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PROTECTIVE EFFECT OF CUMIN ON SODIUM ARSENITE INDUCED TOXICITY IN CHARLES FOSTER RATS

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ABSTRACT

Arsenic in the present times has caused serious health hazards in the human population. In India, the arsenic poisoning in ground water in Gangetic plains has increased many folds. The exposed population are suffering from various arsenicosis symptoms such as hyperkeratosis, melanosis, and other internal organ diseases of gastrointestinal, cardiovascular, hormonal and others. Hence, the present study is focused to combat the deleterious effect of arsenic toxicity in animal models utilizing medicinal plant extract. The animals (Charles Foster rats) were treated with Sodium arsenite at the dose of 8 mg per kg body weight for 16 weeks to make arsenic model and upon these arsenic pre-treated rats seed extract of Cumin at the dose of 25 mg per kg body weight was administered for 4 weeks to study the ameliorative effects of this plant extract. After the entire treatment, rats were sacrificed and their blood samples were obtained and analysed for haematological and biochemical study. The study shows that arsenic induced toxicity caused deleterious effect on the rats at the haematological and biochemical levels and there was significant normalisation in the animal at all the respective levels. Hence, Cumin possesses ameliorative properties against arsenic induced toxicity and can be used for human purpose after dose titration.

KEYWORDS: Sodium arsenite, *Cumin*, protective effect, Charles Foster Rats.

1. INTRODUCTION

Arsenic menace in the recent years has become a major challenge in the terms of its health impact. About more than 300 million people are affected with the arsenic poisoning due to its contamination in ground water (Hassan 2018). In the state of Bihar about 10 million

population are exposed to arsenic due to drinking of contaminated water causing serious health related problems (Singh et al., 2014; Kumar et al., 2019). The Gangetic plains are the most affected area in the state where the exposed population along with common disease burden are also suffering from the non-communicable disease like cancer (Saha 2009; Rahman et al., 2019; Kumar et al., 2020). The trivalent form of arsenic is more toxic than the pentavalent form and unfortunately, in exposed population groundwater mostly trivalent form has been observed (Saha and Sahu 2016; Kumar et al., 2010).

The enters the human body through drinking water via human blood and causes various changes at the level of biochemical, hormonal, tissue and gene level disrupting the normal function of the cells (Vahidnia et al., 2008; Smith et al., 1998; Kannan et al., 2001). The also cause skin manifestations according to the magnitude of the exposure. The long duration exposure of arsenic causes damage to the vital organs of the body such as lungs, urinary bladder, liver, kidney etc. (Hartwig et al., 2002; Andrew et al., 2006). Arsenic usually binds with the sulfhydryl groups of enzymes which in turn causes changes in the epidermal keratinocytes leads to cause skin manifestations such as keratosis, melanosis, rain drop pigmentation etc. (Patlolla et al., 2005).

Use of medicinal plants for the ailments of various diseases has attracted researchers to decipher novel plants against the disease as it has very good impact and very least side effects (Maurya et al., 2011). Plethora of medicinal plants are available as antioxidants but, study on antidote for arsenic induced toxicity is very less. One such potential plant source is seed extracts of *Cuminum cyminum (Cumin).* The phytochemical investigations of *cumin* seeds revealed that it contained bioactive constituents such as cuminaldehyde, cymene and terpenoids (Singh et al., 2017; Hajlaoui et al., 2010).

Thus, the present study was undertaken to evaluate the antidote and antitoxic potential of the ethonolic extract of cumin seeds on sodium arsenite induced hepatotoxicity and nephrotoxicity in rats.

2. Experimental procedures

2.1 Animals

Adult and healthy male Charles Foster strain rats (24) of 8 weeks old weighing around 160g to 180g were used for the present study which were provided by the animal house of Mahavir Cancer Sanasthan and Research Centre, Patna, India (CPCSEA Reg-No.

1129/bc/07/CPCSEA). Ethical approval was obtained from the Institutional Animal Ethics Committee (IAEC) with IAEC No. IAEC/2012/12/04. The rats were acclimatized to laboratory housing conditions under 12 h light and dark cycles (room temperature maintained at 22 ± 2°C) for 15 days prior to the beginning of the treatment under standard laboratory conditions. These experimental rats were housed in conventional polypropylene cages with stainless steel grill top and the diet including food (self prepared laboratory) and water to rats were provided *ad libitum*.

