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Research Article

Spleno Protective Activity of *Argemone mexicana* Linn. against Aniline Induced Splenic Toxicity in Rats

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Abstract:

The protective effects of the aqueous extract of *Argemone mexicana* (Linn.) whole plant, against Aniline induced spleen failure in male albino rats was investigated. The animal was divide in to four groups. Group I kept control. Group II was treated by Aniline hydrochloride (2 mmol/kg) intoxicated for 7 days. Group III was treated by AMEE (*Argemone mexicana* Ethanolic extract) (250 mg/kg p.o) treated for 7 days & AH, Group IV: AMEE (500 mg/kg p.o) treated for 7 days & AH, Group V:Livfit (150 mg/kg p.o) for 7 days. The blood was collected (5 ml) by cardiac puncture. Spleen weight, Relative spleen weight and morphological characteristics of spleen was recorded along with hematological parameters such as hemoglobin, R.B.C, Total W.B.C, platelet count, reticulocyte conc., serum albumin and methemoglobin conc. were determined in blood samples.

KEY WORDS: Spleno protective, Linn., Haematological, Argemone *Mexicana*

1. Introduction

The aim of the present context is to evaluate the Spleno protective potential of the aqueous extract of *Argemone mexicana* whole plant in a conventional animal model of Spleno toxicity. The plant *Argemone mexicana* Linn, Belonging to the family papaveraceae, is a widely distributed plant throughout the subtropical and tropical regions of the world. It is commonly known as 'Mexican prickly poppy'. The plant Argemone mexicana traditionally used as a potent diuretic agent. Along with the plant shows anti anthelmintic, anti inflammatory, wound healing, anti bacterial, anti fungal [3-6]. The plant is bitter, acrid, cooling, vulnerary, purgative, anti- inflammatory, expectorant, aphrodisiac, emetic, depurative, anodyne, anthelmintic, antipyretic, ophthalmic, stomachic and sedative. It cures leprosy, skin diseases, inflammation and bilious fevers. Roots are useful in guinea-worm infestation, skin diseases, leprosy, pruritus, blennorrhagia, inflammations. All type of poisoning, constipation, flatulence, colic, malarial fever and vestibular calculus. The leaves are useful in cough, wounds, ulcers and in skin diseases. Juice is used to cure opthalmia and opacity of cornea. Seeds are purgative and sedative. Seeds resemble

mustard seeds and in Indian it is used to adulterate mustard seed. Seed yield non edible toxic oil and causes lethal dropsy when used with mustard oil for cooking. Seeds are also useful in vitiated conditions of cough, asthma, pertussis, skin diseases, leprosy, wounds, odontalgia, dentalcaries, constipation, rheumatalgia, colic and flatulence. The latex is useful in dropsy, jaundice, skin diseases, leprosy, blisters, conjunctivitis, inflammation, burning sensation and malarial fever. The oil is useful in indolent ulcers, wounds, leprosy and skin diseases, constipation, flatulences, colic and rheumatalgia. In Homeopathic system of medicine the drug prepared from this herb is used to treat the problem caused by tape worm. The plant contains alkaloids as berberine, protopine, sarguinarine, optisine, chelerytherine etc. The seed oil contains myristic, palmitic, oleic, linoleic acids etc. The yellow juice containing small quantities of berberine, potassium nitrate was identified among the salts naturally existing in the plant [1-7].

Aniline, a toxic aromatic amine, is a widely used industrial chemical, particularly in the manufacture of dyes, pigments, herbicides, explosives, isocyanates, hydroquinones and rubber chemicals, with an annual production of over 900 million pounds in the united states. The haematopoietic system is the primary target of aniline insult in rats which is characterized by methemoglobinemia, hemolysis and hemolytic anemia and by the development of splenic hyperplasia, siderosis, fibrosis, a variety of sarcomas on prolonged exposure. Many characteristics of splenotoxicity in rats, such as hyperplasia, hyperpigmentation, and/or formation of highly malignant tumors when animals were exposed to substituted anilines such as chloroaniline, p-nitroaniline, o-toluidine and substituted phenyl urea herbicides.

