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Effect of *Hibiscus esculentus* fruit on arsenic induced oxidative changes in albino rats

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Abstract

Objective: The study was undertaken to assess the antiperoxide effect of *Hibiscus esculentus* fruit on various tissues, blood cell counts and haemoglobin of arsenite exposed albino rats. Materials and methods: *H.esculentus* fruit extract (200 mg/kg body wt/day) obtained by continuous hot percolation in soxhlet apparatus and was administered orally to albino rats. Its protective effect was determined on sodium arsenite induced lipid peroxidation (LPO), superoxide dismutase (SOD) levels of various tissues, blood cell counts and haemoglobin of rats. Results: A significant reduction in arsenic induced LPO in brain, heart, lungs and liver was observed after the administration of *H.esculentus* fruit extract. The blood lactate and SOD levels in tissues decreases when the extract was given with arsenite. There was a significant increase in RBC, WBC, neutrophils, lymphocytes, haemoglobin and percentage of body weight in rats administered with *H.esculentus*. Conclusion: These results indicate that people exposed to higher levels of arsenite can increase their intake of *H.esculentus*, as the quercetin 3-O-glucoside which is the major antioxidant of *H.esculentus* has a possible protective role on LPO status of certain tissues and blood cell counts of arsenic exposed rats.

Key words: *Hibiscus esculentus*, antioxidants, lipid peroxidation, arsenic exposure, Thiobarbituric acid reacting substances.

1.Introduction

Inorganic arsenic is considered the most potential human carcinogen and humans are exposed to it from soil, water, air and food [1]. The principal natural reservoirs of arsenic are rocks. Mobilization of arsenic from these sources constitute the availability of this element in soil, water and air in various forms. Soil may contain arsenic levels between 0.1 and 40 ppm [2, 3]. In seven districts of West Bengal arsenic has been found in ground water above maximum permissible limit (0.05 mg/l) recommended by WHO [4]. Arsenic is a pro-oxidant and thus may cause lipid peroxidation [5]. In traditional societies, nutrition and healthcare are interconnected and many plants are consumed as food in order to benefit health [6]. *H. esculentus* fruit (Fam. Malvaceae) contains pectin which helps to lower the blood

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cholesterol levels [7]. In Unani system of medicine, *H. esculentus* fruit is used to treat blood disorders, strangury and diarrhoea. *H.esculentus* was also proved to have an antiulcerogenic effect [8]. Ansari *et. al* [9] reported that *H. esculentus* showed a *in vitro* non-enzymatic inhibition of bovine brain lipid peroxidation. Linn *et. al* [10] found that quercetin 3-O-glucoside is the major antioxidant in *H. esculentus*. But the *in vivo* antioxidant effect of *H. esculentus* and its effects on blood cells of arsenic exposed rats have not yet been investigated. So, it was decided to study the effect of *H. esculentus* fruit on LPO and SOD status of different tissues, lactate levels in blood, haemoglobin and blood cell counts of arsenite exposed rats. This would be useful to persons exposed to arsenic chronically.

2. Materials and Methods

2.1 Animals

The experiments were designed and conducted according to the Institutional Animal Ethical Committee (IAEC) and guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), after obtaining clearance from the committees. The adult Wistar strain male albino rats weighing 280 ± 2.9 g, were used for the study, food and water were given *ad libitum*. The animals were fed with commercial pellet rat chow (calcium 1%; phosphate 0.6%) obtained from Gold Mohur Animal Feeds (Bangalore, India).

2.2 Chemicals

Trichloroacetic acid (TCA), Tris-HCl, thiobarbituric acid (TBA) and sodium arsenite were purchased from Sigma Chemical Co. (St. Louis, Mo.USA), Lactate Kit (Dia Sys Diagnostic Systems, Germany).

2.3 Extract of Hibiscus esculentus

Fresh fruits of *H. esculentus* were procured from a vegetable shop in Peelamedu, Coimbatore. The fruits were washed with fresh water and dried in shade at room temperature. The dried fruits were ground into fine powder and 500 g of dried powder was packed in soxhlet apparatus and extract was prepared with 300 ml of acetone by continuous hot percolation method for 6 hours. Acetone was distilled off to yield the total extract of *H. esculentus*. It was dried to constant weight at 80°C and kept in a desiccator [11]. The yield of *H. esculentus* was found to be 1.8%.

2.4 Experimental procedures

The animals were divided randomly into three groups consisting of 10 rats in each group.

Group I - Control group.

Group II - Sodium arsenite 2.5 mg/kg body weight was administered intra-peritoneally for 20 days.

