INTERNATIONAL JOURNAL OF BIOASSAYS
ISSN: 2278-778X
CODEN: IJBNHY
ORIGINAL RESEARCH ARTICLE
OPEN ACCESS

Studies on the remedial effects of coriander and cumin on arsenic exposed Charles Foster Rats

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Received: April 12, 2016; **Accepted**: April 25, 2016

Abstract: A wide exploration to achieve an easy and effective remedy for the chronic arsenic poisoning has been carried out in the present study. The study constitutes the treatment of arsenic treated rats with the ethanolic extracts of cumin seeds and coriander leaves respectively. Rats were fed with sodium arsenate through gavage method for 45 days and then were fed with ethanolic extracts of cumin seeds and coriander leaves. The rats were observed recovering themselves after exposure of ethanolic extracts of cumin seeds and coriander leaves which was confirmed by the biochemical assays (KFT and LFT) carried out for the well testing of Liver and Kidney. A substantial recovery was estimated in the blood cells count, activity of liver enzymes and decrease in the level of malondialdehyde.

Key words: Arsenic; Sodium Arsenate; Cumin Seeds and Coriander Leaves.

Introduction

Arsenic is a metal which forms various compounds, inorganic or organic. It is classed as metalloids because it complexes with metals. Inorganic arsenic is widely distributed in nature in trivalent form (As 3+) and also found in pentavalent form (As 5+). The arsenic form includes arsenic trioxide, sodium arsenite, and arsenic trichloride.

The less toxic organic arsenite is produced in a biomethylation process by many organism including human and shellfish. Though, arsenic occurs naturally in rocks and soil, majority of arsenic released into the environment is from Industrial smelting.

Human exposure to arsenic is primarily by food, air and water. Drinking water may be contaminated with arsenic from arsenical pesticide, natural mineral deposits or improperly disposed arsenical chemicals. However, the most effected regions are the river basin of Ganga, Brahmaputra and Meghna in India and Bangladesh.

Arsenic is one of the most dangerous occupational and environmental toxins. Both natural and anthropogenic sources are responsible for the distribution of many toxicants, mainly heavy metals throughout the environment. Arsenic is abundant in the crust of the earth and is found in all environments. It is found in soil, minerals, surface and groundwater (Antman, 2001). The arsenic contamination was also observed in three districts Ballia, Varanasi and Gazipur of UP in the upper and middle Ganga plain, India (Ahamed *et al.*, 2006). Globally, over 130 million people are now estimated to be potentially exposed to arsenic in drinking water at concentrations above the WHO guideline value of 10 ppb, a number that is likely to grow as more areas are affected now. The two worst affected areas in the world are Bangladesh and West Bengal, India. In 42 districts in southern Bangladesh and in nine adjacent districts in West Bengal, 79.9 million and 42.7 million people respectively are exposed to groundwater arsenic concentrations that are *above* the World Health Organization maximum permissible limit of 50 ppb. Approximately 20 incidents of groundwater arsenic contamination have been reported from all over the world (Mukherjee *et al.*, 2006). Due to groundwater contamination, a large number of populations in Bangladesh are suffering from melanosis, leuco-melanosis, keratosis, hyperkeratosis, dorsum, non-petting oedema, gangrene, skin cancer and skin lesions in sole and palm (Karim, 2000).

India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy. These systems have rightfully existed side by side with Allopathic and are not in 'the domain of obscurity'. Millions of Indians use herbal drugs regularly, as spices, home-remedies, health foods as well as over-the-counter (OTC) as self-medication or also as drugs prescribed in the non-allopathic systems.

A. Crude drugs are usually the dried parts of medicinal plants (roots, stem wood, bark, leaves, flowers seeds, fruits, and whole plants etc.) that form the essential raw materials for the production of traditional remedies of Ayurveda, Siddha, Unani, Homeopathy, Tibetan and other systems of medicine including the folk, ethno or tribal medicines. The crude drugs are also used to obtain therapeutically active chemical constituents by

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specialised methods of extraction, isolation, fractionation and purification and are used as phytochemicals for the production of modern allopathic medicines or herbal/ phytomedicines.

B. Phytoremediation has been focused on two important plant extracts; Cumin *(Cuminum cyminum)* and Coriander *(Coriandrum sativum)*, Dhandapani *et al.,* (2002) have well reported the hypolipidemic effect of *Cumin* while Chauhan *et al.,* (2010) have observed the immunomodulatory effect. *Derakhshan et al.,* (2008 & 2010), Allahghadri *et al.,* (2010) and Pajohi *et al.,* (2011) have well studied on the antibacterial properties of *Cumin.* On the other hand, similar studies have been also reported by Samojlik *et al.,* (2010) who have well studied on the antioxidant and hepato-protective properties of *Coriander.* Jagtap *et al.,* (2004) and Wu *et al.,* (2010) have also studied the anti-inflammatory effect of *Coriander* while Otoom *et al.,* (2006) have well reported the anti-diabetic effects of it. Jabeen *et al.,* (2009) have reported the diuretic properties of *Coriander* while Rahman *et al.,* (2011) have reported about its antibacterial properties.