In the present study, we have screened the splenoprotective components from whole plant of *Argemone mexicana* Linn Against aniline induced hypersplenism in rats.

2. Materials and Methods

2.1 Extraction of plant material and preparation of test dose

About 200 gm of coarse dried powder of stem of the Argemone mexicana was taken in the soxhlet apparatus and extracted successively [8] using the selected solvent (i.e. Ethanol). The extraction for carried out for 18 to 20 hours. The extract was collected by evaporating the solvents by slow heat treatment. Total 1.4kg of pulverized stem was subjected under solvent extraction to produce the required amount of test extract. Calculated amount of dried ethanolic extract was suspended in 0.5% w/v of sodium-CMC in normal saline solution to get the test doses (250mg/kg and 500mg/kg per ml.). The dose limits were selected on the basis of previously performed oral acute toxicity.

Studies in mice, in accordance with the OECD guideline 420. [9]

2.2 Splenoprotective activity

Group I: Normal Control

Group II: Aniline hydrochloride (2 mmol/kg) intoxicated for 7 days

Group III: AMEE (250 mg/kg p.o) treated for 7 days & AH

Group IV: AMEE (500 mg/kg p.o) treated for 7 days & AH

Group V: Livfit (150 mg/kg p.o) for 7 days & AH

After completion of treatments, all animals were anesthetized by diethyl ether and then sacrificed. The blood was collected (5 ml) by cardiac puncture and kept in test tubes containing EDTA anticoagulant for evaluation of haematological parameters. The spleen was removed immediately, blotted, weighed and preserved in 10 % formalin for histological studies. Spleen weight, Relative spleen weight and morphological characteristics of spleen was recorded along with hematological parameters such as hemoglobin, R.B.C, Total W.B.C, platelet count, reticulocyte conc., serum albumin and methemoglobin conc. were determined in blood samples.

3. Results and Discussion

Aniline and several substituted anilines is known to cause splenic toxicity in rats. It is generally thought that the initial compound induced damage to erythrocytes results in their scavenging by the spleen which subsequently shows a series of toxic events in the spleen. These events potentially include specific accumulation of aniline or its metabolites, deposition of erythrocyte debris resulting in vascular congestion, induction of splenic hyperplasia as a result of erythrocyte overload and most importantly, release of iron which might catalyze tissue damaging free radical reactions in the spleen. The data of present study clearly emphasized that, the aniline treatment leads to hypersplenism associated with haematological abnormalities in experimental animals. The changes in blood parameters were closely associated with simultaneous enlargement in the spleen i.e. splenomegaly appears to be due to excessive deposition of chemically damaged erythrocytes. This deposition would also increase aniline and/or its metabolites in the red pulp of spleen which on subsequent breakdown from damaged erythrocytes could bind with splenic mesenchymal tissues, causing injury and/ or deleterious effects. These haematological changes were retrieved toward normalcy by the administration of hydroalcoholic extract of *Argemone maxicana* Linn.

In conjugation with the accumulation of aniline metabolites within the spleen, could lead to the transformation of mesenchymal cells of the spleen. An association between increased iron deposition and development of fibrotic lesions in the aniline treated rats presumably due to iron mediated production of ROS which might act as a stimulus for increased collagen production in splenic tissue, leading to fibrosis. The increased red pulp cellularity of the spleen noted here was most likely due to scavenging of damaged RBCs by macrophages with consequent sinusoidal dilation and fibrosis that may also play a role in the aniline-induced splenic injury. 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 and H_2O_2 within the cells. H_2O_2 and superoxide radical either may be damaging directly or could lead to the formation of highly reactive species, such as hydroxyl radicals and ferryl cation, which results in the observed injury.