Group III - Fed with extract of *H.esculentus* fruit 200 mg/kg body weight intra-gastrically with Ryle's tube followed by i.p. administration of sodium arsenite (same dose) one hour later for 20 days. All the rats were weighed both before and after the experiment to see if there was any alteration in the weight. At the end of 20 days blood was collected by retro-orbital puncture by anaesthetizing the animals using ether. Blood was collected in bottles containing ethylene diamine tetra acetic acid (EDTA). The skull, thorax and abdomen were opened by a midline incision and brain, lungs, heart, liver and kidney were excised, washed with chilled saline (0.9%) and stored in a deep freezer [12].

2.5 Estimation of thiobarbituric acid reacting substances (TBARS)

Tissues were homogenized in Tris-HCl buffer, pH 8.6 (0.1M). The lipid peroxidation was estimated by Ohkawa *et. al* [13] method. LPO was estimated by the formation of TBARS. The tissue homogenate was prepared in 0.1 M acetate buffer at pH 5 in Erlenmeyer flask and incubated for 1 hour (37°C).

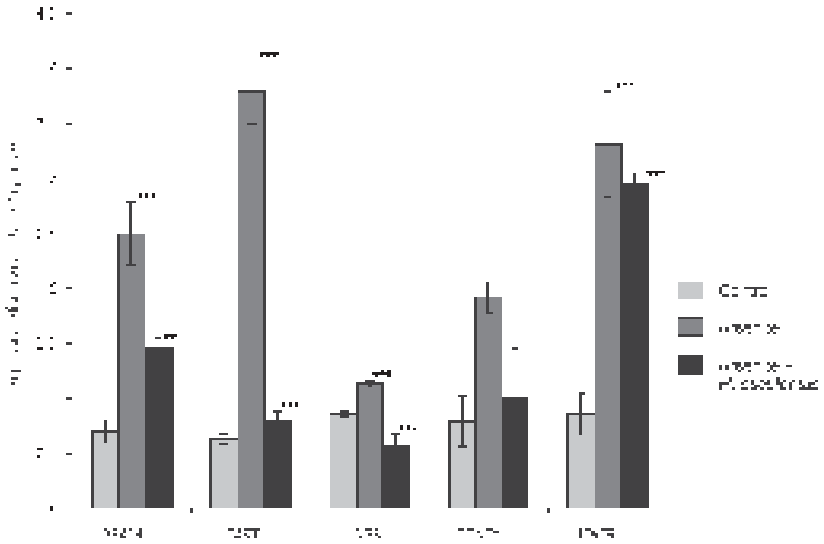

Fig. 1. Effect of *Hibiscus esculentus* fruit on superoxide dismutase. Values are mean ± SEM (n=10), **p<0.01,***p< 0.001.

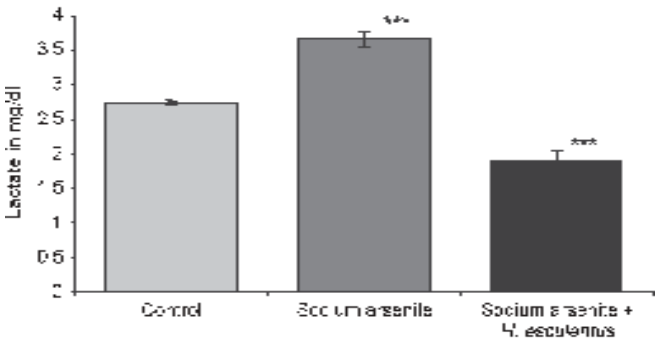

Fig. 2. Effect of *Hibiscus esculentus* fruit on blood lactate levels. Values are mean ± SEM (n=10), ***p<0.001.

Table 1. Effect of *Hibiscus esculentus* fruit on sodium-arsenite induced changes in LPO status of different tissues of rats.

Group	Treatment	Tissues (TBARS in nmol of MDA formed / gm of tissue)				
		Brain	Heart	Lungs	Kidney	Liver
I	Control	0.11 ± 0.00	0.06 ± 0.00	0.08 ± 0.01	0.15 ± 0.02	0.15 ± 0.03
II	Arsenite-treated (2.5 mg /kg body wt/day)	0.14 ± 0.02	0.19± 0.03***	0.20 ± 0.03**	0.19 ± 0.02	0.19 ± 0.02
III	Arsenite+*Hibiscus esculentus* (200 mg/kg body wt/day)	0.02 ± 0.01***	0.04 ± 0.00***	0.02 ± 0.00***	0.09 ± 0.02	0.03 ± 0.00***
	F ratio	17. 30[a]	18.42[a]	17.09[a]	4.46[a]	6.40 [a]

Values are expressed as mean ± SEM; n=10. ***p<0.001, **p<0.01, a denotes significance at 1% level. (One way ANOVA followed by Student's unpaired '*t*'- test).

