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Arsenite induced genotoxic effect and its phytoremediation by *Acacia catechu* leaf extract in freshwater fish, *Channa punctatus* (Bloch)

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Abstract
Present experiment design to investigate the As (III) induced genotoxic stress and its phytoremediation by ethanolic leaf extract of *Acacia catechu*, using micronucleus assay as genotoxic marker in freshwater teleostean fish, *Channa punctatus* (2n=32). After 10 days acclimatization, fish were divided into four groups. Group I, served as control. Fish of group II were exposed to 96 h-LC_{50}/10 of As (III). Fish of group III and IV were exposed to 96 h-LC_{50}/10 of As (III) along with different ethanolic leaf extract of *A. catechu* for dose standardization. A significant increase in frequency of micronuclei was observed in group II. This increased frequency of micronuclei was found to be reduced in group III and IV after simultaneous exposure of As (III) along with ethanolic leaf extract of *A. catechu* on comparing with group ll. Finding depict that As (III) is a potent genotoxic agent and *A. catechu* considerably reduces As (III) induced genotoxic stress in dose dependent manner. The outcome of present study is helpful in exploring the efficacy of *A. catechu* leaf extracts in combating the ill effect of As (III). Result is also significant for fish formers who can use *A. catechu* as a safeguard against As (III) contamination of water.

Keywords: *Acacia catechu*, Genotoxicity, Micronucleus assay, Antioxidant, Teleostean, Phytoremediation

1. Introduction
Arsenic a common environmental contaminant exists in 4 oxidative stages (+V, +III, 0, -III), out of which As (V) is most stable state and As (III) is predominantly present in reduced environment (Hasegawa et al., 2010) [8]. In an aquatic environment, biomethylation of inorganic As (III) to methyl arsenic, with an intermediate stage of As (III) is done by photosynthetic microorganism through biotransformation which also contributes significantly to the biogeochemical cycling of As species in aquatic systems (Navratilova et al., 2011; Price et al., 2012; Rahman and Hasegawa, 2011) [14, 15, 16]. Moreover, being higher at trophic level accumulation of arsenic more than the permissible limit in fishes cannot be ruled out as such arsenic ultimately reaches to human being through food chain.
There are several chemical solutions present against arsenic toxicity but with side effects and limitation. The ethanolic extract of leaves of *A. catechu* of family Febaceae is rich in bioflavonoid antioxidants, *viz.*, Rutin and Quercetin, (Lakshmi et al., 2012) [11]. It has been used separately in many traditional medicines and pharmaceutical products for a variety of uses including anti-inflammatory, antiviral, antibacterial, anticancer, and cardiovascular applications (Singh et al., 1976; Razina et al., 1989; Mahmood et al., 1993; Van Loon, 1992; Cowan, 1999; Lee et al., 2000; Shigeta, 2000; De Clercq, 2000; Huang et al., 2005) [21, 18, 13, 22, 3, 12, 19, 4, 9]. Keeping the abovementioned facts in view *A. catechu* is used in the present study as natural, mitigating agent against arsenic trioxide induced genotoxicity (Halliwell 2011) [6].

2. Material and Method
2.1 Test animal and test chemical
Healthy and live fresh water teleostian fish *Channa punctatus* (14.5 ± 1.0 cm and weight 30 ± 2.0 g) were obtained from local lentic habitats in the vicinity of Lucknow. They were given prophylactic dip in formalin (0.4%) for 15 min followed by 1 h $KMnO_4$ (1 mg l^{-1}) to keep away dermal infections. Then prior to experiment fish were acclimatized for 10 days in large glass aquaria (100x40x40 cm^3), during which they were fed minced goat liver and artificial

fish food Tokyo. During acclimatization fish were maintained by following standard fish maintenance procedure by APHA et al., (2012) [1] (Temperature of 14 to 22 °C, dissolved oxygen- 6.62 to 6.76 mg/ l, alkalinity- 62 to 68 mg/ l).

2.2 Collection and preparation of plants extract
Leaf of *A. catechu* was collected, washed and identified by experts. Leafs were washed, air dried and powdered with the help of grinder. Powdered plant material was pre-extracted in 50% ethanol by soxhlet apparatus for 72 h. Extract was stored in freeze. The liquid extract was filtered and then concentrated using rotary flash evaporator at a temperature less than 45°C to get semisolid residue which was dried under vaccum. The concentrated ethanolic leaves extract of *A. catechu* were subjected for further studies.

2.3 Determination of sub lethal concentration of CPF, (LC_{50})
The value of 96 h-LC_{50} of ATO was used as 69.36 mg/l previously determined by Yadav and Trivedi, (2009) [23] by using Trimmed Spearman–Karber Method (Hamilton et al., 1977) [7]. Based on 96 h- LC_{50} value, sub-lethal concentrations of 96 h-LC_{50}/10 was used for further studies.

2.4 Experimental design
After 10 days acclimatization fish were divided into 4 groups having 10 fish. Group 1 served as control. Fish of group 2 were exposed to 96 h-LC_{50}/10 of ATO only. In group 3 and group 4 fish were exposed in combination with both 96 h-LC_{50}/10 of As_2O_3 ATO along with 2 ppm concentration *A. catechu* and 4 ppm concentration of *A. catechu* respectively. At the end of stipulated periods (24, 48, 72, and 96 h) of exposure, fish were randomly selected and blood samples were collected from control as well as treated groups. Three replicate were used for each group.

