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Research Article

Splenoprotective Effect Of Kudzu Root Extract In Aniline Induced Spleen Toxicity In Rats

Sumitkumar Sharma, Md. Aakif Bagwan, Mangesh Tatar, Dr. Aman Upaganlawar*, Dr. Chandrashekar Upasani

Department of Pharmacology, SNJB's Shriman Sureshdada Jain College of Pharmacy, Chandwad Dist: Nashik, India

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ABSTRACT

Introduction: This study was designed to evaluate the Splenoprotective of Kudzu Root Extract on biochemical and hematological changes in aniline induced spleen toxicity in rats**. Materials and Methods:** Wistar rats of either sex (250-250g) were used and divided into five groups each group contains six rats. In rats spleen toxicity was induced by administration of Aniline Hydrochloride (200ppm) in drinking water for a period of 30 days. Control group received water as vehicle and Treatment group (iii, iv and v) received Kudzu Root Extract (25/50/100mg/kg/day, p.o.) along with Aniline Hydrochloride. At the end of treatment various serum and tissue parameters were evaluated. **Results:** Rats administered with AH (200 ppm) in drinking water for 30 days showed a significant alteration in general parameters (body weight, organ weight, feed consumption, water intake and fecal matter appearance), hematological parameters (red blood cell (RBC), white blood cell (WBC), and hemoglobin content), and biochemical parameters(total iron content, protein content, lipid peroxidation, reduced glutathione (GSH), Superoxide dismutase (SOD) Catalase (CAT) and nitric oxide (NO) content) of spleen. Treatment with KRE (50 and 100 mg/kg/day, p.o.) for 30 days along with AH showed significant recovery in aniline-induced splenic toxicity. **Conclusion:** The present result showed the involvement of oxidative and Nitrosative stress in aniline-induced splenic toxicity and KRE protects the rats from the toxicity, which might be due to its antioxidant property and the presence of different phytochemicals.

INTRODUCTION

Aniline is a prototypical aromatic amine.C6H5NH2 is the chemical formula for the organic material aniline. With its two groups—an amino and a phenyl—it is the most basic aromatic amine. In 2020, the global aniline market will

***Corresponding Author: Dr.** Aman Upaganlawar
Address: *Department of Pharmacology, SNJB's Shriman Sureshdada Jain College of Pharmacy, Chandwad Dist: Nashik, India.*
Email ✉: amanrxy@gmail.com

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reach 8.4 million tons. Aniline and its derivatives are used as intermediaries in a wide range of operational fields, such as the synthesis of dyes like fuchsine, safranin, and induline; plasticizers; pharmaceuticals like paracetamol and acetanilide; pesticides; herbicides; polymers including polyurethane and rubber additives; photographic chemicals and varnishes; and precursors of amino aromatic derivatives. [1, 2]

Conversely, exposure to environmental and occupational contaminants may pose a health risk caused by aniline. It enters the human body through the skin, digestive system, and respiratory tract. Exposure to aniline can result in clinical symptoms such as stupor, cyanosis, headache and dizziness, loss of coordination, weakness and coma.

In addition to its widespread use in industrial chemicals that cause hemolytic anaemia, methemoglobinemia, and hemolysis, aniline may also have other effects such as neuron toxicity. Rats are exposed to high doses of the substance over an extended period of time, which damages the spleen and causes fibrosis, hyperplasia, splenomegaly, and eventually the formation of mesenchymal tumors or highly malignant soft tissue and sarcomas [3, 4].

The spleen is the largest lymphoid tissue, the spleen, is involved in immunological responses, iron metabolism, blood filtration, phagocytosis, removal of damaged red blood cells, and removal of infectious organisms, among other critical functions [5, 6]. Consequently, any harm or injury to the spleen results in a decrease in its function. Increases in red pulp cellularity, an increase in macrophages and fibroblasts, and other alterations like iron overload are linked to aniline-induced splenotoxic responses. Oxidative and nitrosative stress then trigger the induction of redox-sensitive transcription factors in the spleen [7].

The main causes of aniline-induced splenic toxicity are (1) oxidative stress, which is demonstrated by increased oxidation (lipid peroxidation, DNA oxidation, protein oxidation, and nitrotyrosine production) (2) excess free and total iron content in the spleen and (3) ethryocyte damage [9–11].

Due to the spleen's production of reactive oxygen species (ROS), iron overload is another notable effect of aniline exposure [11, 13]. Many diseases, including rheumatoid arthritis, ageing, cardiovascular disease, and cancer, are thought to be largely influenced by ROS [14, 15].

Reactive oxygen species (ROS) are generated as byproduct of respiration and oxidative metabolism. [16]

Antioxidants prevent or lessen oxidative damage by scavenging free radicals from bodily cells.

Antioxidant-rich natural products are becoming more and more important in the prevention of diseases where oxidative stress has been implicated.

