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INFLUENCE OF *MORINGA OLEIFERA* (DRUM-STICK) FRUIT EXTRACT ON HAEMATOLOGICAL PROFILE FOLLOWING REPEATED EXPOSURE TO LOW LEVELS OF ARSENIC THROUGH FEED ON RATS

Vaibhav R. Pachade [2], Aruna Kumari Singh [1], Lokesh Verma [2], K.M. Koley [2] and V.P. Vadlamudi [2].

ABSTRACT: Effect of *Moringa oleifera* fruits hot methanolic extract (MFE), if any, in minimizing the adverse reactions of repeated exposure to arsenic trioxide (AT) in feed was investigated in Wistar rats with reference to haematological profile. Three groups of rats each containing 10 (5male+5female) were used. The group I served as negative control. Rats of group II were fed arsenic trioxide (AT) alone @ 100 ppm in feed while those of group III simultaneously received AT (@100 ppm) and MFE (50 mg/kg/day) for 28 days. Blood samples were collected from retroorbital plexus for estimation of hematological parameters (haemoglobin, PCV, TEC, MCH, MCHC, MCV) of different groups on 0 day, 15th day and 29th day respectively. Exposure to AT through feed in group II resulted in significant (P<0.05) decrease in haemoglobin, TEC and MCHC, accompanied by increased MCV, with no significant alteration of PCV or MCH of the rats. While rats of group III treated with AT (@100 ppm) and MFE (50 mg/kg/day) also resulted in same consequences as it was in group II but it was slightly less than that of group II suggesting of mild non significant protective effect.

Key words: Albino rats, *Moringa oleifera* fruits, Arsenic trioxide, Haematological profile.

INTRODUCTION :

Deterioration of environmental quality due to pollution of heavy metals, especially by arsenic, is well recognized. Arsenic pollution of the environment has become a major public health concern globally, including several states of our country (Mukherjee *et al.* 2006). Arsenic is one of the most potent bio-toxic agents, having multiple potent adverse reactions such as carcinogenicity, cardiovascular toxicity, dermatological effects, genotoxicity, haematological disorders, hepatoxicity, mutagenecity, neurotoxicity, pulmonary effects, renal toxicity etc. (Tchounwou *et al.* 2003). The toxicity due to arsenic depends on several factors such as exposure dose, duration and frequency, species, age and gender, as well as individual susceptibility, genetic and nutritional status (Chen and Lin 1994). Most of the toxic actions/effects at the

[1] *Department of Pharmacology and Toxicology, West Bengal University of Animal and Fishery Sciences, Belgachia- 700 037, West Bengal, India.*

[2] *Department of Veterinary Pharmacology & Toxicology College of Veterinary Science & AH, IGKVV, Anjora, Durg-491001, Chhattisgarh, India*.

cellular level are due to ability of arsenic to interact with sulfhydral (-SH) groups and thus inactivating a variety of proteins including many enzymes. One of the most valuable indigenous plants, *Moringa oleifera* (the drumstick tree, also popular as "the mothers best friend or miracle tree of hope") possessing varied medicinal values has recently been demonstrated to be a source of potent, hitherto chemically unidentified, antioxidants (Perumal and Klaus 2003, Gupta *et al*. 2007, Pari *et al.* 2007). Therefore in the light of the above the present investigations were carried out to evaluate the beneficial effect of *Moringa oleifera* fruits hot methanolic extract (MFE), if any, following repeated exposure to low levels of arsenic through feed on Wistar rats with reference to haematological profile.

MATERIALS AND METHODS :

Plant Material : The fresh, ripe fruits of *M. oleifera* were purchased from the local market were properly washed and cleaned and then cut into small pieces and shade-dried, were ground to a fine powder with the help an electric grinder. The powder was kept in air-tight containers and stored at $4^{O}C$ for use whenever required for further processing.

Preparation of Extract : The powder of *M. oleifera* fruits was subjected to continuous hot methanolic extraction using the Soxhlet's apparatus (Raaman, 2006). After complete extraction the extract was transferred over a hot water bath for complete evaporation of the solvent. The extract was transferred into screw cap glass vials and stored in refrigerator for use whenever required. The extract henceforth referred as "Moringa fruit extract" (MFE).

