285###	7.646±0.197###	7.884±0.224###
WBCs ($\times 10^3$/cells)	1	10.310±0.225	10.380±0.214	10.688±0.192	10.290±0.332	10.460±0.198
	30	10.560±0.189	15.130±0.336***	14.088±0.217#	12.940±0.251###	1.962±0.218###

Values are expressed as mean±SEM, (n=6). One way ANOVA and Dunnett's test, Level of significance is considered as *P<0.05. **P<0.01, ***P<0.001 compared to control group. #P<0.05, ##P<0.01, ###P<0.001 compared to AH treated group. RBC: Red blood cells, WBCs: White blood cells, GG: *Glycyrrhiza glabra*, AH: Aniline hydrochloride

Table 3: Effect of GG on total iron content and total protein

Parameters	Days	Control	AH (100 ppm)	AH+GG (50 mg)	AH+GG (100 mg)	AH+GG (200 mg)
Iron content (µg/dL)	1	276.433±0.555	272.134±0.355	285.535±0.579	286.738±0.564	273.463±0.842
	30	276.334±0.551	777.136±2.345***	695.135±15.551##	594.539±16.453###	348.200±21.030###
Total protein (g/dL)	1	7.848±0.311	8.156±0.216	8.315±0.222	8.272±0.270	8.302±0.109
	30	8.014±0.216	3.112±0.192***	4.974±0.241##	5.887±0.283###	7.154±0.20###

Values are expressed as mean±SEM, (n=6). One way ANOVA and Dunnett's test, Level of significance is considered as *P<0.05. **P<0.01, ***P<0.001 compared to control group. #P<0.05, ##P<0.01, ###P<0.001 compared to AH treated group, GG: *Glycyrrhiza glabra*, AH: Aniline hydrochloride

with AH for showed significantly (P < 0.001) increased in GSH level in spleen as compare to AH-treated group. Antioxidant treatment showed significant (P < 0.001) improvement in the GSH level compared to AH-treated rats. GG (200 mg/kg/day, p. o) of antioxidant showed better recovery in GSH in AH-treated group. In spleen, the NO level were significantly (P < 0.001) increased in AH-treated rats as compared to control animals. The treatment with GG (50 mg/kg/day, p. o) and GG (100 mg/kg/day, p. o) for 30 days showed significantly (P < 0.001) decrease in NO level in spleen as compare to AH-treated group. GG (200 mg/kg/day, p. o) was found to be most effective compared to treated group, as shown in Table 4. A significant (P < 0.001) decrease in the activities of mitochondrial enzymes (Na^+/K^+ ATPase) of spleen and AH-treated rats as compared to control animals. The treatment with GG (50 mg/kg/day, p. o) and GG (100 mg/kg/day, p. o) for 30 days showed significantly (P < 0.001) increase mitochondrial enzyme level in spleen as compare to AH-treated group. GG (200 mg/kg/day, p. o) significantly attenuated the alteration and restored the altered levels to near normal levels. In spleen, the Ca^+ ATPase level was significantly (P < 0.001) decrease in AH-treated rats as compared to control animal. The treatment with GG (50 mg/kg/day, p. o) and GG (100 mg/kg/day, p. o) for 30 days showed significantly (P < 0.001) increased in Ca^+ ATPase level in spleen as compare to AH-treated group. GG (200 mg/kg/day p. o) antioxidant showed better recovery in Ca^+ ATPase in AH-treated group. The activity of Mg^+ ATPase in spleen was significantly (P < 0.001) decreased in AH-treated rats as compared to control animals.

The treatment with GG (50 mg/kg/day, p. o) and GG (100 mg/kg/day, p. o) for 30 days showed significantly ($P < 0.001$) increased in Mg^+ ATPase level in spleen as compare to AH-treated group. GG (200 mg/kg/day p. o) antioxidant showed better recovery in Mg^+ ATPase in AH-treated group Table 5.

DISCUSSION

Exposure to aniline and substituted aniline is known to cause selective splenic toxicity in rats.[16] Aniline, a toxic aromatic amine, which is widely used industrial chemical, for the preparation of dyes, resins, varnishes, perfumes, pigments, herbicides, fungicides, explosives, etc. An earliest sign of aniline toxicity is the formation of MetHb, which interferes with the oxygen-carrying capacity of the blood (Gupta, 2001). The aniline does not cause MetHb formation when it is incubated with erythrocytes (*in vitro*) in concentrations associated with *in vivo* methmoglobinemia, this methmoglobinemia is mediated by a metabolites formed during hepatic clearance of aniline. N-oxidation of aniline results in the formation of PHA, which can be further oxidized to NB. These two metabolites are interconvert in the blood through an enzyme-mediated redox cycle. On the other hand, ring hydroxylation in aniline results in the formation of 2-and 4-aminophenols. Even though PHA is a minor metabolite of aniline, it is most potent in causing methmoglobinemia and hemolysis. AH in rats causes an association between erythrocyte damage and the severity of the splenotoxicity.[1] The major function of spleen is to remove damaged red cells; aniline damaged erythrocytes are expected to be scavenged by splenic phagocytes. As rat and human shows similar toxic responses in blood, so in humans aniline-damaged, erythrocytes will also be taken up by the spleen for degradation, resulting in splenic toxicity.[7] It is possible that during scavenging of damaged erythrocytes, the phagocytes, especially the macrophages, themselves get activated, and thus lead to an increased production of superoxide radical either may be damaging directly or could lead to the formation of highly reactive species, such as hydroxyl radical and ferrylcation, which results in the observed injury.[1] The present study demonstrates the splenoprotective role of GG against aniline-induced splenic toxicity. In the present study, splenic toxicity was induced by administration of Aniline HCl (AH) (100 ppm) through drinking water. Toxicity of spleen was developed after 30 days of Aniline HCl administration and marked changes in the, body weight, feed consumption, water intake, and blood parameters such as hemoglobin level, RBC and WBC count, bleeding time, clotting time, total iron content, and total protein were observed. The above changes are in line with the previous report of.[1,7] Significant decreased in body weight, feed consumption, and water intake in aniline treated