In traditional Chinese medicine, kudzu root (Pueraria lobata (Willd.) Ohwi) is frequently used as an adjuvant treatment for fever, inflammatory disorders and diarrhea. The primary active ingredient in kudzu root, Puerarin, has significant nutritional and physiological benefits [1-3]. Puerarin has a range of bioactive effects, including antioxidant, blood glucose lowering and blood pressure regulating, anti-inflammatory and cancer prevention, according to contemporary pharmacological research [17].

The current investigation was planned to evaluate various biochemical parameters in order to determine the impact of (kudzu root) on aniline exposure-induced spleen damage in rats, taking into account its strong antioxidant activity and historical use.

MATERIAL AND METHODS

ANIMALS

The study employed 200–250g wistar rats of both sexes, with three male and three female rats in each group. The animals were purchased from AUNDH

(Pune) LACSMI Bio farms. Separate groups of four rats each were housed in polypropylene cages with bedding made of paddy husk. Throughout the experiments, the animals were kept in standard laboratory conditions with a temperature of 23 ± 1°C, relative humidity of 45–55, and a 12-hour light/dark cycle. The SSDJ College of Pharmacy's Institutional Animal Ethics Committee (IAEC) in Neminagar, Chandwad approved the experimental protocol (approval no. SSDJ/IAEC/22-23/03).

Experimental protocol

The animals were divided into six groups (n=6)

Group I Animals served as normal control and received water.

Group II Animals received Aniline Hydrochloride (200ppm) in drinking water for 30days.

Group III Animals, received Aniline Hydrochloride (200ppm) in drinking water and dose of Kudzu root extract 25mg/kg/day, p.o.

GROUP IV Animals, received Aniline Hydrochloride (200ppm) in drinking water and dose of kudzu root extract 50mg/kg/day, p.o.

Group V Animals, received Aniline Hydrochloride (200ppm) in drinking water and dose of kudzu root extract 100mg/kg/day, p.o.

General Parameter

General parameter like Feed consumption, Water intake, body weight and spleen weight as well as fecal matter appearance was also noted

Serum was separated after blood was taken from the retroorbital plexus using a glass capillary at the conclusion of the treatment period.

Blood sample was used for estimation of red blood cell (RBC) and White blood cell (WBC) using hemocytometer and hemoglobin (sahil's hemometer method) and serum sample was used for the estimation of protein content and iron content using span Diagnostic kit.

Assessment of Tissue parameters.

Tissue homogenization

At the end of experimental period, rats were sacrificed by decapitation and isolated spleen was quickly washed with ice-cold tris hydrochloric buffered saline (pH 7.4). The spleen were cross-chopped with surgical scalpel into fine slices, suspended in chilled 0.25M sucrose solution & quickly blotted on filter paper. The tissues were then minced and homogenized in chilled tris hydrochloric buffer (10mM, pH7.4) to a concentration of 10% w/v (Pawar et al., 2021).

Prolong homogenization under hypnotic condition was designed to disrupt, as far as possible, the structure of cells so as to release soluble proteins. The homogenate was centrifuged at 10,000 rpm at 0°C for 15minutes using high speed cooling centrifuged. The clear supernatant was used for the determination of lipid peroxidation (LPO), nitric oxide (NO), superoxide dismutase (SOD), reduced glutathione (GSH), and catalase (CAT) for antioxidants and sediment was used for determination of ATPases.

Assessment of lipid peroxidation (LPO)

After adding two milliliters of the tissue homogenate (supernatant) to two milliliters of freshly made 10% w/v trichloroacetic acid (TCA), the mixture was placed in an ice bath and left for fifteen minutes. The precipitate was separated after 15 minutes by centrifugation (2,000 rpm for 10 min), and 2.0 ml of freshly made thiobarbituric acid (TBA) was combined with 2.0 ml of the clear supernatant solution.

For ten minutes, the resultant solution was heated in a bath of boiling water. After that, it was quickly cooled for five minutes in an ice bath. At 532 nm, the colour developed was measured in comparison to the reagent blank. Different concentrations (0–23 nM) of standard malondialdehyde (MDA; prepared from 1,1,3,3-tetraethoxypropane, obtained from Sigma Chemicals, St Louis, MO, USA) were taken and processed as above for standard graph. The values were expressed as nM of MDA/mg tissue.

Assessment of Nitric oxide (NO)

Add 1 milliliter of Griess reagent to 1 milliliter of tissue homogenate, and then incubate it for 15 minutes at 37°C. Read the absorbance at 540 nm against a Griess reagent blank. The standard was a solution of sodium nitrite. The obtained standard curves were used to estimate the amount of nitrite present in the samples.