Experimental Animals : Weanling Wistar rats (90-140 gm) of either sex were procured from a registered lab animal breeder. The animals were housed in poly propylene rat animal cages and maintained on balanced lab animal feed in climatized Lab. Animal House attached to the Department of Veterinary Pharmacology and Toxicology under standard conditions of management. After a 10-day period of acclimatization to local conditions, they were randomly assigned to different groups for conduct of the toxicity trial.

Toxicity Trial : Thirty rats were randomly divided into three groups (I, II and III), each consisting of five male and five female rats. The Group I rats served as healthy control, which received normal feed and deionized drinking water. The rats in Group II received feed added with 100 ppm of arsenic trioxide and deionized water. The Group III animals received arsenic added feed, deionized water and in addition were orally administered methanolic extract of *M. oleifera* fruit (MFE) @ 50 mg/kg/day. The plant extract thoroughly mixed in distilled water was administered in Group III rats, daily through a gastric needle in volumes not exceeding 0.5 ml/rat/day. The rats in Groups I and II were like-wise administered distilled water. The oral dose of MFE as 50 mg/kg was selected on earlier studies in the lab, where the dose was found to produce significant analgesic and anti-inflammatory effects in rats. The trial lasted for 28 days.

Toxicity Signs and Symptoms: All the experimental rats were regularly observed for appearance of visible toxicity manifestations, if any.

Collection of Blood Samples: Blood samples were drawn from retro-orbital plexus with the help of capillary tubes as described by Sorg and Buckner (1964). Heparin was added @ 2 IU/ml to the blood samples as anticoagulant for haematological study. The haematological estimations (Dacie *et al.* 1968, Jain 1986) includes haemoglobin (cyanmethaemoglobin method), packed cell volume (microhaematocrit method) by Coles (1986) and total erythrocyte (haemocytometer method) using Gower's solution (SD Fine Chemicals Limited, Mumbai). The above haematological estimations were used for determination of the indices like mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and mean corpuscular volume (Jain 1986).

The haemogram was determined at pre-treatment, mid-treatment (15th day) and post-treatment (29th day) intervals of the trial.

STATISTICAL METHODS :

The results were expressed as mean standard error (S.E.). The data were analyzed statistically using general linear model with univariate data in SPSS 10.0 version of software.

RESULTS AND DISCUSSION:

Arsenic Toxicity Study: The rats fed on diet containing 100 ppm of arsenic trioxide (Group II) did not reveal any significant visible toxic sign or symptom, except that they were dull and inactive, especially after the third week of treatment. The rats in Group III (Arsenic trioxide + MFE) and control group (I) remained apparently healthy during the feeding trial.

Haemogram:

Table 1 shows the haemoglobin levels of rats in the three groups. The haemoglobin levels among the three groups at pre-treatment were statistically similar (14.10 ± 0.43 to 14.77 ± 0.12 gm/dl). The Group II rats fed on arsenic added diet (100 ppm) showed significantly lowered haemoglobin at both the intervals (12.02 ± 0.48 to 12.06 ± 0.31 gm/dl) as compared to their pre-treatment level and the respective levels of control group. Whereas Group III showed significantly lowered levels at 15th day of post-treatment (13.15 ± 0.42 gm/dl) and 29th day of post-treatment (12.46 ± 0.48 gm/dl) intervals as compared their pre-treatment level. However, among these rats the haemoglobin level only at the later interval was significantly lesser that the respective level of control group.

Table 2 shows the total erythrocyte count (TEC) of rats in the three groups. The counts among the three groups at pre-treatment were statistically similar (5.67 ± 0.33 to 5.82 ± 0.29 millions/cumm). The TEC (5.66± 0.5 to 5.80 ± 0.42 millions/cumm) in control group on 15th day post-treatment and 29th day post-treatment were also statistically similar to the pre-treatment count. The Group II rats fed on arsenic added diet (100 ppm) showed significantly

Table 1: Effect of arsenic ingestion (100 ppm/day for 28 days) through feed on haemoglobin of Wistar rats

