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Protective Effect of Ferulic Acid Alone and in Combination with Ascorbic Acid on Aniline Induced Spleen Toxicity

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Abstract

The present study was design to evaluate the protective effects of ferulic acid and ascorbic acid on aniline induced spleen toxicity in rats. Wistar rats of either sex were used in the study. Spleen toxicity was induced by aniline hydrochloride (100 ppm) in drinking water for 30 days. Treatment group received ferulic acid (40 mg/kg/day, p.o) and ascorbic acid (40 mg/kg/day, p.o) alone and in combination for 30 days. At the end of treatment period serum and tissue parameters were evaluated. Aniline hydrochloride treated rats showed a significant alteration in body weight, spleen weight, feed consumption and water intake, haematological parameters (Haemoglobin, Red Blood Cells, White Blood Cells and Total Iron Content), biochemical parameters (total iron content, total protein, Lipid peroxidation, Reduced glutathione, nitric oxide) and Membrane bound phosphatase (ATPase). Treatment with combination of ferulic acid and ascorbic acid 40 mg/kg respectively showed a significant recovery in aniline induce spleen toxicity. Combination of ferulic acid and ascorbic acid showed better effects than alone antioxidants in aniline hydrochloride induced spleen toxicity.

Keywords: Aniline hydrochloride; Spleen toxicity; Ferulic acid; Ascorbic acid; Antioxidant

Introduction

Exposure to aniline is reported to produce spleen toxicity in rats. Aniline is reported to produce iron overload, protein oxidation, oxidative stress and methemoglobin in spleen [1]. The clinical symptom of aniline exposure includes cyanosis, weakness, dizziness, headache, stupor, loss of coordination, and coma [2]. Protection against damage by free radicals can be enhanced by the intake of antioxidants. Ferulic acid (4-hydrooxy-3-methoxycinnamic acid, FA) is a polyphenolic compound with strong antioxidant property [3]. Ascorbic acid (vitamin C, AA) is a monosaccharide antioxidant found in both animals and plants .The enolic form of AA (3-keto L gluco furanolactone) involved in tissue metabolism and is connected with numerous electron transport processes. AA is reducing agent can reduce and neutralize reactive oxygen species (ROS) such as hydrogen peroxide [4]. Oxidative stress is one of the contributing factors in spleen toxicity. FA and AA acts as potent antioxidant. Studies reported that the use of combination of antioxidants gives better/synergistic effects in disease management [5]. Till date no such study been carried out to evaluate the effects of combine antioxidant therapy in aniline induced toxicity. Based on this hypothesis the present study was design to screen the effects of FA and AA alone and in combination in aniline hydrochloride (AH) induced spleen toxicity.

Material and Methods

Drugs and Chemicals

Ferulic acid was procured from (Otto Kemmi PVT LTD Mumbai, India). Ascorbic acid was procured from Sigma Aldrich, USA. Aniline hydrochloride (AH), 2, 2-dipyridyl, 5, 5-dithiobis-(2-nitrobenzoic acid) and N-(1-Napthyl) ethylenemine dihydrochloride were purchased from Hi Media Lab. Pvt Ltd, Mumbai. All the other chemicals used in the study were of analytical grade and procured from standard supplier.

Preparation of drug solutions

Ferulic acid was suspended in carboxyl methyl cellulose (CMC) 1%, solution prepared in distilled water, ascorbic acid was dissolved in distilled water and 100 ppm (100 mg/liter) of Aniline hydrochloride was prepared in distilled water. All the drug solutions were freshly prepared before starting experiment.

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Table 1: Effect of FA and AA alone and in combination on body weight, spleen weight, water intake and feed consumption in AH-treated rats.

Parameters	Gr.I	Gr.II	Gr.III	Gr.IV	Gr.V	Gr.VI
Body weight (g)	306.2±9.711	217.0±4.779***	258.9±0.785##	259.7±2.605 ##	294.8±14.45###	257.4±3.959 ##
Spleen weight (g)	0.709±0.027	1.331±0.036***	1.001±0.076##	0.997±0.081##	0.650±0.055###	0.999±0.084##
Water intake (ml)	38.00±0.730	17.18±0.718***	20.34±0.399#	20.88±0.416##	39.21±0.747###	21.17±0.670##
Feed consumption (g)	19.35±0.448	12.37±0.419***	15.24±0.924#	15.39±0.997#	20.35±0.458###	16.26±0.408##

The results were expressed as mean ± SEM (n=6). The data was analyzed using ANOVA followed by Dunnett's't' test (Graph Pad Prism 5). *p<0.05, **p<0.01, ***p<0.001compared with control group. #p<0.05, ##p<0.01, ###p<0.001compared aniline hydrochloride treated group.