Table 2. Effect of *Hibiscus esculentus* fruit on sodium arsenite induced changes in blood cells counts and body weight of rats.

Groups	RBC (millions/ cumm)	WBC (thousands/ cumm)	Differential Count			Hb (gm%)	% ↑or ↓in weight
			Neutrophil	Lymphocyte	Monocyte		
I Control	8.72 ± 0.08	6350 ± 138.44	65.40 ± 0.65	32.50 ± 0.60	1.00 ± 0.00	17.86 ± 0.16	1.61 ± 0. 50
II Arsenite (2.5 kg/body wt of Sodium arsenite)	6.79 ± 0.14 ***	3080 ± 268.66***	50.30 ± 0.88***	24.30 ± 0.36***	2.50 ± 0.42**	14.68 ± 0.13***	3.15 ± 0.77
III Arsenite+ *Hibiscus esculentus* (200mg/kg body wt)	8.28 ± 0.93***	7200 ± 1014.06***	78.90 ± 0.73***	31.10 ± 0.27***	3.50 ± 0.65	19.98 ± 0.20***	8.06 ± 1.05**
F ratio	109.49[a]	225.44[a]	351.14[a]	1.25	7.80[a]	248.36[a]	25.20[a]

Values are expressed as mean ± SEM; n=10. ***p<0.001, **p<0.01,a denotes significance at 1% level. (One way ANOVA followed by Student's unpaired '*t*'- test).

Aliquots of 1ml were taken into 1.5 ml of 20% cold TCA and centrifuged at 3000 rpm for 10 min. To 2 ml of the supernatant, 2 ml of 0.67% aqueous TBA reagent was added, mixed well and kept in a boiling water for 10 min. After cooling the pink colour obtained was measured at 535 nm. Molar extinction co-efficient was 1.56 x 10^5/cm/M.

2.6 Estimation of superoxide dismutase

Superoxide dismutase was estimated by the method described by Marklund and Marklund [14]. The principle of this method is the inhibition of autooxidation of pyrogallol by superoxide dismutase. Protein estimation was done by Biuret method [15]. The values of superoxide dismutase are expressed in units per mg of protein.

2.7 Estimation of lactate

Lactate was estimated by using the Lactate kit purchased from Dia Sys Diagnostic Systems, Germany.

2.8 Determination of blood parameters

Red blood cell (RBC), white blood cell (WBC) and differential leucocyte counts were determined by using Haemocytometer consisting of Neubauer's counting chamber with appropriate diluting fluids, and stain. Haemoglobin was determined by using Sahli's haemoglobinometer.

2.9 Statistical analysis

Data are expressed as Mean ± SEM. One way ANOVA test was performed to find whether or not scores of different groups differ significantly. To test the intergroup significant difference Student' s unpaired '*t*' test was performed. Statistical probability of $p<0.05$ was considered to be significant.

3. Results

3.1 TBARS

Table 1 shows that compared to control, group II rats on exposure to arsenite showed significant increase in MDA production in heart ($p<0.001$) and lungs ($p<0.01$). Administration of *H. esculentus* fruit extract along with arsenite had shown to effectively reduce such arsenite generated increase in MDA production in brain, heart, lungs and liver of rats ($p<0.001$).

3.2 Superoxide dismutase

Superoxide dismutase levels increased significantly ($p<0.001$) in brain, heart, lungs and liver after treatment with arsenite. Administration of *H. esculentus* decreased the superoxide dismutase levels significantly in brain ($p<0.01$), heart, lungs and liver ($p<0.001$) when compared with rats treated with arsenite alone. (Fig 1).

3.3 Blood lactate levels

Lactate levels in blood increased in group II rats significantly ($p<0.001$). These levels decreased in group III animals ($p<0.001$) when compared with rats treated with arsenite alone (Fig 2).

3.4 Blood cell counts and Haemoglobin

Arsenite treatment caused a significant ($p<0.001$) decrease in RBC and WBC cell counts. Differential count also showed a significant ($p<0.001$) decrease in neutrophils, lymphocytes, monocytes ($p<0.01$) after arsenite treatment. Group II animals also showed a decrease in haemoglobin ($p<0.001$) when compared with group I animals. In group III animals there was a significant ($p<0.001$) increase in RBC, WBC, neutrophils, lymphocytes and haemoglobin. (Table 2).