The histopathological study showed the recovery of damaged splenic architecture in the extract treated group. The greatly separated area of white pulp in intoxicated spleen was reformed. The degree of vascular and cellular congestion of red cells was also reduced as compared to the aniline treated

groups. The normal architecture was restored to the same as that of standard drug (Livfit) treated spleen. This clearly indicates that the hydroalcoholic extract *Argemone maxicana* Linn. posses very good splenoprotective activity. The splenoprotective effect of above stated extract could be due to its antioxidant property which deactivated the increased production of free radicals in the splenic tissues.

Acknowledgment

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Table 1. Effect of AMEE on haematological parameters of normal and experimental animals after 7 days treatment against aniline induced splenic toxicity.

Groups	Treatment	Hemoglobin (gm%)	R.B.C (mill/cumm)	Total W.B.C ($X\ 10^3$ /cumm)	Platelets (Lck/cumm)
I	Control	18.76 ± 0.12	6.45 ± 0.099	18.6 ± 0.073	2.61 ± 0.07
II	Aniline treated	9.76 ± 0.19[a]	3.33 ± 0.076[a]	10.53 ± 0.071[a]	1.6 ± 0.07[a]
III	Aniline + AMEE 250 mg/kg	15.33 ± 0.16[b]	4.31 ± 0.079[b]	14.33 ± 0.13[b]	2.14 ± 0.04[b]
IV	Aniline + AMEE 500 mg/kg	16.63 ± 0.12[b]	5.33 ± 0.076[b]	15.28 ± 0.12[b]	2.51 ± 0.05[b]
V	Aniline + Livfit 150 mg/kg	17.63 ± 0.12[b]	5.58 ± 0.094[b]	15.7 ± 0.10[b]	2.48 ± 0.02[b]

Values are represented as mean ± S.E.M (n=6). One-way ANOVA followed by Student-Newman-Keuls post test (P< 0.001) is used. a-*vs* group I and b-*vs* group II.

Table 2. Effect of Ethanolic extract of *Argemone mexicana*, Linn. on Reticulocyte, Methemoglobin & Albumin conc. in blood of normal and experimental animals after 7 days treatment against aniline induced hypersplenism.

Groups	Treatment	Reticulocyte (%)	Methemoglobin (mg%)	Serum Albumin (%)
I	Control	3.4 ± 0.12	0.37 ± 0.009	3.63 ± 0.12
II	Aniline treated	10.86 ± 0.21[a]	4.48 ± 0.06[a]	1.71 ± 0.06[a]
III	Aniline + MAEE 250 mg/kg	6.03 ± 0.14[b]	1.64 ± 0.03[b]	2.16 ± 0.03[b]
IV	Aniline + MAEE 500 mg/kg	5.3 ± 0.07[b]	1.00 ± 0.04[b]	2.91 ± 0.01[b]
V	Aniline + Livfit 150 mg/kg	3.8 ± 0.07[b]	0.58 ± 0.01[b]	3.25 ± 0.06[b]

Values are represented as mean ± S.E.M (n=6). One-way ANOVA followed by Student-Newman-Keuls post test (P< 0.001) is used. a-*vs* group I and b-*vs* group II.

Table 3. Effect of Ethanolic extract of *Argemone mexicana*, Linn. on Reticulocyte, Methemoglobin & Albumin conc. in blood of normal and experimental animals after 7 days treatment against aniline induced hypersplenism.

Groups	Treatment	Reticulocyte (%)	Methemoglobin (mg%)	Serum Albumin (%)
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IV	Aniline + MAEE 500 mg/kg	5.3 ± 0.07[b]	1.00 ± 0.04[b]	2.91 ± 0.01[b]
V	Aniline + Livfit 150 mg/kg	3.8 ± 0.07[b]	0.58 ± 0.01[b]	3.25 ± 0.06[b]

Values are represented as mean ± S.E.M (n=6). One-way ANOVA followed by Student-Newman-Keuls post test (P< 0.001) is used. a-*vs* group I and b-*vs* group II