Table 2: Effect of FA and AA alone and in combination on haemoglobin, RBCs and WBCs in AH-treated rats.

Blood content	Gr.I	Gr.II	Gr.III	Gr.IV	Gr.V	Gr.VI
Hb (gm/dl)	13.25±0.172	4.917±0.200***	7.554±0.506##	7.346±0.4263##	15.55±0.291###	8.744±0.736###
RBCs (×10⁶/cells)	8.060±0.259	4.644±0.202***	6.294±0.259##	6.371±0.326##	9.652±0.284###	7.177±0.409###
WBCs (×10⁶/cells)	10.26±0.220	17.29±0.751***	15.18±0.507#	15.07± 0.478#	10.54±0.216###	14.34 ±0.668##

The results were expressed as mean ± SEM (n=6). The data was analyzed using ANOVA followed by Dunnett's't' test (Graph Pad Prism 5). *p<0.05, **p<0.01, ***p<0.001compared with control group. #p<0.05, ##p<0.01, ###p<0.001compared aniline hydrochloride treated group.

Animals

Adult albino rats of either sex (200-250 gm) were divided in different groups, each group contains six rats. The animals were procured from LACSMI Biofarm, AUNDH (Pune). Rats were placed separately in polypropylene cages with paddy husk as bedding. The animals were maintained under standard laboratory condition as suggested by CPCSEA. Animals had free access to water and standard laboratory feed (Nutrivet Lab, Pune, India) prior to the dietary manipulation. The experimental procedures and protocols used in this study was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of SSDJ College of pharmacy, Neminagar, Chandwad.

Experimental design

The rats were divided into different groups. Group I: served as normal control and received CMC (1%), orally as vehicle, group II: rats received AH (100 ppm) in drinking water for 30 days, group III: rats received AH (100 ppm) via drinking water and FA (40 mg/kg/day, p.o), for 30 days, group IV: rats received AH (100 ppm) via drinking water and AA (40 mg/kg/day, p.o), for 30 days, group V: rats received AH (100 ppm) and FA+AA (40+40 mg/kg/day, p.o), for 30 days, group VI: rats received AH (100 ppm) and FA+AA (20+20 mg/kg/day, p.o), for 30 days.

Assessment of biochemical parameters

General parameter like body weight, spleen weight, water intake, and feed consumption were studied in between and at the end of experiment. Blood was withdrawn and used for the estimation of hemoglobin (Sahli's haemometer method), red blood cell (RBCs) and white blood cells (WBCs) count using hemocytometer [6]. Serum was used for the estimation of iron content [7], 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, p^H 7.4) to a concentration of 10% w/v. The homogenate was centrifuged at 10,000 rpm at 0^0C for 15 min using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the determination of lipid peroxidation [8], reduced glutathione (GSH) [9] and nitric oxide (NO) [10] level whereas the sediment was used for the estimation of membrane bound phosphatase such as Na^+/K^+ ATPase [11], Ca^{++}ATPase [12], and Mg^{++}ATPase [13].

Statistical analysis

The results were expressed as mean ± SEM. The data was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's'*t*' test as appropriate using computer based fitting program (Graph Pad Prism 5). Differences were considered to be statistical significant when $p<0.05$.

Results

Effect of FA and AA alone and in combination on body weight, spleen weight, water intake and feed consumption in AH-treated rats.

As shown in Table 1, AH treatment for 30 days showed a significant alteration in body weight, spleen weight, water intake and feed consumption as compared to control rats. Treatment with FA, AA, FA+AA (40+40 mg/kg/day, p.o each)and FA+AA(20 mg/kg day, p.o each) showed significant recovery in alteration body weight, water intake, feed consumption and spleen weight as compared to AH-treated rats. The combination of FA+AA (40+40 mg/kg/day, p.o each) showed better effect as compare to other treatment (Table 1).

