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Histopathological effects of coriander and cumin on arsenic exposed liver of Charles Foster Rats

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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 histological studies on the liver of rats.

Key words: arsenic; sodium arsenate; cumin seeds; 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 include 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

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therapeutically active chemical constituents by specialized 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

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.

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.

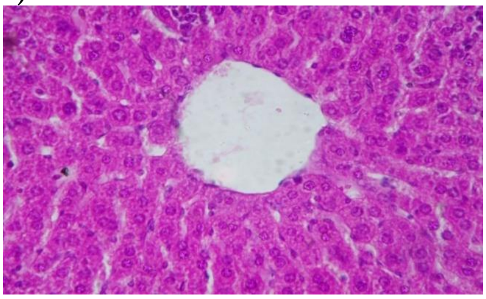
The spice materials were cut into small pieces; 20 g of each were soaked in 100 ml of 95% ethanol, and shaken at 150 rpm for 4 days at ambient temperature. The filtrates was then evaporated using vacuum rotary evaporator, and frozen at -80°C before freeze drying.

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.

Histopathological Study

Histopathological study supports the findings of hepatic irregularity analysed by the biochemical parameters. The control group of animal showed normal histological liver organization and architecture with granulated cytoplasm and small uniform nuclei. In the positive control groups, the liver tissue structure were observed with cellular disorganization which confirms the irregularity in the liver cells of the rats.

Microphotograph section of control Liver, stained with eosin and haematoxyline. (X× 400)



Figure(1N): T.S of liver showing normal architecture of central vein (CV) and hepatocytes.

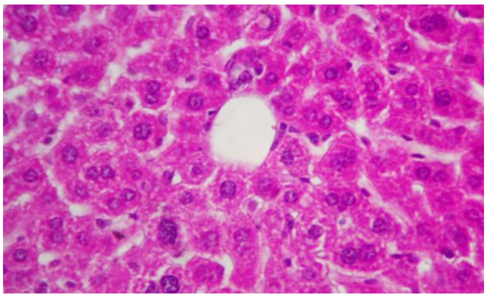


Figure (2N): T.S of liver showing normal architecture of few kupffer cell and portal vein, well arranged hepatocyte in sinusoid.

Microphotograph section of Liver, 60 days arsenic treated @ 9mg/kg b.w. (x×400).

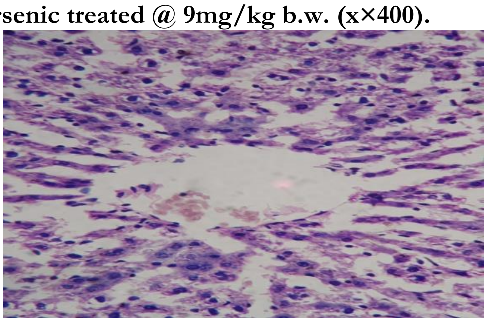


Figure (1A). Haemmorhage in central veins clearly observed.The number of kupffer cells has increased denoting the level of toxicity.The sinusoid are also disorganized .

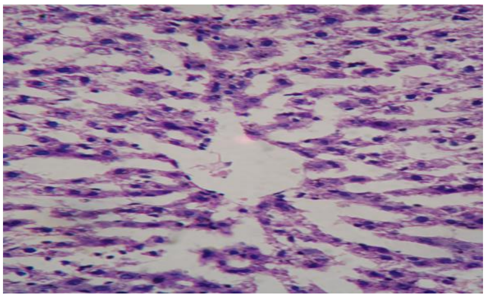


Figure (2A): T.S of liver showing high degree of degeneration in hepatocytes and central vein.The sinusoid are disorganized. The number of kupffer cells has increased denoting the level of toxicity.

Microphotograph section of Liver of pre-treated arsenic rats followed by 45 days cumin (200 mg /kg b.w) (×400), showing

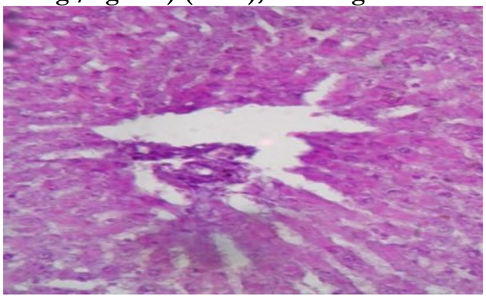


Figure 1LCU: T.S section of liver showing regeneration in liver as central vein is in normal shapes about 70% is clearly visible. The hepatocytes are normal and well arranged in the sinusoid

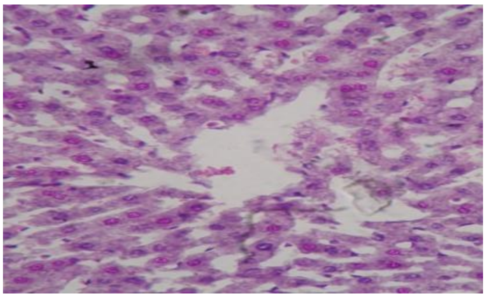


Figure 2LCU: T.S section of liver showing normal architecture of hepatocytes, although few vacuolation in the hepatocytes still persists and has not been restored completely.