Assessment of Glutathione (GSH)

Tissue homogenate (supernatant) and 20% TCA were mixed in equal volumes. Centrifuge the precipitated fraction and add 2ml of DTNB reagent to 0.25ml of supernatant. The final volume was increased to 3ml by adding phosphate buffer. The colour developed was measured at 412 nm against a reagent blank. A graph was created using standard glutathione from Sigma Chemicals, St Louis, MO, USA. Protein content in the spleen was determined using Lowry C and Folin's phenol reagent. GSH levels were expressed as micrograms per milligram of protein.

Assessment of Superoxide dismutase (SOD).

5ml of tissue homogenate was diluted with 0.5ml of distilled water, to which 0.25ml of ice-cold ethanol and 0.15ml ice cold chloroform was added. Mixed the solution properly and centrifuged at 2500rpm for 15 minutes. Then 0.5ml of supernatant was mixed with 1.5ml of carbonate buffer and 0.5ml of EDTA solution. The reaction was initiated by adding 0.4ml of Epinephrine and the change in optical density/minute was measured at 480nm against blank.

SOD concentration was expressed as units/ml. tissue. Change in optical density per minute at 50% inhibition of Epinephrine to adrenochrome transition the enzyme is taken unit. Prepared the calibration curve by 10-125 units of SOD.

Assessment of catalase (CAT)

Mixed 2ml diluted sample with 1ml of Hydrogen peroxide to initiate the reaction. Prepared the blank by adding 2ml of diluted sample in Iml of phosphate buffer (50 mM. pH 7.0). The dilution should be such that the initial absorbance should be approximately 0.50. The decrease in absorbance was measured at 240nm.Catalase concentration was expressed as μmoles of H_2O, consumed/min/mg/protein.

Assessment of Membrane bound phosphate $Na^+/k+$ ATPase, Ca^{++} ATPase and Mg^{++} ATPase.

The membrane fraction is estimated to remain after centrifugation of tissue homogenates. The activities of Na+/K+ ATPase [32], Ca++ ATPase [33], and Mg++ ATPase [34] were assessed. The supernatant's phosphorus content was estimated using Fiske and Subbarow's method [35]. Enzyme activity was measured as μM of inorganic phosphorus liberated per mg protein/min. Potassium dihydrogen orthophosphate (4-20 μg/mL) was used as a phosphorus standard.

Statistical Analysis.

All the values are presented as mean ± SEM. Statistical significance between more than two groups was tested using one-way analysis of variance (ANOVA) followed by Dunnett's test as appropriate using computer based fitting program (Prism 5). Differences were considered to be statistically significant when $p < 0.05$.

RESULTS

Effect of KRE on body weight, food intake, water intake and spleen weight.

Table 1: Effect of KRE on body weight, Feed consumption and water intake.

Parameters	Control	AH (200ppm)	AH+KRE (25mg)	AH+KRE (50mg)	AH+KRE (100mg)
Body weight	272.3 ±1.022	232.8 ±0.792***	237.0 ±0.930###	256.0 ±0.894###	262.8 ±0.600###

Feed Consumption (gm)	19.55 ±0.105	9.54 ±0.135***	14.46 ±0.111###	17.17 ±0.232###	16.12 ±0.257###
Water intake (ml)	40.45 ±0.109	15.74 ±0.111***	25.47 ±0.191###	30.26 ±0.254###	32.24 ±0.184###

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. Treatment with KRE (25/50/100mg/day, p.o.) showed a significant alternation in body weight, water intake and feed consumption as compared with AH treated group.

Table 2. Effect of KRE on spleen weight

Parameters	Control	AH (200ppm)	AH+KRE (25mg)	AH+KRE (50mg)	AH+KRE (100mg)
Weight of spleen (gm)	0.590 ±0.018	1.266 ±0.016***	0.995 ±0.143#	0.990 ±0.012#	0.753 ±0.020###

Spleen weight was monitored at the end of study. A significant ($p<0.001$) increased in spleen weight was observed AH treated group as compared to control group. Treatment with KRE (25/50mg/day, p.o.) showed significantly ($p<0.05$) restored the weight of spleen as compared to AH – treated group. Treatment with KRE100mg/day, p.o.) Showed significant ($p<0.001$) improvement in weight of spleen as compared with AH – treated group.

Effect of KRE on biomarkers of oxidative stress

Effect of KRE on Nitric Oxide (NO) level.

The Nitric Oxide level (Figure 1a) was monitored in all group. It was observed that the concentration of NO in homogenate of spleen tissue was significantly ($p<0.001$) elevated in AH - treated rats as compared to control rats. AH treated rats administered with KRE (25mg/kg/day, p.o.) showed a significant ($p<0.01$) reduction in NO level in spleen and treatment with KRE (50/100mg/kg/day, p.o.) was found to be more effective as compared to AH - treated rats.

Effect of KRE on Lipid Peroxidation (LPO) level.