2.2. Chemicals

Arsenic was used as Sodium Arsenite (98.5%) manufactured by Sigma-Aldrich, USA (CAS Number: 7784-46-5; S7400-100G), Lot# SLBH5736V, PCode 1001683292. It was purchased from the Scientific store of Patna, Bihar, India.

Preparation of *Cumin* seeds ethanolic extract: In the present study Cumin (*Cuminum cyminum)* seeds were purchased from local market of Patna, Bihar and identified by Dr. Ramakant Pandey (Botanist), Department of Biochemistry, Patna University, Patna, Bihar, India. The plant seeds were washed through running tap water and incubated at 37°C temperature. The dried seeds were grinded to fine powder which was further soaked in 100% ethanol for 48 hours, and finally extracted using Rota vapour apparatus after continuous drying for 4 hours at pressure 4psi. The ethanolic extract dose was calculated after LD_{50} estimation and the final dose was titrated to 25 mg/kg body weight. Previously, for LD_{50} estimation, 8 groups of rats (n=4 each) were set up and different doses of plant extract were orally administered for 7 days. The LD_{50} dose was found to be 3000mg/Kg body weight.

2.3. Experimental design

Rats were randomly divided into four groups, each group comprising six rats and categorized as following. Group I: Normal control group. Group II: Arsenic treated- Rats were orally sodium arsenite induced (8mg/kg body weight/day) for 16 weeks and were sacrificed after the completion of the experiment. Group III: Arsenic treated control- Rats were orally treated with sodium arsenite (8mg/kg body weight/day) for 16 weeks and left without any dosing for further 4 weeks. Group IV: *Cumin* seed extract administration- Rats were pretreated with sodium arsenite (8mg/kg body weight/day) followed by administration of cumin seeds ethanolic extract (25mg/kg body weight/day) for 4 weeks. After the end of dosing, rats were anaesthetized by diethyl ether and sacrificed. Blood samples were collected through the orbital puncture from all group of rats. Serum were then separated for the various

biochemical estimation.

Biochemical assay: Biochemical analysis were performed of the serum by standard kit process (Coral crest) through (UV - Vis) spectrophotometer (UV-10, Thermo Fisher, USA). The Liver Function Test as Serum Glutamic Pyruvate Transaminase (SGPT) and Serum Glutamic Oxaloacetate Transaminase (SGOT) were measured according to the method (Reitman & Frankel, 1957), Alkaline Phosphate (ALP) by method (Kind & King, 1954), total bilirubin activity by method (Jendrassik & Grof, 1983) while albumin level measured by BCG method. The Kidney Function Test (KFT) were analysed through Urea by (Fawcett, 1960 and Berthelot, 1859), Creatinine by (Toro, et al 1975), and Uric acid by (Bones, 1945).

2.6. Lipid peroxidation (LPO)

Thiobarbituric acid reactive substances (TBARS), as a marker of LPO, were evaluated through the double heating method (Draper and Hadley, 1992) based on the principle of spectrophotometric measurement of color reproduced during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA). For this study, 2.5 ml of 100gm/L solution of Trichloroacetic acid (TCA) were mixed with 0.5 ml serum in a centrifuge tube and heated in the water bath at 90°C for 15 minutes. After cooling, at room temperature, the mixture was further allowed to centrifuge at 3000 rpm for 10 minutes, and 2 ml of the supernatant was mixed with 1ml of 6.7gm/L TBA solution in a test tube which was further heated in water bath at 90°C for 15 minutes and left for cooling at the room temperature. Thereafter, further absorbance was measured by UV - Visible spectrophotometer (Thermo Scientific UV-10 USA) at 532 nm.

2.7. Statistical analysis

Results are presented as mean ± Standard Deviation (SD) for six rats individual groups and total variation represented in a set of data was analyzed through one-way Analysis of Variance (ANOVA). Differences among mean variance has been analyzed by applying Dunnett's 't' test at 99.9% ($p < 0.05$) confidence level. Calculations were performed with the GraphPad Prism Program (GraphPad Software, Inc., San Diego, USA).

3. RESULTS

3.1 Haematological study

In the present study, the data of haematological parameters are shown in Table.1, and the study shows significant decrease $P < 0.0001$ in the erythrocyte counts (RBCs), haemoglobin

percentage, haematocrit percentage, MCV, MCH but significant increase in leukocyte count (WBCs) in comparison with control group after 16 weeks of exposure. But, after administration of Cumin seed extract there was significant reversal in the haematological values. ANOVA showed that the sodium arsenite has more deleterious effect on time duration of exposure ($P < 0.0001$) (Table 1).

