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AMELIORATIVE EFFECT OF THREE MEDICINAL PLANTS (*P. FRATERNUS, TERMINELIA A., AND MORINGA OLEIFERA*) ON ARSENIC TRIOXIDE INDUCED ALTERATION OF LIPID PEROXIDATION AND PROTEIN CONTENTS IN CHICKEN LIVER HOMOGENATE: AN *IN VITRO* STUDY

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Abstract The ameliorative effect of the aqueous extract of three medicinal plants *P. fraternus, Terminalia a.,* and *Moringa oleifera* (PF, TA, and MO) on arsenic trioxide (As_2O_3) induced alteration in lipid peroxidation (LPO) and protein contents was studied *in vitro*. Liver from healthy chicken (*Gallus domesticus*) weighing 1.2 to 1.5 kg was bought to laboratory in frozen condition from local slaughter house and used for study. When 0.2 mL of liver homogenate was treated with 1-5 μg/mL of As_2O_3, it caused significant alterations in LPO and total protein content of chicken liver. The maximum alteration was observed at 3 mg/mL concentration of As_2O_3. Addition of each plant extract to liver homogenate did not caused significant alteration in LPO and protein contents. However, concurrent addition of As_2O_3 and plant extract (25-100 μg/mL) caused significant ameliorative effect. Ameliorative effect of each plant extract was studied separately. The maximum amelioration of PF, MO, and TA was observed at 50 mg/mL, 100 μg/mL, and 100 μg/mL respectively. Thus it is concluded that aqueous extract of PF was observed to have better ameliorative effect.

Keywords: *P. fraternus, Terminalia a., Moringa oleifera*, chicken, LPO, protein, liver, arsenic trioxide.

Arsenic is widely distributed in nature in many forms and their compounds are being used extensively. Occupational exposure to arsenic mainly occurs in the production and use of pesticides, buring of coal and wood treated with arsenic (1). The main source of arsenic exposure for the general population is food and ingestion of drinking water with high level of arsenic (2,3). Arsenic also accounted for nearly one third of the homicide poisonings in France (4). Even in the 21st century arsenic contaminated water is consumed by millions of poor villagers in part of west Bengal, India, in Bangladesh as well as in China (5). The most important information about the occurrence of arsenic in air is from US Environmental Protection Agency (6), which revealed that the atmosphere is a significant channel for the recycling of arsenic to water and soil via fallout or precipitation. Concha et al. (1998) has studied inorganic metabolism of arsenic in children and revealed that arsenic trioxide is more potent toxic than arsenic pentoxide (7). The major lesions are profound gastrointestinal damage (1), inhibiting various enzymatic pathway of energy metabolism (8 – 10). Biochemical assays revealed that the functions of nearly 200 enzymes were affected by arsenic exposure (8). Previous experiments also revealed that arsenic could induce lipid peroxidation and Fe-dependent formation of reactive oxygen species (ROS) which leads to arsenic carcinogenesis in human (11, 12).

TA is belonging to *Conbretacea* family and commonly known as arjuna. Previous study also revealed its anti-carcinogenic activity, specifically in the case of hepatocellular carcinoma and protective effects against genotoxicity *in vitro* (13, 14). PF is belonging to family *Euphorbiacea* and commonly known as bhumyamalaki. Ayurvedic practioners are commonalty prescribes the juice of PF for the treatment of jaundice. Several studies revealed protective effect of PF against allyl alcohol, CCl_4, and ethanol induced oxidative stress in liver mitochondria (15-17). MO is belonging to family of *Moringacea* and commonly known as drumstick. It has been used since ancient time in India because of its medicinal properties like anti-microbial, anti-carcinogenic, anti-spasmodic, diuretic, and anti-inflammatory (18-21).

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The present study was planned to evaluate the individual effect of three medicinal plants on arsenic trioxide induced toxicity *in vitro*. The toxicity was accompanied by measuring changes of lipid peroxidation and total protein content in chicken liver.

MATERIALS AND METHODS

Chemicals

Analytical grade arsenic trioxide was procured from Hi-Media Laboratory Pvt Ltd, Mumbai, India. All other chemicals used in present study were of analytical grade.

Liver sample

Fresh liver sample of healthy adult chicken (*Gallus domesticus*) weighing approximately 1.2-1.5 kg obtained from local slaughter house was brought to laboratory under frozen condition and used immediately.

As_2O_3 solution

Stock solution: 20 mg of arsenic trioxide was dissolved in 0.5 mL HCl (0.005%) and final volume was made up with 50 mL of distilled water.

Working solution: 1 mL of stock solution was taken and diluted to 10 mL with distilled water and used for experiment.

Plant extracts preparation

The extract was prepared according to WHO protocol CG- 06 (1983). Shade dried a whole plant of *P. fraternus* (PF), was grind with the motor and pestle, grinded material was suspended in 100 mL of 50% ethanol and stirred with magnetic stirrer at 40°C for 3 hours. After cooling, the content was filtered successively through ordinary and then through Whatman filter paper no 1. The filtrate was collected and evaporated in the water bath at 40°C to dryness. 2 mg of the filtrate was dissolved in 8 mL of distilled water and used for the present investigation.