Lipid peroxidation (Figure 1b) in spleen homogenate was significantly ($p<0.001$) increased in AH –treated rats as compared to Control animals. Treatment with KRE (25mg/kg/day, p.o.) showed a significant ($p<0.01$) marginal reduction in LPO level in spleen as compare to AH – treated group. Treatment with KRE (50/100mg/kg/day, p.o.) was found to be more effective in maintaining LPO level as compared to AH -treated rats.

Effect of KRE on Reduced Glutathione (GSH) level.

After extensive administration of hemolytic compounds depleted erythrocyte – reduced glutathione. GSH level (Figure 1 c) was significantly ($p<0.001$) decreased in AH - treated rats as compared to control animals. Treatment with KRE (25/mg/kg/day, p.o.) for 30 days along with AH showed significantly ($p<0.05$) slight increase and treatment with KRE (100mg/kg/day, p.o.) for 30 days along with AH showed significant ($p<0.01$) increase in GSH level in spleen as compare to AH - treated rats. Treatment with KRE (50mg/kg/day, p.o.) showed significantly ($p<0.001$) improvement in the GSH level as compared to AH - treated rats.

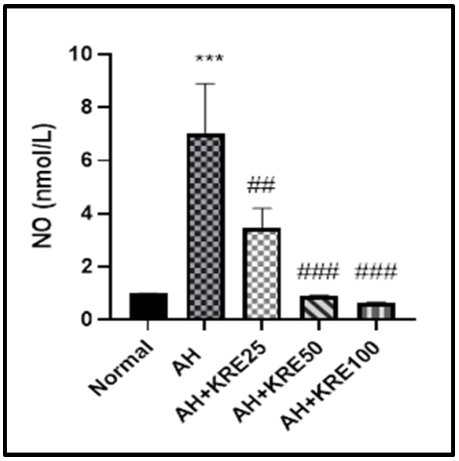

Figure 1 a

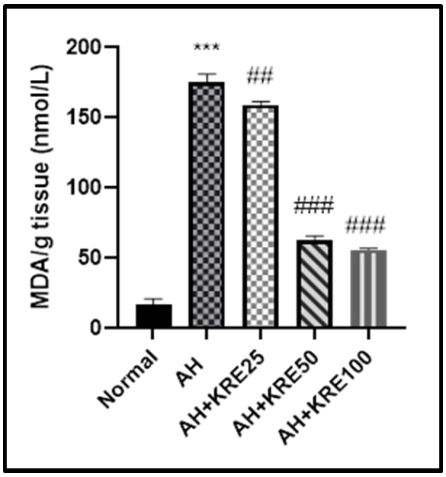

Figure 1 b

Values are expressed as mean ± SEM, (n=6). One way ANOVA and Dunnett't' 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.

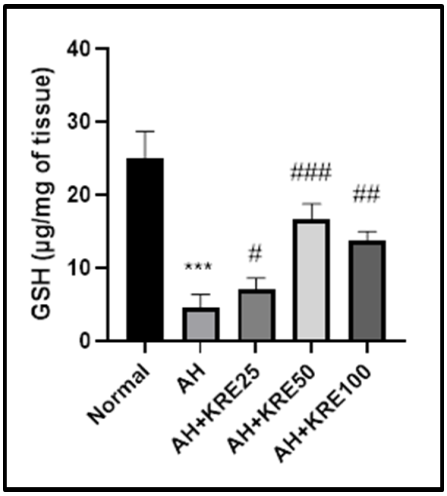

Figure 1 c

Values are expressed as mean ± SEM, (n=6). One way ANOVA and Dunnett't' 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.

Effect of KRE on Catalase (CAT) level.

The catalase level (Figure 1 d) in the homogenate of spleen tissue was monitored in all groups. AH – treated rats showed significant (p<0.001) reduction in level of CAT due to alternation in oxidative stress markers when compared to the control groups. Treatment with KRE (25/50mg/kg/day, p.o.) showed significant (p<0.01) increase in concentration of SOD. Whereas treatment with KRE (100mg/kg/day, p.o.) showed significant (p<0.001) elevation in concentration of catalase as compared to AH - treated group.

Effect of KRE on Superoxide Dismutase (SOD) level

SOD level (Figure 1 e) was significantly (p<0.001) decreased in AH - treated rats as compared to control animals. Treatment with KRE (25/50mg/kg/day, p.o.) for 30 days showed significant (p<0.01) increase in SOD level as compare to AH - treated group. Treatment with KRE (100mg/kg/day, p.o.) showed better recovery in SOD level as compared to AH - treated group.