Table 1: Changes in the haematological parameters of Charles foster rats exposed to Sodium arsenite at the dose of 8 mg/Kg body weight daily for 16 weeks and its amelioration Cumin seed extract at the dose of 25 mg/Kg body weight for 4 weeks.

Blood Parameters	Control	Arsenic treated	*Cumin seed extract* treated
RBC Counts (10^6 /mm^3)	6.753 ± 0.25	2.98 ± 0.498	5.32 ± 0.225
Hb (percentage)	14.22 ± 0.56	5.33 ± 1.365	12.1 ± 0.549
Haematocrit percentage (Hct) (%)	43.10 ± 1.20	16.0 ± 2.276	36.02 ± 0.329
MCV (fL)	63.8 ± 1.67	53.7.21 ± 2.13	67.7 ± 1.51
MCH (pg)	21.1 ± 0.27	17.9 ± 3.62	22.7 ± 1.56
MCHC (g/L)	33.56± 0.036	33.3 ± 2.12	33.6 ± 1.45
WBC (10^3/mm^3)	7240 ± 3.56	3690 ± 8.35	8375 ± 3.90
Platelets (mm^3)	256000 ± 2.33	45000 ± 22.10	180000 ± 2.90

**The data are presented as mean ± S.D, n = 6, significance at P< 0.0001.*

3.2. Biochemical assay

The biochemical assay showed the following findings - in control group of rats, SGPT level was 29.56 ± 1.231 U/ml and after 16 weeks of arsenic treatment was 202.6 ± 27.23 U/ml, while, it was 54.21 ± 23.56 U/ml after 4 weeks of *Cumin seed extract* administration. In control group of rats, SGOT level was 33.51 ± 2.512 U/ml and after 16 weeks of arsenic treatment was 254.1± 14.03 U/ml, while, it was 65.22 ± 1.31 U/ml after 4 weeks of *Cumin seed extract* administration. In control group of rats, ALP level was 7.45 ± 0.539 K.A units and after 16 weeks of arsenic treatment was 35.23 ± 3.421 K.A units, while, it was 12.16 ± 1.09 K.A units after 4 weeks of *Cumin seed extract* administration. In control group of rats, bilirubin level was 0.853 ± 0.234 mg/dl and after 16 weeks of arsenic treatment was 2.45 ± 0.453 mg/dl, while, it was 1.217± 1.20 mg/dl after 4 weeks of *Cumin seed extract* administration. In control group of rats, the kidney function test urea level was 29.3 ± 1.323 mg/dl and after 16 weeks of arsenic treatment was 86.20 ± 2.189 mg/dl, while, it was 32.18 ± 0.47 mg/dl after 4 weeks of *Cumin seed extract* administration. In control group of rats, uric acid level was 2.98 ± 0.332 mg/dl and after 16 weeks of arsenic treatment was 13.90 ± 2.343 mg/dl, while, it was 3.89 ± 1.098 mg/dl after 4 weeks of *Cumin seed extract* administration.

In control group of rats, the creatinine level was 0.902 ± 0.198 mg% and after 16 weeks of arsenic treatment was 2.531 ± 1.001 mg%, while, it was 1.209 ± 0.233 mg% after 4 weeks of *Cumin seed extract* administration. In control group of rats, the lipid peroxidation level was 4.289 ± 0.523 nmol/ml and after 16 weeks of arsenic treatment was 88.23±3.445 nmol/ml, while, it was 11.27 ± 1.224 nmol/ml after 4 weeks of *Cumin seed extract* administration.

Table 2: Changes in the biochemical parameters of Charles Foster Rats exposed to Sodium arsenite at the dose of 8mg/Kg body weight for 16 weeks and its amelioration by *Cumin seed extract* at the dose of 250 mg/Kg body weight for 4 weeks.