Effect of FA and AA alone and in combination on haemoglobin level, RBCs and WBCs count in AH-treated rats.

The RBCs and hemoglobin count was 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 FA, AA, FA+AA (40+40 mg/kg/day, p.o each) and FA+AA (20 mg/kg day, p.o each)along with AH shows significantly (p<0.001) increased in haemoglobin level and RBCs count and significantly (p<0.001) decrease in WBCs count as compare to AH-treated rats. FA+AA (40+40 mg/kg/day, p.o each) showed better effect as compare to alone drug and combination of FA+AA (20 mg/kg day, p.o each) (Table 2).

Effect of FA and AA alone and in combination on total iron and total protein content in AH-treated rats.

A significant ($P < 0.001$) increased in the level of total iron and a significant ($P < 0.001$) decreased in total protein content was observed in AH-treated group as compared to control group. Treatment with FA, AA, FA+AA (40+40 mg/kg/day, p.o each)and FA+AA (20 mg/

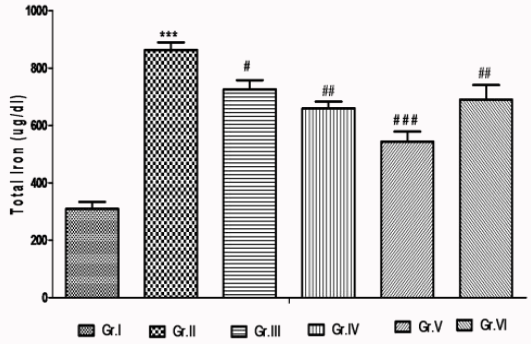

Figure 1: Effect of FA and AA alone and in combination on total iron content in AH-treated rats.
The results were expressed as mean ± SEM (n=6). The data was analyzed using ANOVA followed by Dunnett's't' test (Graph Pad Prism 5). *p<0.05, **p<0.01, ***p<0.001compared with control group. #p<0.05, ##p<0.01, ###p<0.001compared aniline hydrochloride treated group.

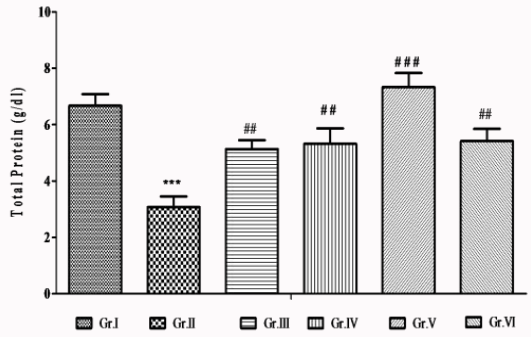

Figure 2: Effect of FA and AA alone and in combination on total protein in AH-treated rats.
The results were expressed as mean ± SEM (n=6). The data was analyzed using ANOVA followed by Dunnett's't' test (Graph Pad Prism 5). *p<0.05, **p<0.01, ***p<0.001compared with control group. #p<0.05, ##p<0.01, ###p<0.001compared aniline hydrochloride treated group.

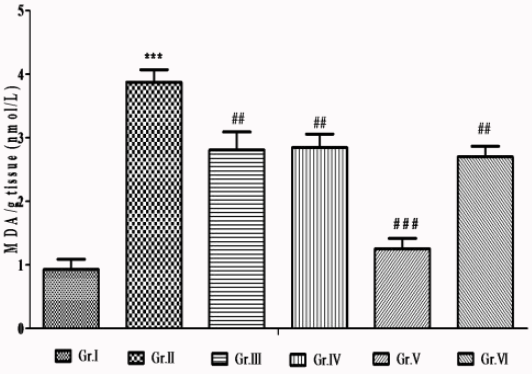

Figure 3: Effect of FA and AA alone and in combination on lipid peroxidation in AH-treated rats.
The results were expressed as mean ± SEM (n=6). The data was analyzed using ANOVA followed by Dunnett's't' test (Graph Pad Prism 5). *p<0.05, **p<0.01, ***p<0.001compared with control group. #p<0.05, ##p<0.01, ###p<0.001compared aniline hydrochloride treated group.

kg day, p.o each) along with AH significantly(p<0.001) lowered total iron content and increased total protein content (Figure 1 and 2).

Effect of FA and AA alone and in combination on tissue lipid peroxidation (LPO), reduced glutathione (GSH), and serum nitric oxide (NO) levels in AH-treated rats.