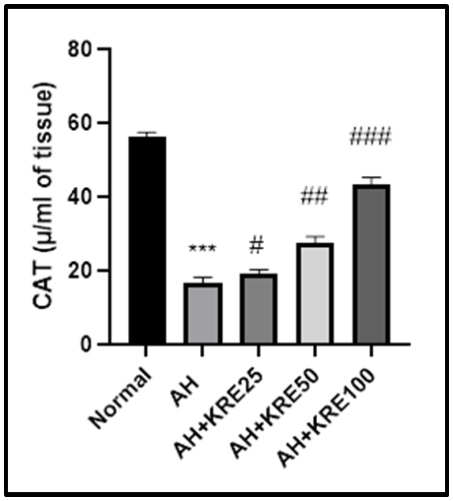

Figure 1 d

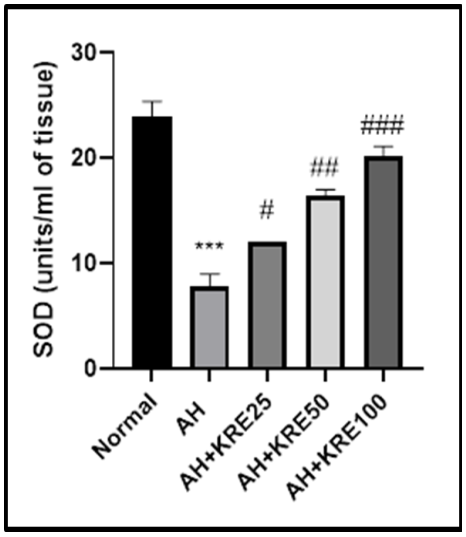

Figure 1 e

Values are expressed as mean ± SEM, (n=6). One way ANOVA and Dunnett't' 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.

Effect of KRE on Na^{+}/K+ ATPase, Ca^{++} and Mg^{++} ATPase.

In AH-treated group the activity of Na^{+}/K+, Ca^{++} and Mg^{++} ATPase level was significantly (p<0.001) decreased as compared to control group animals. Treatment with KRE (25/50/100mg/kg/day, p.o.) showed significant (P<0.001) increase in the level of Na^{+}/k^{+}, Ca^{++} and Mg^{+} ATPase as compare with AH-treated group (Figure 2a, 2b and 2c).

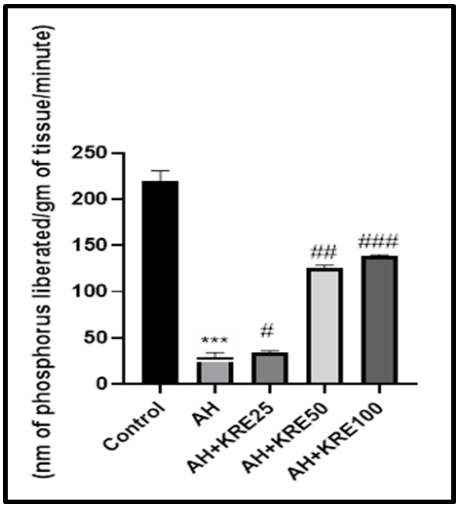

Figure 2a. Effect of KRE on Na^{+}/K^{+}

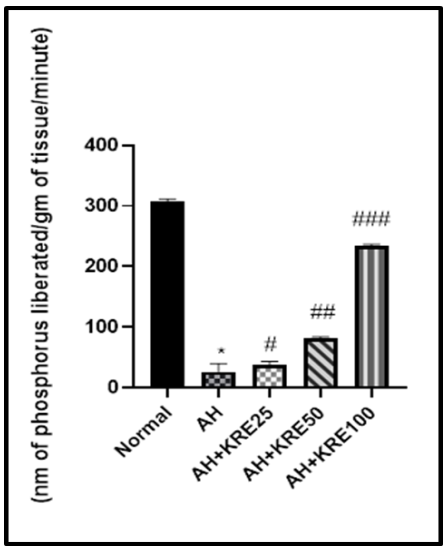

Figure 2b. Effect of KRE on ca^{++}

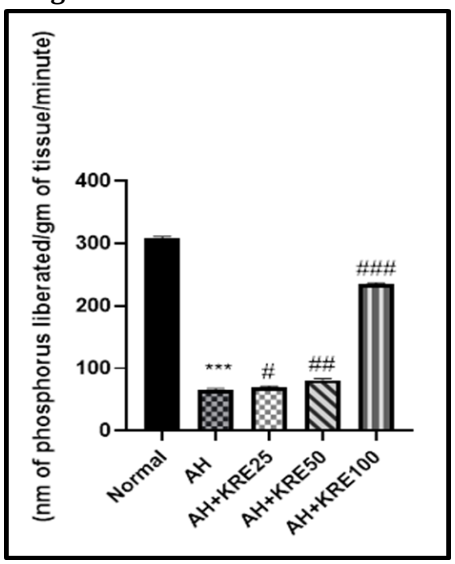

Figure 2c. Effect of KRE on Mg^{++}

Values are expressed as mean ± SEM, (n=6). One way ANOVA and Dunnett't' 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.