The same procedure was followed for the preparation of extract of *Terminalia a.* (TA) and *Moringa oleifera* (MO) plants and used for present investigation.

Liver homogenate

2.5 g of chicken liver was homogenized in 100 mL of chilled distilled water for the estimation of protein and 25 mg of liver was homogenized in 100 mL of chilled phosphate buffer solution for the estimation of LPO.

Methods

Phase I: To determine the effect of arsenic trioxide (1-5 mg/mL) on chicken liver, following sets of tubes were prepared.

1) Control tubes: These tubes contained 0.2 mL of liver homogenate and 0.8 mL of distilled water (D.W.)/ phosphate buffer solution (PBS) for protein and LPO, respectively.

2) Arsenic trioxide treated tubes: Different volumes of As_2O_3 solution (0.1 – 0.5 mL) were mixed with 0.2 mL liver homogenate, and the final volume was made up to 1.0 mL with D.W./PBS for protein and LPO, respectively. The concentration of As_2O_3 in 1.0 mL of final volume therefore ranged from 1 to 5 µg/mL.

Phase II: To determine the ameliorative effect of three medicinal plants extracts on As_2O_3 induced changes on liver homogenate, ameliorative effect of each plant was studied separately. The following sets of tubes were prepared:

1) Control tubes: These tubes again contain 0.2 mL homogenate and 0.8 mL D.W./PBS for protein and LPO, respectively.

2) As_2O_3 treated tubes: 0.3 mL of As_2O_3 was mixed with 0.2 mL of homogenate. The final volume was made up to 1.0 mL with D.W./PBS for protein and LPO, respectively. Therefore the concentration of As_2O_3 in the final volume was 3 µg/mL.

3) Plant extract treated tubes: 0.5 mL of extract of each plant was mixed with 0.2 mL of homogenate in separate tubes. Final volume was made up to 1.0 mL with D.W./PBS for protein and LPO, respectively. Therefore the concentration of each plant extract in each respective tube was 100 µg/mL.

4) As_2O_3 and plant extract treated tubes: 0.3 mL of As_2O_3 solution and 0.1 to 0.4 mL of each plant extract were mixed with 0.2 mL of liver homogenate. The final volume was made up to 1.0 mL with D.W./PBS for protein and LPO, respectively. Therefore the concentrations of As_2O_3 and plant extract in final volume were 3 µg/mL and 25-100 µg/mL, respectively.

All the tubes were subjected to incubation for 30 minutes at 37°C. Estimation of LPO and protein was done by the following methods:

Lipid peroxidation (LPO)

The total LPO in control, toxin treated and toxin + antidote treated samples were measured by quantification of thiobarbaturic acid reactive substance (TBARS) determined by the method of Ohkawa et al. (22).

Protein

The total protein in control, toxin treated and toxin + antidote treated tubes were estimated by the

method of Lowry et al. by using serum albumin as a standard (23).

Statistical analysis of the data was performed using Student's t test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Tables 1 and 2 show the effect of arsenic trioxide on production of thiobarbaturic acid reactive substances (TBARS) and protein content in chicken liver homogenate *in vitro*. The results revealed that H_2O_2 induced lipid peroxidation increases and protein content decreases as the concentration of arsenic trioxide is increased (1-5 mg/mL) in liver homogenate. A maximal increase in LPO and a decrease in protein content was observed at 3.0 mg/mL of arsenic trioxide.

Tables 3 and 4 show that an addition of 3 mg/mL of arsenic trioxide to liver homogenate did not cause significant alteration in LPO and protein content as compared to control. However, concurrent addition of arsenic trioxide (3 µg/mL) and various concentrations (25-100 µg/mL) of 50 % aqueous extract of each medicinal plant (MO, PF, and TA) to liver homogenate caused significant amelioration. The maximum amelioration of MO, PF, and TA was observed at 100 mg/mL, 50 µg/mL, and 100 µg/mL, respectively. Thus it is concluded that aqueous extract of PF was observed to have better ameliorative effect.

Table 1. Arsenic trioxide induced changes in protein content in chicken liver homogenate *in vitro*.

Arsenic (µg/mL)	Protein (mg protein/100 mg fresh tissue weight)	Significant at p <
0	12.14 ± 0.707	—
1	9.888 ± 0.341	0.05
2	10.230 ± 0.342	0.05
3	10.572 ± 0.163	0.05
4	10.643 ± 0.149	N.S.
5	10.964 ± 0.080	N.S.

Values are the mean ± S.E.M, n = 10

Table 2. Arsenic trioxide induced changes in lipid peroxidation in chicken liver homogenate *in vitro*.