Materials and Methods

(a) L.D 50 of sodium arsenite for the test animal

- L.D 50 is a standardized method for expressing and comparing the toxicity of chemicals. It is the dose that kills 50% of the population. In order to avoid the large numbers of killing of test animals a small population of 4 rats per dose were selected.
- A dose dependent experiment was performed to identify the maximum permissible dose of Sodium Arsenite.
- Forty (4*10) rats were grouped into four and reared in 10 different cages administered with consecutive dose of Sodium Arsenite 1-10 mg/kg body weight of rats for 7 days through gavages method.
- The mortality rate of rats among each group was noted.

(b) Preparation of ethanolic extract of cumin seeds and coriander leaves

- The crude extracts of cumin seeds and coriander leaves were prepared using a vacuum rotary evaporator.
- The spice materials were cut into small pieces; 20 g of each were soaked in 100 ml of 95% ethanol, and shaken at 150 rpm for 4 days at ambient temperature. The filtrates were then evaporated using vacuum rotary evaporator, and frozen at -80°C before freeze drying.
- Stock solutions of crude ethanolic extracts was then prepared by diluting the dried extracts with 10% dimethyl sulphoxide (DMSO) solution to obtain a final concentration of 400 mg/ml.

(c) Study on effects of Arsenic on rats through hematological parameters

- Determination of erythrocytes, lymphocytes and platelets were done using a Neubauer chamber after proper dilution of the blood into R.B.C counting fluid, W.B.C fluid and platelets counting fluid. The no of cells and platelets were counted under a compound microscope.
- Blood taken from the sacrificed rats were taken in heparinized tubes for the hematological determination and parallel into anticoagulant ones.
- Cyanmethahemoglobin method were used for the determination of hemoglobin levels.

(d) Effect of Arsenic induced toxicity on liver and kidney of rats

- Liver function test (LFT) and Kidney function test (KFT) were performed to check the toxicity in Liver and Kidney respectively after administration of Arsenic for a particular time period of 60 days.
- All the test was performed using the serum as sample with the commercial kits manufactured by CORAL, India.

(e) Antioxidant study through Lipid peroxidase assay (free radicals) in serum

- Lipid peroxide level were estimated in blood samples by thiobarbituric acid reaction (TBA) with malondialdehyde (MDA), formed due to the peroxidation of membrane lipids compelled by the free radicals (*Ohkawa et al*, 1979). Lipid peroxides are expressed as nM of MDA formed in tissue and accumulated in the serum.

Statistical analysis

Analysis of variance (ANOVA) was used to determine whether significant differences existed between control and treated groups with mean ± standard deviation. A value of $P < 0.05$ was chosen as the minimal level of statistical significance.

Result

General Observation

The Pups were observed slow growing with stability in their weight during the mid-Arsenic administration time period of 60 days. Bleeding eyes and ill behavior were noticed. Male rats were observed with enlarged testis.

L.D. 50 dose of Arsenic for the test animal

L.D. 50 dose for the test animal was determined to be 40-50 mg of arsenic/kg bodyweight of animal, in which 50% of the population of the group i.e two animals out of four survived after 10 days.

Hematological parameters

Hemoglobin concentration (A) and the number of red blood cells (B) can be seen with a significant decrease in the rats treated with arsenic ($F=21.59$, $P<0.01$) compared to the control groups in fig 1). A. Interestingly, when administered with dose of cumin seeds or coriander leaves, a daily dose of 50 mg/kg body weight of the rat, appears to be bit effective in restoration.

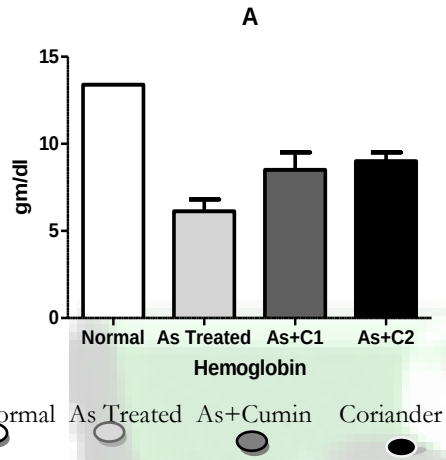

Fig.1. A) Hemoglobin levels (gm/dl). B) RBC count (per mm^3)

Significant decrease of hemoglobin content of approx 90%±5 % was determined in the rats intoxicated with arsenic, Likewise the level of RBC was also calculated to be decreased in number. The administration of cumin or coriander doses in the arsenic treated rats were found to be bit effective in restoration.

Biochemical parameters

Arsenic poisoning in rats led to significant increase in levels of enzymes >90% (fig.2.A) such as pyruvate transaminase and oxaloacetate transaminase which objects the normal functioning of liver whereas the bilirubin levels were also noted to be increased (fig. 2.B). Similarly, the parameters for the kidney testing such as Uric acid and Urea were increased after arsenic administration.