Effect of KRE on RBC, WBC and hemoglobin level

The RBC count was significantly (P<0.001) decreased and WBC count was significantly (p<0.001) increased in AH - treated rats as compared to control animals. The level of hemoglobin was found to be significantly

(p<0.001) decreased in AH - treated rats as compared to control group.

The chronic (30 days) treatment with KRE (25/50mg/kg/day, p.o.) showed a significant (p<0.05) slight decrease in WBC and treatment with KRE (100mg/kg/day, p.o.) showed a significantly (p<0.001) more reduction in WBC count as compared to AH -treated rats (Figure 3b) Treatment with KRE (25/50mg/kg/day p.o.) showed a significant (p<0.01) increase in RBCs and hemoglobin level and treatment with KRE (100mg/kg/day, p.o.) showed significantly (p<0.001) more elevation in RBCs and hemoglobin count as compared to AH - treated rats (Figure 3a and 3c)

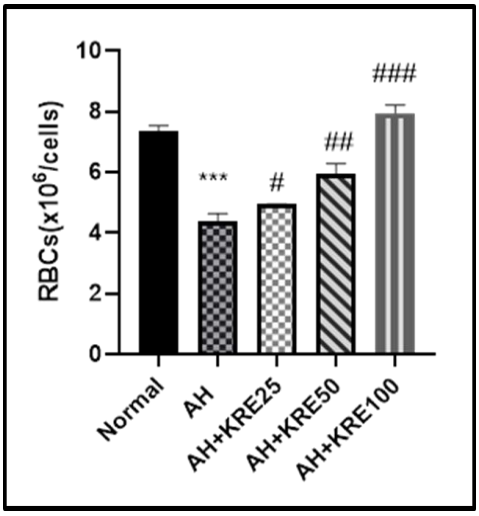

Figure 3a

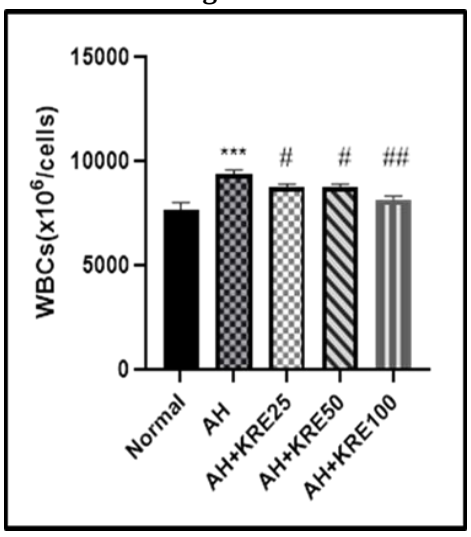

Figure 3b

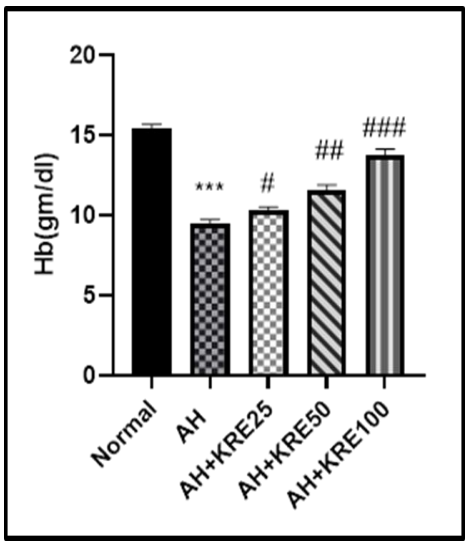

Figure 3c

Values are expressed as mean ± SEM, (n=6). One way ANOVA and Dunnett't' 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.

Effect of KRE on total iron and total protein content.

The total iron content was significantly (p<0.001) increased in AH -treated rats as compared to control animals. Treatment with KRE (25mg/kg/day, p.o.) showed significant (p<0.05) slight reduction in Iron level, whereas treatment with KRE (50/100mg/kg/day p.o.) for 30 days showed a better reduction in total iron content as compared to AH - treated group (Figure 4a).

The level of protein content was also monitored. The level of total protein was significantly (p<0.001) reduced in AH - treated rats as compared to control animals. Treatment with KRE (25/50mg/kg/day, p.o.) showed a significantly (p<0.01) elevation in protein content and treatment with KRE (100mg/kg/day, p.o.) caused significant (p<0.001) improvement in protein content compared to AH - treated rats (Figure 4b).