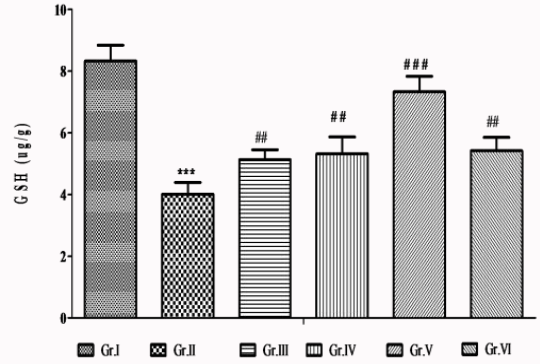

Figure 4: Effect of FA and AA alone and in combination on reduced glutathione in AH-treated rats.
The results were expressed as mean ± SEM (n=6). The data was analyzed using ANOVA followed by Dunnett's't' test (Graph Pad Prism 5). *p<0.05, **p<0.01, ***p<0.001compared with control group. #p<0.05, ##p<0.01, ###p<0.001compared aniline hydrochloride treated group.

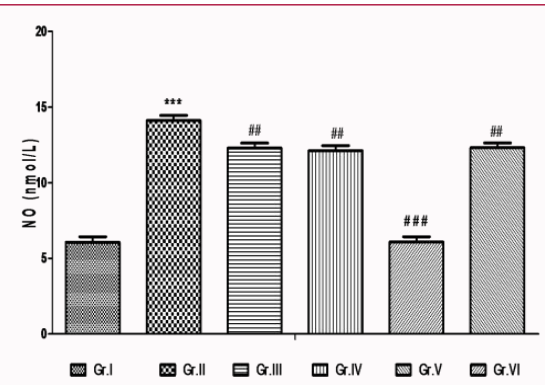

Figure 5: Effect of FA and AA alone and in combination on nitric oxide in AH-treated rats.
The results were expressed as mean ± SEM (n=6). The data was analyzed using ANOVA followed by Dunnett's't' test (Graph Pad Prism 5). *p<0.05, **p<0.01, ***p<0.001compared with control group. #p<0.05, ##p<0.01, ###p<0.001compared aniline hydrochloride treated group.

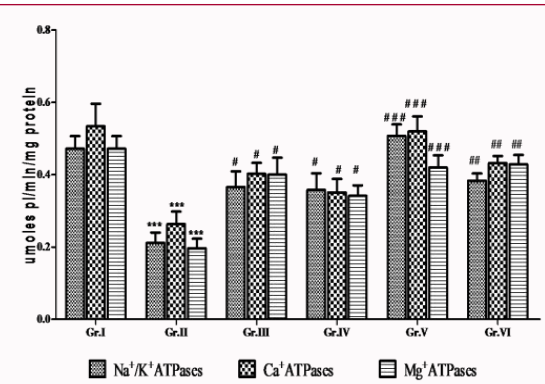

Figure 6: Effect of FA and AA alone and in combination on Na^+/K^+ ATPase, Ca^{++} ATPase andMg^{++}ATPase in AH-treated rats.
The results were expressed as mean ± SEM (n=6). The data was analyzed using ANOVA followed by Dunnett's't' test (Graph Pad Prism 5). *p<0.05, **p<0.01, ***p<0.001compared with control group. #p<0.05, ##p<0.01, ###p<0.001compared aniline hydrochloride treated group.

Aniline treated group showed a significant (p<0.001) elevation of LPO, nitrite content and a significant (p<0.001) reduction of GSH activity, compared to normal control rats. Ttreatment with FA alone and in combination FA+AA (40+40 mg/kg/day, p.o each)

and FA+AA (20 mg/kg day, p.o each) along with AH significantly ($p<0.001$) lowered LPO level and NO whereas GSH content was found to be increased ($P < 0.001$) (Figure 3-5).

Effect of FA and AA alone and in combination on membrane bound phosphatases (Na^+/K^+, Ca^{++}, Mg^{++} ATPase) in AH-treated rats.

The activity of Na^+/K^+, Ca^{++} and Mg^{++} ATPase level was significantly ($p<0.001$) decreasedin AH-treated group as compared to control group.Treatment with FA, AA, FA+AA(40+40 mg/kg/day, p.o each)and FA+AA (20 mg/kg day, p.o each) showedsignificant ($p<0.001$) increase in the level of Na^+/K^+, Ca^{++} and Mg^{++} ATPase as compared with AH-treated group (Figure 6).