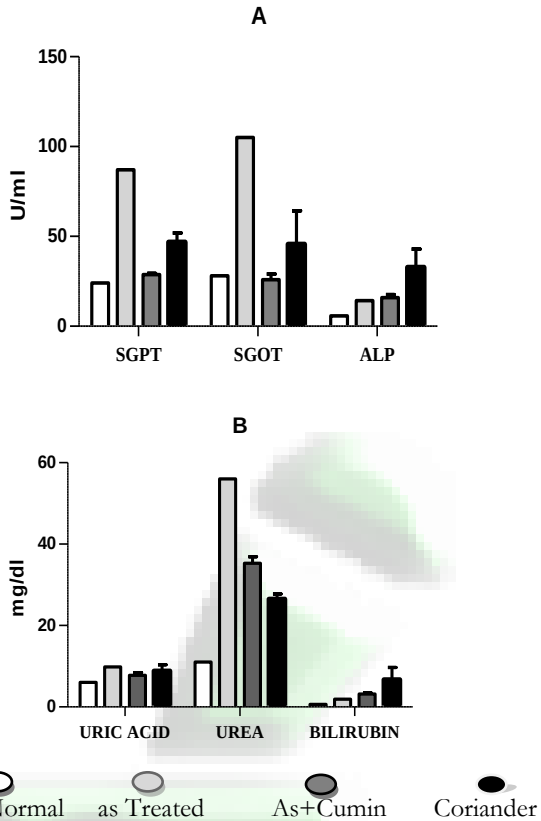

Fig.2: Biochemical Parameters. **A)** SGPT (Serum Glutamate Pyruvate Transaminase) SGOT (Serum Glutamate Oxaloacetate Transaminase) and ALP (Alkaline Phosphatase) levels (U/ml) in Serum. **B)** Uric Acid, Urea and Bilirubin levels (mg/dl) in serum.

Therefore, administration of the herbal doses of cumin or coriander in arsenic treated rats were seen with restoring ability to normal values of the liver enzymes and the kidney functioning parameters. Only the bilirubin levels did not show up with any change and was observed increased on 90^{th} day till the sacrifice of the rat was done.

Antioxidant assay

The assay which indicates the blood toxicity in the form of free radicals obtained from the oxidation of lipid present in the cell membrane leading to the destabilization of the bilayer membrane. The assay indicated a steep increase in the levels of malondialdehyde (MDA) in the serum of blood (fig. 3.A).

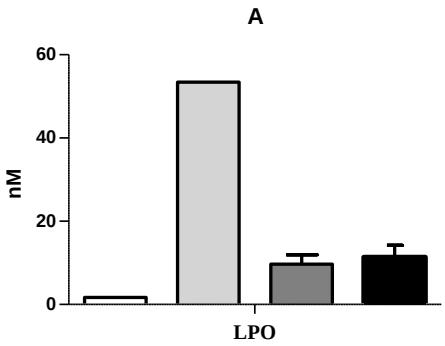

Fig. 3.A) Lipid peroxide (MDA level) (nM)

The levels of free radicals have not been restored and lipid peroxide was estimated as not so efficiently recovering to normal level after 45 days administration of herbal doses of cumin and coriander.

Discussion

It is well estimated that the nutritional status along with the herbal doses of cumin or coriander has a remarkable value on the development and might have a significant ameliorating effect against poisoning of arsenic as well as certain toxic agents in the body of rats.

These herbal doses treatment in the arsenic treated rats also supports the notion that these are of special importance in development of rats exposed to arsenic, as because no change in the feeding habit was observed and they were feeding on normal diet for the time period.

The present study on rats showed alteration in the hematological and biochemical parameters with arsenic exposure of 9 mg/kg body weight. Decreased number of red blood cell, hemoglobin and increase in the levels of liver enzymes, kidney function testing, and MDA level indicated the arsenic poisoning in our experimental model. And in the same model, ameliorating effect of the herbal doses of cumin and coriander were also observed.

Decrease in the level of hemoglobin can be inferred with anemia: For instance, arsenic exposure is reported with decrease in heme production due to inhibition of blood δ-aminolevulinic acid dehydratase (ALAD) activity (Modi *et al*., 2006).

In this study, we observed a significant recovery related to Liver function test and Kidney function test after treatment with the herbal doses of cumin or coriander on the arsenic treated animal models. We have investigated this phenomenon related to the disorganization of cell membranes at tissue levels through histopathology. There are reports supporting the experiment that exposure of arsenic leads to steep increase of free radicals in the cytoplasm, which thereafter leads to react with the membrane lipids in order to stabilize them self. The phenomena lead to disturbance in the orientation of the cell membrane. Our histopathology data that includes sectioning and staining of the liver tissues had revealed the phenomena of arsenic exposure. Likewise, the ameliorating effect of the herbal doses was also revealed with the restoration of the structure of tissues of the kidney and the liver.

Acknowledgement

Authors are thankful to Department of Zoology, Ranchi College, Ranchi and Mahavir Cancer Sansthan, Patna for providing the facilities for carrying out the experiments and DBT, Pune for the funds.

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Cite this article as:
Karan Kumar Kant and Namita lal. Studies on the remedial effects of coriander and cumin on arsenic exposed Charles Foster Rats. *International Journal of Bioassays* 5.5 (2016): 4552-4556.

Source of support: Nil
Conflict of interest: None Declared