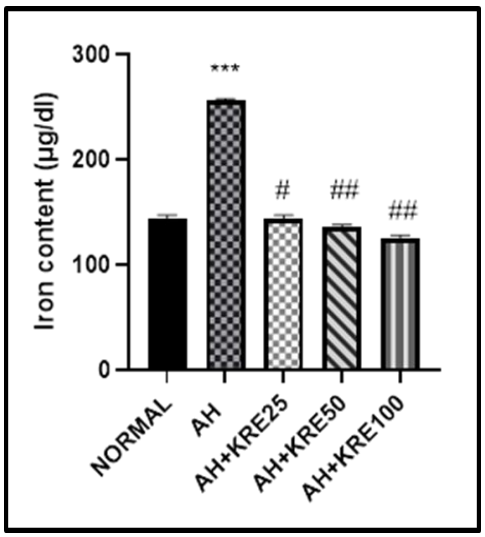

Figure 4a

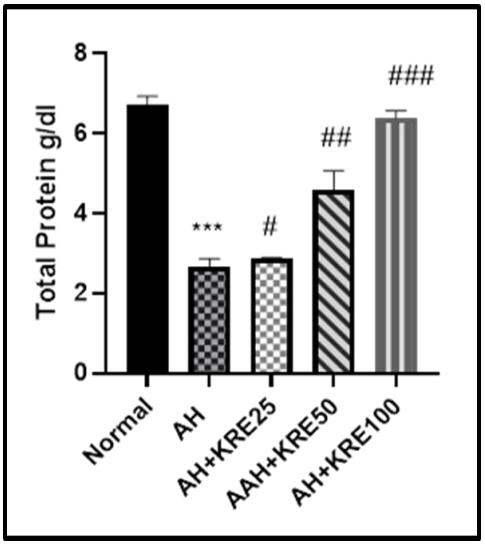

Figure 4b

Values are expressed as mean ± SEM, (n=6). One way ANOVA and Dunnett't' 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.

DISCUSSION

Exposure to aniline and substituted aniline is known to cause selective splenic toxicity in rats. In the present study spleen toxicity was induced by administration of aniline hydrochloride (AH 200ppm) via drinking water. Spleen toxicity was developed after 30 days of aniline hydrochloride administration and marked changes such as body weight, feed consumption, water intake and blood parameter such as Methemoglobin level, hemoglobin level, Red blood cells (RBCs) and White blood cells (WBCs) count, total iron content and total protein were observed. Significant decreased in the body weight, feed consumption, water intake in AH treated rats might be due to toxicity of AH which decreased the food consumption which can directly co-related to decreased body weight. One of the most important features of this study was increased in the spleen weight (splenomegaly) in AH treated rats and changes in the blood parameters. The splenomegaly, presumably due to excessive deposition of PHA-modified erythrocyte, which increased the life of blood of release of the metabolite in the red pulp during erythroclasia.

In present study AH administrated rats shows a significant increase in iron load and decrease in total protein contents. Lipid peroxidation and protein oxidation are the important early biochemical event in AH induced spleen toxicity. It is also apparent that iron may play a significant role as a mediator of AH-induced splenotoxic. Aniline treatment causes remarkable accumulation of iron in the spleen in the time dependent manner. This accumulated iron may catalyze excessive formation of reactive oxygen species (ROS), which can react and damaged protein, nucleic acid, and lipids, leading to cellular dysfunction.

In present study makers of oxidative stress such as lipid peroxidation (LPO), reduce glutathione (GSH) and nitric oxide (NO) Superoxide Dismutase (SOD) and Catalase (CAT) were evaluated. AH-induced group showed a significant increase in the LPO and NO whereas a significant decrease in GSH, SOD and CAT level in the spleen was observed. Oxidative stress plays vital role in spleen 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.

Chronic supplement with Kudzu root extract showed a significant recovery in alternation water intake, body weight, feed consumption and spleen weight as compared to aniline treated rats.

Treatment with kudzu root extract showed significantly increased in hemoglobin level and RBCs count significantly decrease in WBCs count as compare to aniline treated rats.

The level of endogenous antioxidants such as LPO, GSH and NO were measured spleen tissue homogenate. LPO level and NO was found to be significantly increased and GSH level was significantly decreased in spleen of aniline treated rats as compared to control group. NO levels were significantly increased in aniline treated group as compared to control. Chronic treatment with kudzu root extract showed a significant decreased in LPO and NO levels and a significant increase in GSH level as compared to aniline hydrochloride treated group. The level of antioxidants such as SOD and catalase also were measured. SOD and catalase level was found to be significantly decrease in Aniline treated rats as compared to control group rats. Chronic treatment with kudzu root showed a significant increase in SOD and Catalase level as compared to aniline treated rats.

The activity of Na^{+}/K+, Ca^{++} and Mg^{++} ATPase level was significantly decreased in aniline group as compared to control group. Treatment with Kudzu root extract showed a significant increase in the level of Na+/K+, Ca2+ and Mg2+ ATPase as compared aniline hydrochloride treated group.