Discussion

Aniline and substituted aniline exposure leads to the development of selective spleen toxicity in rats. Studies have shown that exposure to aniline produces substantial increase in oxidative stress in rats as well as it leads to enlargement of spleen due to excess deposition of damaged RBC [1,2]. In present study, spleen toxicity was induced by chronic administration of AH (100 ppm) via drinking water. Not only increased in the level of lipid peroxidation, nitric oxide but also decrease in the level of reduced glutathione showing consequence of oxidative stress which occurs when the dynamic balance between pro-oxidant and antioxidant mechanism is impaired in the body. The present study showed an increased in the concentration of mitochondrial thiobarbituric acid reactive substances in aniline intoxicated rat indicating increased lipid peroxidation, which could be attributed to a deficiency of antioxidant defense mechanism. Significant decreased in body weight, feed consumption and water intake in AH-treated rat might be due to toxicity of aniline which decreased food consumption and can be directly correlated to decrease body weight [14]. One of important feature of this study was that the spleen in AH-treated rat showing increase in spleen weight. FA is reported to play a major role in treatment of various conditions such as, lipid lowering activity, anticarcinogenic [15], antidiabetic [16] anti-hypertensive [17]. Scurvy is a vitamin C deficiency disease which is well known treated with AA or Vitamin C, AA is also used in various diseases like anemia, bleeding gum, and wound healing, dry and separated strands in hair [18]. FA and AA in combination show the synergistic activity and it was reported to exhibit antioxidant activity which can modify serum lipid level. In the present study, the combination of the FA and AA reverse the changes in body weight, water intake and feed consumption in AH-treated animals. The changes in the general parameters suggested the positive effect of FA and AA in the AH-toxicity.

Alteration in the level of hemoglobin, RBCs and WBCs were observed. These changes are due to excessive deposition of phenyl hydroxylamine (PHA)-modified erythrocytes [1]. The changes in WBCs count might be due to excessive generation of oxidative and nitrosative stress. The changes observed in the blood parameter were similar to previous studies on aniline and its related derivatives [2].Treatment with FA and AA showed significant alteration of hemoglobin level, RBCs and WBCs content, which might be due to the strong antioxidant or free radical scavenging activity of FA and AA. In the present study AH-treated rats showing a significant decrease in the protein content and significantly increase in iron load. Iron plays important role in spleno toxicity. Aniline administration causes remarkable aggregation of iron which may catalyze the excessive formation of reactive oxygen species, which reacts and damaging proteins, nucleic acid, and lipids, leading to cellular dysfunction [19]. Lipid peroxidation and protein oxidation are at least two important early biochemical events in aniline induced toxicity.

AH-treated group showed a significant increase in LPO and NO (it forms a part of reactive nitrogen species); whereas, a significant decrease in GSH level in spleen. Oxidative stress plays a vital role in toxicity induced by aniline. Aniline induces lipid peroxidation and protein oxidation suggested the role of oxidative stress in spleen toxicity. AH-treatment resulted in significant formation in LPO, suggested that lipid peroxidation produces structural modification of native protein, which can alter their functional properties and thus contributing the spleen toxicity. Total nitric oxide an indicator of nitrosative stress, is increased in the aniline induced spleen toxicity [1,19,20]. Na^+/K^+ ATPase, Ca^+ ATPase and Mg^+ ATPase play a significant role in ionic movement. These enzymes are located in the outer cell membrane and could have been affected by the excessive production of free radical induced by aniline which may affects or alter the energy production [11-13].

In the present study, we have observed that the combination of FA and AA offered better protection to the spleen when compared with individual treatment of FA as well as AA. We suggest that a possible mechanism for the observed synergistic effect could be due to the AA quenching the radical itself and thereby protecting the FA. The another possible mechanism to attenuate the spleen toxicity induced by aniline which might be due to its inhibitory potential of reactive oxygen species as well as potent free radical scavenging activity. In conclusion, present study demonstrate the combined splenoprotective effect of FA and AA the spleen toxicity induced by aniline.