2.5 Micronuclei test
Peripheral blood samples were smeared on pre cleaned microscopic slides and fixed with absolute methanol for 5 min. After fixation, slides were stained with May-Grunewald's solution 1 and 2 for 3 and 5 min, respectively followed by 5% Giemsa staining for 30 min. After overnight drying DPX mounted slides were observed under oil immersion microscope (Nikon Corporation K 12432) using 40/100X objective lenses. Micronuclei were scored by following the criteria of Fenech et al. (2011) [5]. A minimum 1000 erythrocytes for each specimen were examined.

3. Result and Discussion
Erythrocyte of healthy *C. punctatus* is elliptical with centrally located nucleus. While a small round structure with 1/3rd size of the main nucleus having similar in colour as that of main nucleus without any connection with the cytoplasm, is considered as micronuclei. The frequency of micronuclei in erythrocyte of *C. punctatus* exposed to different groups were recorded and summarised in Table.1. It is evident from our study that arsenic trioxide at tested concentration induces genotoxic effect in *C. punctatus* in exposure dependent manner. The micronuclei induction was recorded highest at 96 h exposure period. Thus, the present study revealed that arsenic trioxide is responsible for inducing micronuclei formations in erythrocytes of fish, *C. punctatus*. On the other hand in group 3 and 4 the induction of micronuclei was comparatively less than that of group 2. As it is evident that fish in group 3 were exposed to arsenic trioxide along with *A. catechu* leaves extract thus the recorded reduction in micronuclei frequency is somehow related with the presence of *A. catechu* leaves extract. Since extract of *A. catechu* is known for its antioxidative properties from years back. Ray et al., 2006 [17] used *A. catechu* as antipyretic antidiarrhoeal, hypoglycemic and hepatoprotective agent in albino rat. The presence of verity of phytochemicals in leaves extract of *A. catechu* is related with its ethnomedicinal properties of this plant (Singh et al., 1976; Lakshmi et al., 2012; Kilani−Jaziri et al., 2011; Burnett et al., 2007) [21, 11, 10 2]. Keeping all the above mentioned facts in view *A. catechu* leaves extracts can be used as an excellent mitigating agent against arsenic trioxide. As arsenic trioxide induces genotoxicity by disturbing antioxidant homeostasis in biological system while antioxidant rich leaves extract of *A. catechu* might restore this disturbed antioxidant homeostasis (Singh et al., 1976; Lakshmi et al., 2012; Shila et al., 2005) [21, 11, 20].

Table 1 Micronuclei frequency in erythrocyte of *C. punctatus* in different experimental groups.

Conc. (mg/l)	Exposure period(h)	Total cells scored	Total no. of MN	Frequency of MN mean (%)±S.D
Control	24	6123	9	1.46±0.12
	48	6050	8	1.32±0.14
	72	6070	8	1.31±0.09
	96	6133	7	1.14±0.13
96 h-LC_{50}/10 of ATO	24	6100	29	4.75±0.20
	48	6120	31	5.06±0.45
	72	6130	39	6.36±0.68
	96	6109	44	7.20±0.12
96 h-LC_{50}/10 of ATO+2ppm *A. catechu*	24	6106	25	4.09±0.24
	48	6103	19	3.11±0.75
	72	6101	15	2.45±0.78
	96	6130	10	1.63±0.32
96 h-LC50/10 of ATO+4 ppm *A. catechu*	24	6206	21	3.38±0.49
	48	6207	17	2.73±0.85
	72	6102	13	2.13±0.73
	96	6101	9	1.47±0.24

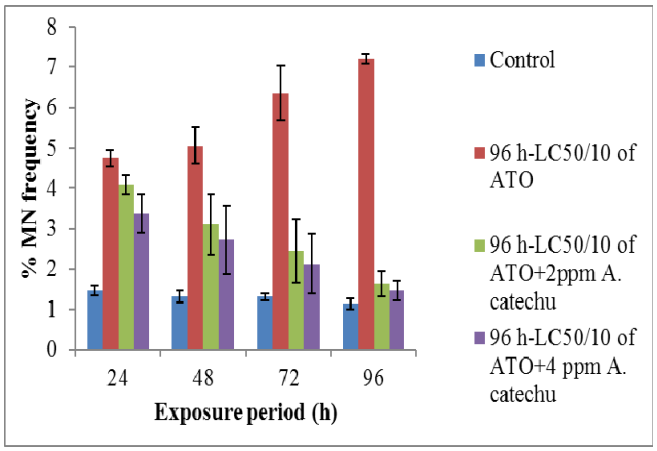

Fig. 1 Change in frequency of micronuclei in different groups at 96 h exposure period.

4. Conclusion
Plant and their products are known for their antioxidative properties and used from ancient time in the ethnomedicines. Findings from the present study shows the antioxidative potential of *A. catechu* leaf extract against arsenic trioxide

induced toxicity in terms of reduction in frequency of micronuclei in fish, *C. punctatus*. As fishes are excellent source of protein and omega-3 fatty acids thus they are very important food source for population. Thus the antioxidant property associated with A. *catechu* can be used for good health of fish even in the toxic medium.

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