Arsenic (µg/mL)	Lipid peroxidation (nanomoles MDA/mg protein/60 minutes)	Significant at p <
0	4.390 ± 0.080	—
1	4.918 ± 0.151	0.01
2	6.058 ± 0.142	0.001
3	6.558 ± 0.105	0.001
4	5.957 ± 0.058	0.001
5	5.264 ± 0.131	0.001

Values are the mean ± S.E.M, n = 10

DISCUSSION

The present study was planned to evaluate toxic effect of arsenic trioxide by a biochemical parameters indicative of oxidative stress and its amelioration by three medicinal plants (MO, PF, and TA) *in vitro*. Arsenic is a known toxicant and it leads to a large number of hematological, hepatic, renal, and neurological disorders (23). Increased free radical level is reported during arsenic exposure (24, 25). The reactive oxygen species (ROS) are implicated as important pathological mediators in many disorders. Study conducted in our laboratory also indicated that arsenic trioxide might generate ROS causing cellular toxicity (26). Our result revealed that an addition of arsenic trioxide (1-5 µg/mL) to chicken liver homogenate *in vitro* shows significant alteration in LPO and protein content. The maxi-

Table 3. Effect of arsenic trioxide induced changes in protein (mg%) content in liver homogenate and its amelioration by *P. fraternus, Terminalia a.* and *Moringa oleifera*.

Treatment As_2O_3 (µg/mL)	Plant extract (µg/mL)	*P. fraternus*		*Terminalia a.*		*Moringa oleifera*	
		Protein	Significant at p <	Protein	Significant at p <	Protein	Significant at p <
0	0	12.371 ± 0.233	—	12.275 ± 0.95	—	12.235 ± 0.145	—
3	0	12.345 ± 0.197	—	10.566 ± 0.75	—	11.978 ± 0.171	—
0	100	11.618 ± 0.075	—	12.10 ± 0.34	—	11.460 ± 0.122	—
3	25	12.084 ± 0.118	0.001	11.210 ± 0.131	0.001	11.837 ± 0.041	0.01
3	50	12.097 ± 0.074	0.001	11.283 ± 0.105	0.001	11.965 ± 0.062	0.001
3	75	12.384 ± 0.051	0.001	10.944 ± 0.147	0.001	11.934 ± 0.057	0.001
3	100	12.065 ± 0.087	0.001	10.660 ± 0.269	0.001	11.377 ± 0.82	N.S.

Values are the mean ± S.E.M, n = 10

Table 4. Effect of arsenic trioxide induced changes in lipid peroxidation (nanomoles of MDA/mg protein/ 60 min.) in liver homogenate and its amelioration by *P. fraternus, Terminalia a.* and *Moringa oleifera.*

Treatment As_2O_3 (µg/mL)	Plant extract (µg/mL)	*P. fraternus*		*Terminalia a.*		*Moringa oleifera*	
		LPO	Significant at p <	LPO	Significant at p <	LPO	Significant at p <
0	0	4.374 ± 0.146	—	4.157 ± 0.221	—	4.399 ± 0.340	—
3	0	4.284 ± 0.209	—	7.277 ± 0.389	—	4.353 ± 0.269	—
0	100	6.558 ± 0.105	—	4.028 ± 0.237	—	6.382 ± 0.393	—
3	25	4.275 ± 0.307	0.05	5.52 ± 0.314	0.05	5.820 ± 0.445	N.S.
3	50	3.986 ± 0.238	0.001	5.046 ± 0.474	0.01	5.653 ± 0.426	N.S.
3	75	3.925 ± 0.216	0.001	4.539 ± 0.229	0.01	5.333 ± 0.262	0.05
3	100	3.870 ± 0.292	0.001	4.077 ± 0.170	0.001	4.570 ± 0.375	0.001

Values are the mean ± S.E.M, n = 10

mum alteration was observed at 3 µg/mL concentration of arsenic trioxide (Table 1 and 2). The alteration in lipid peroxidation and protein content might be due to the production of ROS by arsenic trioxide (26).

PF, TA, and MO are three medicinal plants which are widely used in ayurvedic practice as hepato-protective medicine. Recent studies of TA revealed anticancer potency against hepatocellular carcinoma in rats, the action is due to the strong free radical scavenging activity comparable to that of vitamin C (14, 27-28). Our result revealed max. retardation of arsenic trioxide induced toxicity by TA at 100 mg/mL concentration *in vitro*. Hepato-protective effect of medicinal plant MO was studied extensively, the protective effect of plant is mainly because of the presence of vitamin A, C, and B along with calcium and potassium (29). L. Pari and N. Ashok Kumar (2002) have studied the hepato-protective activity of MO on anti-tubercular drug-induced liver damage in rats. (30) Our result revealed max. retardation of arsenic trioxide induced toxicity by MO at 100 µg/mL concentration *in vitro*. PF is known as liver tonic in ayurveda. It carries specific activity in energy metabolism and protects mitochondrial dysfunction (16, 31). *In vitro* and *in vivo* experiment for the protective effect of PF revealed its specificity towards liver and antioxidative properties. The result revealed max. retardation of arsenic trioxide induced toxicity by PF at 50 µg/mL concentration *in vitro*. Hence our results revealed that PF may have better hepato-protective action against arsenic trioxide induced toxicity than MO and TA *in vitro*.

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