Parameters	Control	Arsenic Treated	Cumin seed extract Treated
SGPT (U/mL)	29.56 ± 1.231	202.6 ± 27.23 ***	54.21 ± 23.56 ###
SGOT (U/mL)	33.51 ± 2.512	254.1± 14.03***	65.22 ± 1.31 ###
ALP (KA Unit)	7.45 ± 0.539	35.23 ± 3.421 ***	12.16 ± 1.09 ###
Total Bilirubin (mg/dL)	0.853 ± 0.234	2.45 ± 0.453*	1.217± 1.20 ##
Urea (mg/dL)	29.3 ± 1.323	86.20 ± 2.189**	32.18 ± 0.47 ###
Uric acid (mg/dL)	2.98 ± 0.332	13.90 ± 2.343**	3.89 ± 1.098 ##
Creatinine (mg/dL)	0.902 ± 0.198	2.531 ± 1.001 **	1.209 ± 0.233 ##
LPO (nmol/ml)	4.289 ± 0.523	88.23±3.445 *	11.27 ± 1.224 #

*# Effect of different treatments on liver and kidney biomarker parameters in the studied groups (n=4, Significant ***$P < 0.0001$, *$P < 0.05$ compared with control group, ### $P <$ 0.0001 compared with arsenic treated group, values are expressed as mean ± SEM).

4. DISCUSSION

In the present study there has been significant changes observed in the studied parameters. There was significant decrease in the RBC indices parameters such as haemoglobin percentage, hematocrit percentage and MCHC as well as in WBC counts and platelets counts. The present result speculates that there has been significant myelosuppression causing the serious failure of the defense mechanism. In the present study there has been significant increase in the lipid peroxidation (ROS) levels. This confirms the breach in the defense mechanism. Moreover, this causes the depletion of lipids from the membrane which is the onset of loss of cellular integrity (Dwivedi et al., 2015; Sankar et al., 2016; Sharma et al., 2018).

Arsenic usually enters the system through the gastrointestinal tract and interacts with the sulfhydryl moieties and causes disruption in the metabolic functions associated with it (Lu et al., 2007). However, the excess arsenic concentration is eliminated through the urine and bile (Helleday et. al, 2000). But the arsenic absorbed in the liver gets accumulated in the vital

organs of the body, interfering the signalling pathways (Soni, et al. 1993). It also interferes with the cellular apoptosis pathways causing abrupted function causing the onset of carcinogenesis of the vital organs such as kidney, bladder, liver etc. (Mallikarjuna et al., 2003; Yamanaka et al. 2004). The methylated DMA^{+5} acts as tumour promoter (Yamamoto et.al, 1995 & Wanibuchi, et. al, 1996; Cohen, et al. 2006; Miller et. al., 2002; Huang et al., 2004; Duker et al.,2005).

In the present study there has been remarkable changes observed as there was significant increase in the SGPT, SGOT, ALP, bilirubin, urea, uric acid and creatinine levels. Recent study speculates that the arsenic in the form of Dimethyl arsenic acid (DMA) is converted which is still a carcinogen category I, which disrupts the functions of the liver as well the kidney (Chen et al., 2011; Hsueh et al., 2009; Zheng et al., 2013 & 2014; Jha et al., 2013). The biochemical changes observed could be due to the deleterious effect caused by the DMA.

In the present study, there has been significant restoration due to cumin extraction administration especially the haematological parameters and the biochemical parameters. Cumin bears active ingredients like cuminaldehyde, cymene and terpenoids. The major compounds of terpenoids are monoterpenes beta-pinene, p-cymene and gamma-terpinene and the terpenoid aldehydes cuminic aldehyde and the isomeric menthadien carboxaldehydes. Apart from it also contains phenolic acids, flavonoids which possesses antioxidant activity play a vital role in inhibiting the lipid peroxidation as well as various types of oxidizing enzymes (Li and Jiang 2004; Gallo et al., 2010; Morshedi et al., 2015). However, the other functions of Cuminaldehyde has also been studied by various researchers such as antiplatelet (Sekin et al., 2007), antibacterial (Lee 2005), antifungal (Morshedi and Aliakbari), antidiabetic (Bennet et al., 1982), anti-Parkinson's (Johri 2011), while it also activates the salivary glands and facilitates the primary digestion of the food and produce carminative effects. The protective effect of cumin seed extract has been merely reported (Alizadeh et al., 2019; Wanner et al., 2010). Hence, the active ingredient Cuminaldehyde might have played the vital role to control the arsenic induced hepato-renal toxicity, normalizing the metabolic functions of the liver and kidney.

CONCLUSION

From the entire study it can be concluded that arsenic causes deleterious effect on the studied parameters especially at the haematological and biochemical levels in the rats disrupting the normal functioning of the body. But, after the administration of the Cumin seed in them.

Hence, it indicates that Cumin seed extract possesses hepato-renal protective effect against arsenic induced toxicity. Therefore, it can be targeted as novel and safe antitoxic drug against arsenic.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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