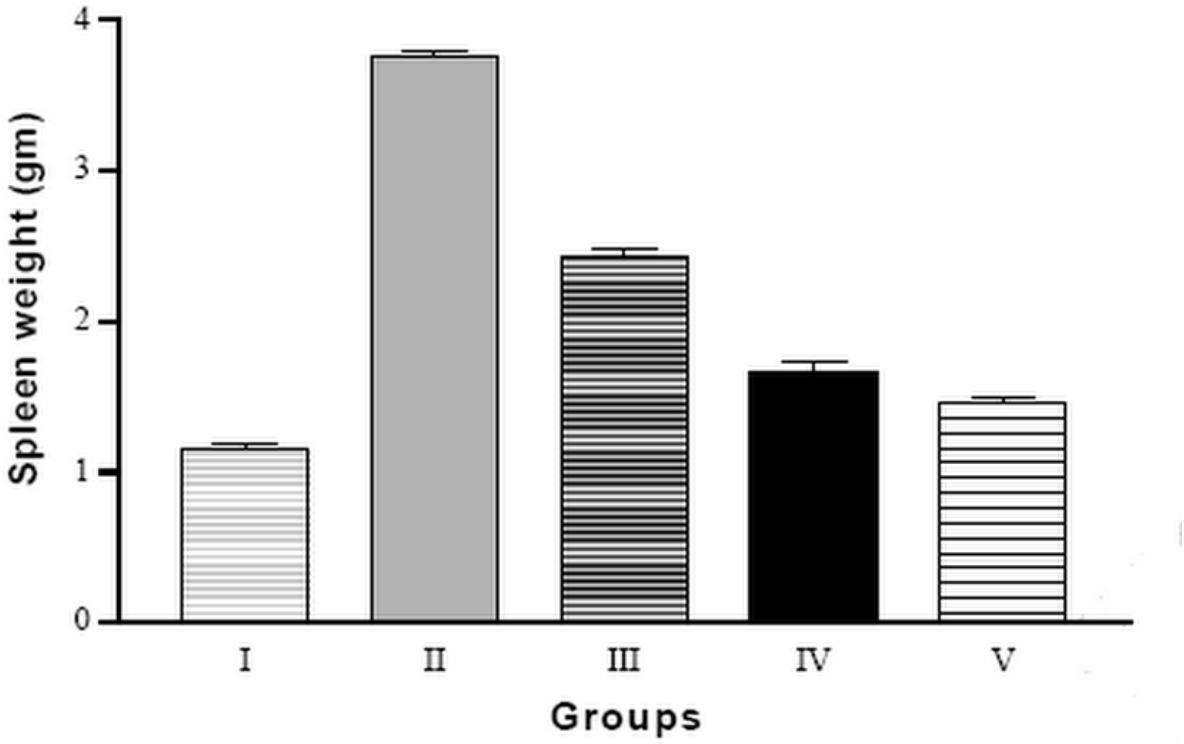

Figure 1. Effect of AMEE on Spleen weight of normal and experimental animals after 7 days treatment against aniline induced splenic toxicity