3.5 Body weight

There was a decrease in the weight in group II rats, though it was not statistically significant. But *H. esculentus* treatment showed a significant ($p<0.01$) increase in weight in group III rats. (Table 2).

4. Discussion

Large number of man made arsenic compounds are used in agriculture as effective agents against weeds and pests [2]. Arsenic present in metal ores or coal is released during melting process or in coal burning and this contaminates the soil, air and water [16]. So human beings are unavoidably exposed to this toxic metalloid. The interest in search for new natural antioxidants has grown over past years because reactive oxygen species production and oxidative stress have been shown to be linked to age related disorders [17]. Synthetic antioxidants can cause problem of toxicity [9].

Sodium arsenite is the main toxic form of arsenic in environment [18]. The significant increase in LPO following administration of sodium arsenite in heart, lungs of rats reported in this paper agrees well with the previous studies [19]. There was increase in LPO in liver, brain and kidneys also though it was not statistically significant.

Ramos *et. al* [20] demonstrated that acute arsenic exposure for 1 hour lead to significant increase in LPO in liver, kidney, lungs and heart with concomitant decrease in tissue glutathione content. Maiti & Chatterjee [21] also showed in their studies that glutathione concentration was significantly depleted and lipid peroxidation was increased after 1 hour of arsenic exposure. It was also found that arsenic generates reactive oxygen species [22, 23, 24].

When *H. esculentus* was given there was a significant decrease in the LPO in brain, heart, lungs and liver. The oxidant effect of arsenite was counteracted by *H. esculentus* by its free radical scavenging activity. Ansari *et. al* [9] by DPPH assay reported that *H. esculentus* has a free radical scavenging activity. They also proved that extract of it has *in vitro* non enzymatic inhibition of lipid peroxidation in liposomes.

Arsenite inhibits pyruvate dehydrogenase (PDH) activity through binding to vicinal dithiols in pure enzyme and tissue extract [25]. Activity loss of PDH was linked to modification of the sulph hydryl groups on lipoic acid, the essential co-factor of PDH. Hence, compounds with sulph hydryl group will prevent the inhibition of PDH by arsenic. *H. esculentus* contains large amounts of quercetin which can convert 30-100% of thiamine into thiamine di sulphide [26] which has sulphydryl group that will prevent the inhibition of PDH. So there is a

significant decrease in blood lactate levels when *H. esculentus* was given along with sodium arsenite. The increase in superoxide dismutase levels after arsenite treatment agrees well with the previous studies of Mylona *et. al* [27]. They showed that superoxide dismutase activity was increased in response to a wide range of arsenic concentration. Arsenite increases the superoxide dismutase levels due to the oxidative stress. When *H. esculentus* is given the antioxidants present in it prevents the oxidative stress and thus the increase in the superoxide dismutase levels is not seen in group III animals.

There was a significant decrease in RBC count and Haemoglobin after arsenite administration. This is in accordance with the previous studies where there was decrease in red cell production after administration of arsenic and arsenious acid [28]. Garcia and Hernemdez [29] found that arsenic interferes with the activities of several enzymes of haem biosynthetic pathway. It was shown that in rats the presence of arsenic is prolonged owing to its accumulation in erythrocytes [30]. RBC are very susceptible to ASH_3 toxicity. This was demonstrated by an immediate intracellular potassium loss and by hemolysis and lactate dehydrogenase leakage [31]. It was also shown that rat haemoglobin specifically binds dimethyl arseinous acid and this greatly increases the biological half life of inorganic arsenic in rats [32]. So arsine is believed to be fixed by haemoglobin in a non-volatile form within red cell after which lysis occurs [33], perhaps as the consequence of the action of compounds formed in oxidation of arsine [34]. Increase in the blood cells and the haemoglobin when *H. esculentus* was administered could be due to its antioxidant effect which prevents the cell lysis caused by arsenite.

The neutrophils and lymphocytes showed a significant increase following *H. esculentus* administration compared with arsenic treated rat. Neutrophils, lymphocytes and monocytes decrease in number following sodium arsenite injection as the inorganic arsenic poisoning causes granulocytopenia [35], anemia and less often thrombocytopenia. There was a significant increase in weight gain in group II and group III animals. This is contradictory to the previous studies which states that arsenic decreases the body weight [36]. *Hibiscus esculentus* also has a tendency to increase the body weight probably because it contains large amounts of protein.

5. Conclusion

The study demonstrates that *H. esculentus* has the potential to reverse some of the biochemical changes produced by arsenic in rats. Further studies may suggest its use in humans.

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