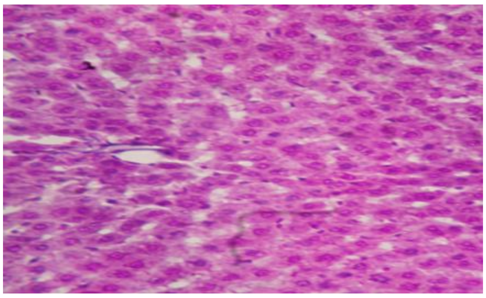


Figure 3LCU: The hepatocytes are well arranged in sinusoid and the restoration of portal vein is quiet apparent denotes the normalization in function of liver.

Microphotograph section of Liver of pre-treated arsenic rats followed by 45 days Coriander leaves (60 mg /kg b.w.) (×400), showing

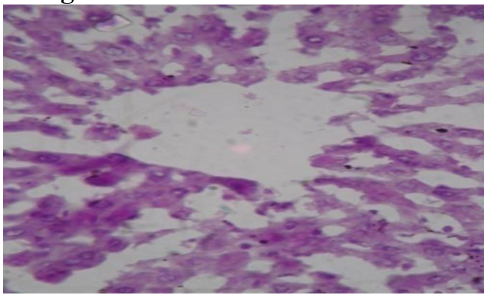


Figure 1LCO: T.S of liver showing regeneration process in the liver as central vein is restoring about 60% is clearly visible.

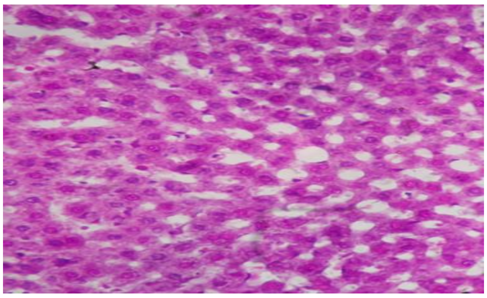


Figure 2LCO:

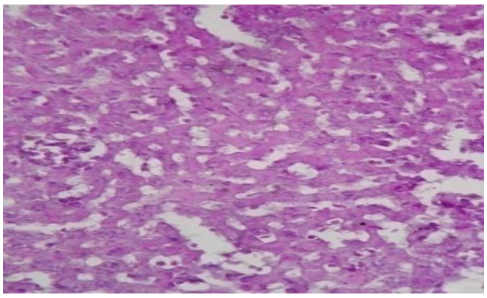


Figure 3LCO:

Figure (2) & (3) LCO: T.S of liver showing hepatocytes is regenerating and are also well arranged in sinusoid, although vaculations in the hepatocytes still persists that shows that restoration need more treatment with coriander.

Result and Discussion

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.

LD_{50} dose of Arsenic for the test animal

LD_{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.

The control group of animals showed normal histological liver architecture having cells with granulated cytoplasm and small uniform nuclei (fig. 2N). On the other hand, the positive control group showed significant loss of liver architecture (fig. 1C). This figure shows significant high degree of degeneration in both hepatic and portal vessels. The histological appearance of Kupfer cell can be also seen. The disoriented cells are seen to grow in nests and thick cords and are separated from one another by disorganized sinusoids. Cytologically, they seem to be like the tumor cells bearing some resemblance to normal hepatocytes, but are slightly larger, have more irregular and prominent nuclei (fig. 2C). In contrast the group treated with antidotes of cumin seeds and coriander leaves ethanolic extracts at a dose of 50 mg/kg b.w. and 500 mg/kg b.w. respectively showed well-defined structures and hepatocytes maintaining near normal architecture [fig. 1(LCU), 1(LCO)], and well-defined aggregation of hepatic and portal veins [fig. 2(LCU), 2(LCO)]. The potential beneficial effects of antidotes on the liver tissue proved to be a visible evident from histological studies which also showed a substantial improvement in the tissue architecture.

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