Table 4: Effect of GG on lipid peroxidation (LPO), reduced glutathione (GSH), and nitric oxide (NO) level

Parameters	Control	AH (100 ppm)	AH+GG (50 mg)	AH+GG (100 mg)	AH+GG (200 mg)
LPO	0.762±0.023	3.361±0.152***	2.739±0.192$^{\#}$	2.509±0.147$^{\#\#}$	1.479±0.182$^{\#\#\#}$
GSH	2.372±0.026	1.247±0.031***	1.519±0.027$^{\#\#\#}$	1.790±0.047$^{\#\#\#}$	2.136±0.029$^{\#\#\#}$
NO	5.782±0.195	13.97±0.250***	11.32±0.318$^{\#\#\#}$	9.946±0.503$^{\#\#\#}$	6.752±0.355$^{\#\#\#}$

Values are expressed as mean±SEM, (n=6). One-way ANOVA and Dunnett's test, Level of significance is considered as *P<0.05. **P<0.01, and ***P<0.001 compared to control group. $^{\#}P$<0.05, $^{\#\#}P$<0.01, and $^{\#\#\#}P$<0.001 compared to AH treated group, GG: *Glycyrrhiza glabra*, AH: Aniline hydrochloride

Table 5: Effect of GG on Na^+/K^+ATPase, Ca^+ATPase, and Mg^+ATPase

Parameters	Control	AH (100 ppm)	AH+GG (50 mg)	AH+GG (100 mg)	AH+GG (200 mg)
Na^+/K^+ATPase	0.447±0.013	0.129±0.010***	0.295±0.027$^{\#\#\#}$	0.322±0.035$^{\#\#\#}$	0.358±0.026$^{\#\#\#}$
Ca^+ATPase	0.693±0.020	0.360±0.010***	0.479±0.022$^{\#}$	0.522±0.032$^{\#\#}$	0.608±0.045$^{\#\#\#}$
Mg^+ATPase	0.559±0.008	0.276±0.017***	0.395±0.015$^{\#\#}$	0.428±0.014$^{\#\#\#}$	0.542±0.039$^{\#\#\#}$

Values are expressed as mean±SEM, (n=6). One-way ANOVA and Dunnett's test, Level of significance is considered as *P<0.05. **P<0.01, and ***P<0.001 compared to control group. $^{\#}P$<0.05, $^{\#\#}P$<0.01, and $^{\#\#\#}P$<0.001 compared to AH-treated group, GG: *Glycyrrhiza glabra*, AH: Aniline hydrochloride