CONCLUSION

The present study showed that involvement of oxidative and Nitrosative stress in aniline induced splenic toxicity and KRE protects the rats from the toxicity, which might be due to its Antioxidant property and the presence of different phytochemicals. The group treated with (100mg/kg/day) showed better effect might of antioxidant generation mechanism. Antioxidant therapy will be helpful in preventing the spleen toxicity, further in-depth study at molecular level is required to confirm the reaction mechanism of the antioxidant.

REFERENCE

1. H. Yen, W. Tsai, C. Chen, and S. Wang, “Fate of anilide and aniline herbicides in plant-materials amended soils,” Journal of Environmental Science Health B, 43(5): 382-389, 2008.
2. Y. Jiang, Y. Shang, J. Zhou, K. Yang, and H. Wang “Characterization and biodegradation potential of an aniline degradingstrainofPseudomonasJA1atlowtemperature,” Desalination and Water Treatment, 57: 25011-25017, 2016.
3. 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(1-2), 86-93.
4. Pauluhn, J. Subacute inhalation toxicity of aniline in rats: analysis of time-dependence and concentration-dependence of hematotoxic and splenic effects. Toxicol. Sci., 2004, 81(1), 198-215.
5. Steiniger, B.; Barth, P. Microanatomy and function of the spleen; Adv. Anal. Embryol. Cell. 1999, 151(iii-ix), 1-101.
6. Chatterjee, C. Human Physiology; Medical allied Agency, 2000, Vol. 2.
7. Khairnar, U.; Upaganlawar, A.; Upasani, C. Ameliorative effect of chronic supplementation of protocatechuic acid alone and in combination with ascorbic acid in aniline hydrochloride induced spleen toxicity in rats. Scientifica (Cairo), 2016, 2016, 1-9.
8. Wang, J.; Wang, G.; Ansari, G.A.; Khan, M.F. Activation of oxidative stress-responsive signaling pathways in early splenotoxic response of aniline. Toxicol. Appl. Pharmacol., 2008, 230(2), 227-234.

9. Khan, M.F.; Wu, X.; Boor, P.J.; Ansari, G.A. Oxidative modification of lipids and proteins in aniline-induced splenic toxicity. Toxicol. Sci., 1999, 48(1), 134-140.
10. Khan, M.F.; Wu, X.; Kaphalia, B.S.; Boor, P.J.; Ansari, G.A. Nitrotyrosine formation in splenic toxicity of aniline. Toxicology, 2003, 194(1-2), 95-102.
11. Wu, X.; Kannan, S.; Ramanujam, V.M.; Khan, M.F. Iron release and oxidative DNA damage in splenic toxicity of aniline. J. Toxicol. Environ. Health A, 2005, 68(8), 657-666.
12. Wang, J.; Ma, H.; Boor, P.J.; Ramanujam, V.M.; Ansari, G.A.; Khan, M.F. Up-regulation of heme oxygenase-1 in rat spleen after aniline exposure. Free Radic. Biol. Med., 2010, 48(4), 513-518.
13. Khan, M.F.; Boor, P.J.; Kaphalia, B.S.; Alcock, N.W.; Ansari, G.A. Hematopoietic toxicity of linoleic acid anilide: importance of aniline. Fundam. Appl. Toxicol., 1995, 25(2), 224-232.
14. Ames, B.N.; Shigenaga, M.K.; Hagen, T.M. Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. USA, 1993, 90(17), 7915-7922.
15. Götz, M.E.; Künig, G.; Riederer, P.; Youdim, M.B. Oxidative stress: free radical production in neural degeneration. Pharmacol. Ther. 1994, 63(1), 37-122.
16. Labont H. Introduction into the study of the antioxidants and the structures of single electrons in biology-1st part-General study. Agressologie, 1960, 1, 63-79.
17. Wu, X., GAO, W., & Liu, G. (2020). Antioxidant properties of kudzu root extract and its protective effects on oxidative stress in vitro and in vivo. Journal of Ethno pharmacology, 248, 112344.
18. 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.
19. Ramsay WN. The determination of total iron-binding capacity of serum. Clin Chim Acta 1957; 2:221-6.
20. Li, L.; Yin, H.Y.; Zhao, Y.; Zhang, X.F.; Duan, C.L.; Liu, J.; Huang, C.X.; Liu, S.H.; Yang, S.Y.; Li, X.J. Protective role of pueraria on LPS/D-Gal induced acute liver injury via restoring autophagy. Am. J. Transl. Res. 2018, 10, 957–965.
21. Wang, J.C.; Zhu, H.L.; Zhang, C.; Wang, H.W.; Yang, Z.J.; Liu, Z.P. Pueraria protects rat liver and kidney against cadmium-induced oxidative stress. Indian J. Anim. Sci. 2019, 89, 927–931.

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