Group No.	Treatment	Mean Haemoglobin (gm/dl ± SE)		
		Pre-treatment	Post-treatment (Days)	
			15th	29th
I	Normal feed and water (Healthy Control)	14.10 ± 0.43	14.29 ± 0.99	13.77 ± 0.48
II	Arsenic trioxide @ 100 ppm in feed and water	14.77 ± 0.12	12.02 ± 0.48[a,b]	12.06 ± 0.31[a,b]
III	Arsenic trioxide @ 100 ppm in feed, MFE orally @ 50 mg/kg/day and water	14.32 ± 0.37	13.15 ± 0.42[a]	12.46 ± 0.48[a,b]

a: Significantly lesser than the respective pre-treatment levels ($P < 0.05$)

b: Significantly lesser than the respective post-treatment levels of control group ($P < 0.05$)

Table 2: Effect of arsenic ingestion (100 ppm/day for 28 days) through feed on total erythrocyte count (TEC) of Wistar rats

Group No.	Treatment	Mean TEC (millions/cumm ± SE)		
		Pre-treatment	Post-treatment (Days)	
			15th	29th
I	Normal feed and water (Healthy Control)	5.67 ± 0.33	5.80 ± 0.42	5.66 ± 0.55
II	Arsenic trioxide @ 100 ppm in feed and water	5.73 ± 0.25	4.63 ± 0.29 [b]	4.29 ± 0.19 [a,b]
III	Arsenic trioxide @ 100 ppm in feed, MFE orally @ 50 mg/kg/day and water	5.82 ± 0.29	5.14 ± 0.17	4.70 ± 0.24 [a]

a: Significantly lesser than the respective pre-treatment level ($P < 0.05$)
b: Significantly lesser than the respective post-treatment levels of control group ($P < 0.05$)

Table 3 shows the data of packed cell volume (PCV) of rats. The variation in PCV values within the groups as well as between the groups at pre-treatment and post-treatment intervals was statistically similar.

Table 3: Effect of arsenic ingestion (100 ppm/day for 28 days) through feed on packed cell volume (PCV) of Wistar rats

Group No.	Treatment	Mean PCV (Per cent± SE)		
		Pre-treatment	Post-treatment (Days)	
			15th	29th
I	Normal feed and water (Healthy Control)	40.80 ± 0.58	40.40 ± 1.22	39.60 ± 1.03
II	Arsenic trioxide @ 100 ppm in feed and water	41.40 ± 2.14	40.14 ± 1.40	41.20 ± 1.32
III	Arsenic trioxide @ 100 ppm in feed, MFE orally @ 50 mg/kg/day and water	39.40 ± 1.50	39.66 ± 1.17	41.40 ± 0.51

lowered TEC at both the post-treatment intervals (4.29 ± 0.19 to 4.63 ± 0.29 millions/cumm) as compared to their pre-treatment level as well as the respective post-treatment levels of control group. Whereas, in Group III, where the rats were exposed arsenic added feed and MFE (50 mg/kg/day) showed significantly lowered TEC on 29th day of post-treatment only (4.70 ± 0.24 millions/cumm) as compared their pre-treatment count.

Tables 4, 5 and 6 give the data of different eryhthrocytic indices such as the mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV), respectively.

The mean MCH values among the three groups at pre-treatment and at both the intervals of post-treatment is shown in Table 4. The variation in MCH values within the groups as well as between the groups at pre-treatment and post-treatment was sta-

Table 4: Effect of arsenic ingestion (100 ppm/day for 28 days) through feed on mean corpuscular haemoglobin (MCH) of Wistar rats

Group No.	Treatment	Mean MCH (micro gm ± SE)		
		Pre-treatment	Post-treatment (Days)	
			15th	29th
I	Normal feed and water (Healthy Control)	25.01 ± 1.10	25.19 ± 2.11	25.37 ± 2.85
II	Arsenic trioxide @ 100 ppm in feed and water	26.26 ± 1.35	26.35 ± 1.82	28.37 ± 1.65
III	Arsenic trioxide @ 100 ppm in feed, MFE orally @ 50 mg/kg/day and water	24.76 ± 0.85	25.65 ± 1.08	26.77 ± 1.38

Table 5: Effect of arsenic ingestion (100 ppm/day for 28 days) through feed on mean corpuscular haemoglobin concentration (MCHC) of Wistar rats

Group No.	Treatment	Mean MCHC (% ± SE)		
		Pre-treatment	Post-treatment (Days)	
			15th	29th
I	Normal feed and water (Healthy Control)	34.59 ± 1.35	35.40 ± 0.74	34.80 ± 0.93
II	Arsenic trioxide @ 100 ppm in feed and water	37.33 ± 1.56	30.25 ± 1.76 [a,b]	29.42 ± 1.33 [a,b]
III	Arsenic trioxide @ 100 ppm in feed, MFE orally @ 50 mg/kg/day and water	36.67 ± 2.19	33.29 ± 1.43	30.12 ± 1.25 [a]

a: Significantly lesser than the respective pre-treatment level ($P < 0.05$)
b: Significantly lesser than the respective post-treatment levels of control group ($P < 0.05$)

tistically insignificant.