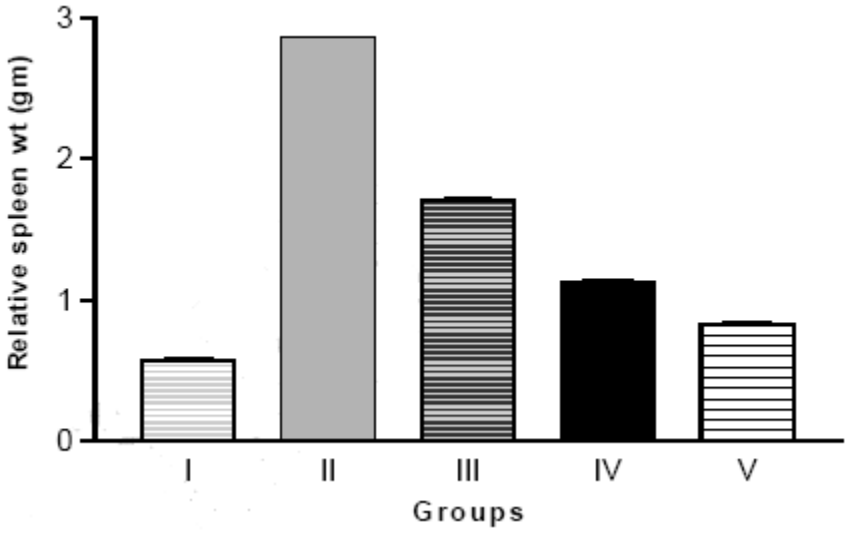

Figure 2. Effect of AMEE on Relative Spleen weight of against normal and experimental animals after 7 days treatment

Figure 3. Effect of AMEE on Spleen morphology against aniline induced splenic toxicity

(a) Normal spleen isolated from rat in the normal control group. (b) Massive splenomegaly was seen in aniline treated group of animal. (c) Spleen isolated from low dose extract treated group of animal retrieved toward normal size. (d) Spleen isolated from higher dose extract treated group of animal which became as normal size. (e) Spleen isolated from Livfit treated group of animal resized like normal.

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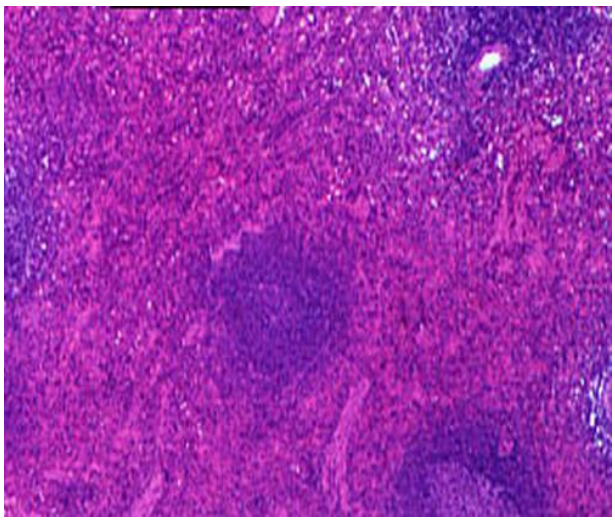
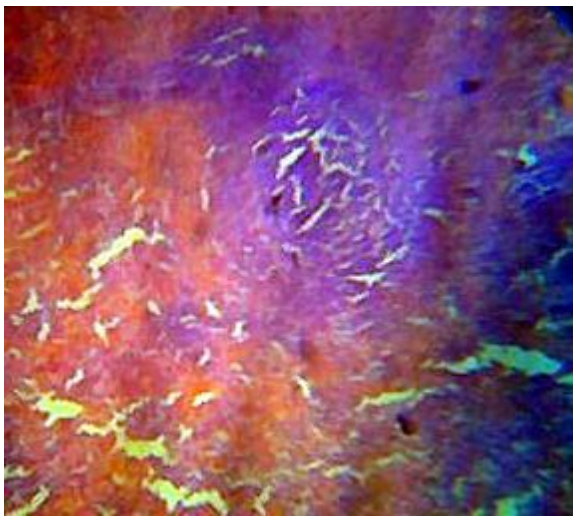
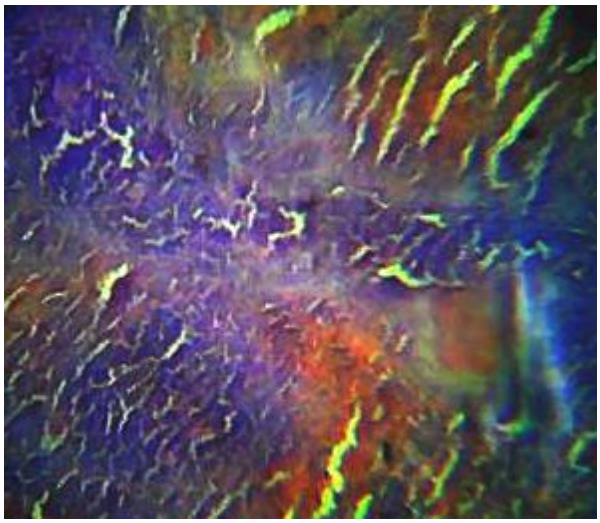