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References

1. Khan R, Upaganlawar A, Upasani C. Protective effect of *Dioscorea Alata* L. in aniline induced spleen toxicity in rats: A Biological Study. Toxicol Int. 2014; 21: 294-299.
2. Khan M F, Kannan S, Wang J. Activation of transcription factor AP-1 and mitogen-activated protein kinases in aniline-induced splenic toxicity. Toxicol Appl Pharmacol. 2006; 210: 86-93.
3. Srinivasan M, Sudheer AR, Menon VP. Ferulic acid: therapeutic potential through its anti-oxidant property. J Clin Biochem Nutr. 2007; 40: 92.
4. Odin A P. Vitamins as antimutagens: Advantages and some possible mechanisms of antimutagenic action. Mutat Res. 1997; 386: 39- 67.
5. Yogeeta S K, Guanapragasam A, Senthi K S, Subashini R, Sathivel A, Devaki T. Synergistic interaction of ferulic acid with ascorbic acid: its cardio protective role during isoproterenol induced myocardial infarction in rats. Mol Cell Biochem. 2006; 283:139-146.
6. Godkar PB, Godkar DP. Determination of haemoglobin. Text Book of Medical Laboratory Technology, 2nd ed. Mumbai, India: Published by Balani Publishing House 2008; 726-731.
7. Ramsay WNM. The determination of total iron-binding capacity of serum. Clin Chim Acta. 1957; 2: 221-226.
8. SlaterT F, Sawyer BC. The stimulatory effect of carbon tetrachloride and other halogen alkane or peroxidative reaction in the rat liver functions *in vitro*. Biochem J. 1971; 123: 805-815.

9. Moron MS, Depierre JW. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochim Biophys Acta. 1979; 582: 67-78.

10. Guevara I, Iwanejko J, Dembinska-Kiec A, Pankiewicz J, Wanat A, Anna P, et al. Determination of nitrite/nitrate in human biological material by the simple Griess reaction. Clin Chim Acta. 1998; 274: 177-188.

11. Bonting SL. Presence of enzyme system in mammalian tissues. Membrane and ion transport. Wiley International Science. 1970: 257-263.

12. Hjerkin S, Pan H. Purification and characterization of two forms of low affinity calcium ion ATPase from erythrocytes membrane. Biochem Biophys Acta. 1983; 728: 281-288.

13. Ohinishi T, Suzuki Y, Suzuki T, Ozawa KA. Comparative study of plasma membrane magnesium ion ATPase activities in normal regenerating and malignant cells. Biochim Biophysica Acta. 1982; 684: 64-67.

14. Barone E, Calabrese V, Mancuso C. Ferulic acid and its therapeutic potential as a hormetin for age-related diseases. Biogerontology. 2009; 10: 97-108.

15. Upasana Khairnar, Aman Upaganlawar, Chandrashekhar Upasani. Ameloriative effect of chronic supplementation of Protocatecheuic acid alone and in combination with Ascorbic acid in aniline hydrochloride induced spleen toxicity in rats. Scientifica. 2016; 1-9.

16. Balasubashini MS, Rukkumani R, Vishwanathan P, Menon VP. Ferulic acid alleviates lipid peroxidation in diabetic rats. Phytother Res. 2004; 18: 310-314.

17. Suzuki A, Kagawa D, Fujii A, Ochiai R, Tokimitsu I, Saito I. Short-and long-term effect of ferulic acid on blood pressure in spontaneously hypertensive rats. Am J Hypert. 2002; 56: 7644-7648.

18. Kojo S. Vitamin-C basic metabolism and its function as an index of oxidative stress. Curr Med Chem. 2004; 11: 1041-1064.

19. Omer M, Upaganlawar A, Upasani C. DL-α-Lipoic Acid Attenuates Acute Aniline Induced Splenic Toxicity in Rats: A Biochemical and Histoarchitecture Study. Asian J Pharmacol Toxicol. 2015; 03: 4-7.

20. Bus JS, Popp JA. Perspectives on the mechanism of action of the splenic toxicity of aniline and structurally related compounds. Food Chem Toxicol. 1987; 25: 619-626.

21. Chojkier M, Houglam K, Olis-Herruzo J. Stimulation of collagen gene expression by ascorbic acid in cultured human fibroblasts. A role for lipid peroxidation. J Biolo Chem. 1989; 264: 16957-16962.