rats might be due to toxicity of aniline which decreased the food consumption which can directly corelated to decreased body weight. One of the important features of this study was increase in the weights of spleen (splenomegaly) in AH-treated rats and changes in blood parameters. The splenomegaly, presumably due to excessive deposition of PHA-modified erythrocytes, will also increase the like hood of release of the metabolite in the red pulp during erythroclasia. The metabolite could bind with splenic mesenchymal tissues, causing injury, and/or deleterious effects.[16] The changes observed in the blood parameters were rather expected and consistent with those of earliest studies on aniline and its derivatives.[17] These changes in the blood parameters were closely associated with simultaneous enlargement in the spleen in a time dependent manner; splenomegaly appears to be due to excessive deposition of chemically damaged erythrocytes.[18] This deposition would also increase aniline and/or its metabolites (PHA-modified) in the red pulp of spleen which on subsequent breakdown from damaged erythrocytes could bind with splenic mesenchymal tissue, causing injury, and/or deleterious effects. In the present study, AH-administered rats displayed significant increase in iron load and decreased in protein contents. Lipid peroxidation and protein oxidation are at least two important early biochemical events in AH-induced splenic toxicity. It is also apparent that iron may play a significant role as a mediator of AH-induced splenotoxicity.[1] Aniline treatment causes remarkable accumulation of iron in the spleen in a time dependent manner. This accumulated iron may catalyze excessive formation of reactive oxygen species (ROS), which can react with and damage proteins, nucleic acids, and lipids, leading to cellular dysfunction. The aniline exposure leads to iron overload and induction of lipid peroxidation (oxidative stress) in the spleen, which are accompanied by such morphological changes as vascular congestion, increased red pulp cellularity due to increased sinusoidal cells and fibroblasts, capsular thickening, and formation of fibrous tissue in the capsule and throughout the parenchyma.[19] The increases in the AH-induced splenic lipid peroxidation and protein oxidation are consistent closely associated with the accumulation of iron in the spleen. Aniline treatment resulted in greater formulation of malonaldehyde (MDA)-protein adducts in the spleen, suggesting that MDA generated as a consequence of lipid peroxidation produces structural modification of native proteins, which can alter their functional properties and, thus, contribute to splenic toxicity induced by aniline.[19] An association between increased iron deposition and development of fibrotic lesions in spleen of the aniline-treated rats presumably due to iron mediated production of reactive oxygen species (ROS) which might act as a stimulus for increased collagen production in splenic tissue, leading to fibrosis. We suspect that the iron deposition in the spleen may result in the formation of ROS, which can react with and damaged protein, nucleic acid, and lipids, particularly the fatty acid component of membrane phospholipids, leading to cellular dysfunction and development of oxidative stress.[19] In the present study, markers of oxidative stress such as lipid peroxidation, GSH, and NO were evaluated. AH-induced group showed a significant increase in LPO and NO, whereas a significant decreased in GSH level in spleen was observed. Oxidative stress plays key role in splenic toxicity induced by aniline. Aniline induces lipid peroxidation and protein oxidation in the spleen suggests that oxidative stress plays a role in the splenic toxicity of aniline. Oxidative stress occurs when there is an excessive production of ROS or when total antioxidant capacity decreases. It suggested that involvement of oxidative stress in the splenotoxicity of aniline may be caused by its reactive metabolite (s) such as PHA. GG is reported to play a major role in the treatment of infectious diseases such as hypertension, diabetes, as an antioxidant, hepatoprotective, anti-inflammatory, as an immunomodulator, etc. GG was reported to exhibit antioxidant activity which can modify serum lipid level.[20] GG treatments reverse the changes in body weight, feed consumption, water intake, and also show the increase in hemoglobin, RBC, clotting time, total protein, and decrease in total iron content. This prevention might be due to the strong antioxidant/free radical scavenging

activity of GG.[21] Increases the level of LPO and NO during toxicity may be damaged to spleen. The process of lipid peroxidation could lead to numerous cytotoxic degradation products including MDA and unsaturated aldehyde such as 4 hydroxy-2-nonenal (4-HNE) that are implicated in mutagenesis, tumorigenesis, and initiated and promotion of carcinogenesis. The reactivity of MDA with proteins has been known for many years, and it has been shown that per oxidative stress leads to increased formation and degradation of MDA-modified proteins. MDA binds to DNA and is mutagenic and, therefore, may initiate tumor formation. Likewise, 4-HNE is also very reactive with proteins, to be responsible for cytopathological effect observed during oxidative stress produced toxic effect on the splenic cell leading to splenic toxicity.[1] The total NO, an indicator of nitrosative stress, is increased in the experimental model of AH-induced toxicity. GG treatment showed the attenuation of increased lipid and NO level which might be due to its ROS inhibitory potential as well as potent free radical scavenging activity. In the present study, we found good antioxidant activity of GG against free radical activity. The elevation of GSH level by GG has important implication due to the fact that the glycyrrhizin, an already known anti-inflammatory compound, has also been found as the first plant-based inhibitor of thrombin. It prolonged the thrombin and fibrinogen clotting time and increased plasma recalcification duration. The thrombin-induced platelet aggregation was found to be inhibited by the action of glycyrrhizin, but platelet aggregating factor or collagen-induced agglutination was not affected by glycyrrhizin, flavonoids play a very important protective role against the damaging effect of toxic oxides radicals such as super oxide (O_2), hydroxyl radical (OH) and toxic peroxides such as hydrogen peroxide, and other peroxides (ROOH). These highly reactive species are thought to be partly responsible for the destruction of the spleen cells of the spleen in splenic toxicity. High phenolic content compounds present in GG. It is responsible for its strong antioxidant activity due to free radical scavenging, metal ion chelating, hydrogen-donating, anti-lipid per oxidative, and reducing activities. GG flavonoids have 100 times strong antioxidant activity when compared with antioxidant activity of vitamin,[22] in animal and human models glycyrrhetic acid plays a protective role when liver cells are challenged. It decreases inflammatory states by reducing cytokines such as tumor necrosis factor-alpha and increasing protective antioxidants like hemeoxygenase-1.[23] The aniline has been reported to give rise to superoxide (O2-) and destroys the splenic cells and causes splenic toxicity. GSH level has been reported to be diminished in toxicity of spleen. GG treatment at varying doses increased the diminished GSH level might be due to attenuated the ROS production by destruction of the spleen cells of the spleen in splenic toxicity.

CONCLUSION

It has been observed that chronic exposure to AH increased the free radical generation and toxicity of spleen in experimental animals. It was also aimed at to explore the possibility of use of GG in AH-induced free radical release and splenic toxicity. It has been observed that GG exhibits a significant antioxidant and splenoprotective effect in experimental animals exposed to AH.

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