The mean MCHC (Table 5) among the three groups at pre-treatment were statistically similar. The levels in control group on 15th and 29th post-treatment days were also similar to their pre-treatment MCHC. The Group II rats fed on arsenic added diet (100 ppm) showed significantly lowered MCHC at both the post-treatment intervals (29.42 ± 1.33 to 30.25 ± 1.76 %) as compared to their pre-treatment level as well as the respective post-treatment levels

Table 6: Effect of arsenic ingestion (100 ppm/day for 28 days) through feed on mean corpuscular volume (MCV) of Wistar rats

Group No.	Treatment	Mean MCV (microns ± SE)		
		Pre-treatment	Post-treatment (Days)	
			15th	29th
I	Normal feed and water (Healthy Control)	72.81 ± 4.34	71.48 ± 6.95	72.62 ± 7.50
II	Arsenic trioxide @ 100 ppm in feed and water	69.63 ± 1.86	88.57 ± 5.35 [a,b]	96.72 ± 5.65 [a,b]
III	Arsenic trioxide @ 100 ppm in feed, MFE orally @ 50 mg/kg/day and water	68.57 ± 5.15	77.40 ± 2.75	88.80 ± 3.61 [a]

a: Significantly lesser than the respective pre-treatment level ($P < 0.05$)
b: Significantly lesser than the respective post-treatment levels of control group ($P < 0.05$)

of control group. Whereas, in Group III, where the rats were exposed to arsenic added feed and MFE (50 mg/kg/day) showed significantly lowered MCHC on 29th day of post-treatment only (30.12 ± 1.25 %) as compared their pre-treatment count.

The mean MCV (Table 6) among the three groups at pre-treatment were statistically similar. The values in control group on 15th and 29th post-treatment days (71.48 ± 6.95 to 72.62 ± 7.50 microns) were also similar to their pre-treatment MCV. The Group II rats fed on arsenic added diet (100 ppm) showed significantly elevated MCV at both the post-treatment intervals (88.57 ± 5.35 to 96.72 ± 5.65 microne) as compared to their pre-treatment level as well as the respective post-treatment levels of control group. Whereas, Group III showed significantly higher MCV on 29th day of post-treatment as compared their pre-treatment count.

From the observation of haemogram it is evident that feeding of diet added with arsenic trioxide @ 100 ppm for 28 consecutive days resulted in significant reduction in haemoglobin, TEC and MCHC, accompanied by increased MCV, with no significant alteration of PCV or MCH of the rats. Therefore, exposure to arsenic might cause anaemia characterized by hypochromic-macrocytic nature. Contradictory as well as inconsistent observations are reported in literature on haematological alterations in asrsenic toxicity. Pandey *et al.*(2005) observed haemoconcentration characterized by increase in haemoblobin and PCV, with low RBS and WBC in induced acute sodium arsenite toxicity in goats. Roy *et al.* (2008) reported dose-dependent reduction in haemoglobin, PCV, RBC, WBC and MCV and increase in MCHC in chronic sodium arsenite toxicity in goats. Therefore, it is apparent arsenic might interfere with

eryhthropoiesis or haeme- biosynthesis. In concurrence with the report of Hernandez-Zavala *et al.* (1999), who observed altered activity of haeme-biosynthesis pathway enzymes in persons chronically exposed to arsenic in Mexico. The haemogram of rats in group III also showed reduction but it was slightly less than that of arsenic treated groups suggesting mild non significant protective effect.

CONCLUSION:

From the above experiment it can be concluded that exposure to arsenic might cause anaemia characterized by hypochromic-macrocytic nature and treatment with MFE is having slight non significant protective effect.

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