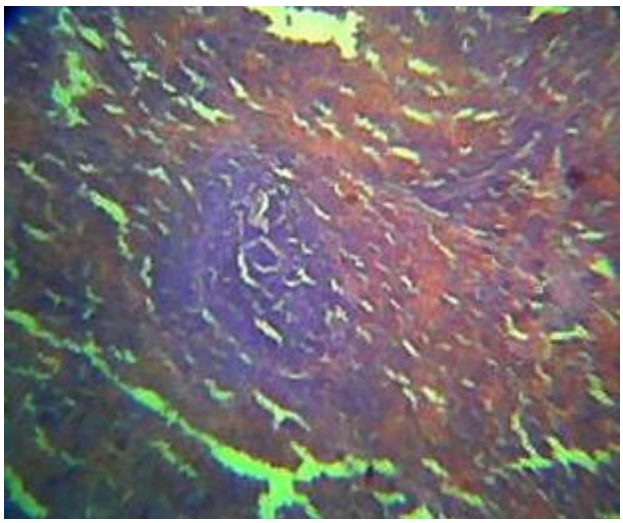
Normal Control Spleen



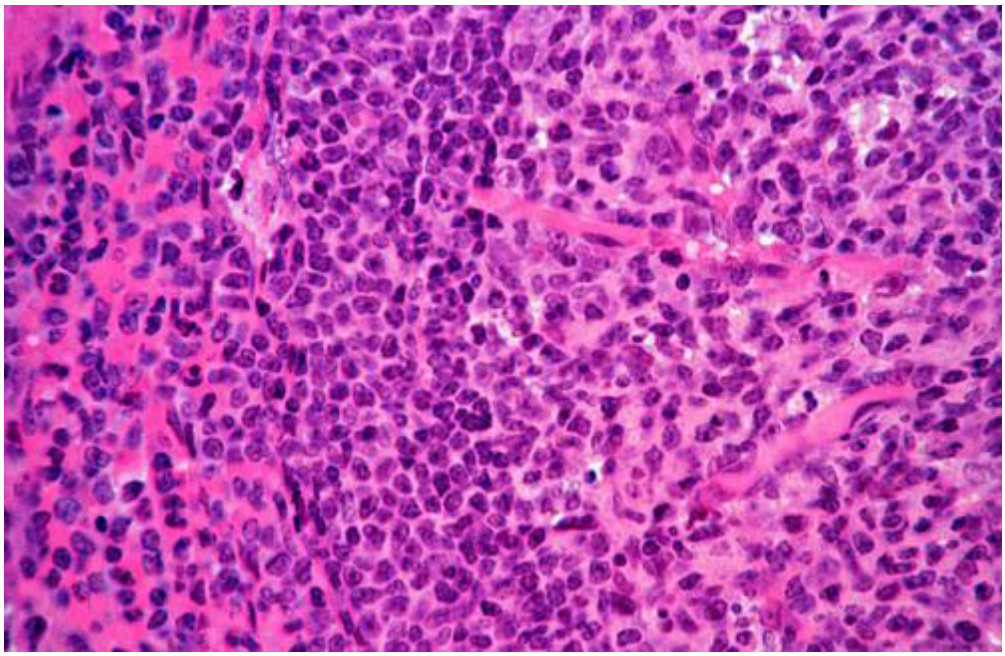
Aniline treated



Aniline + AMEE 250 mg/kg



Aniline + AMEE 500 mg/kg



Aniline + Livfit 150 mg/kg