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RESEARCH ARTICLE

GENOTOXIC EFFECT OF UREA ON FRESH WATER FISH, *Channapuntata*

Sabista Anjum[1] and Dharmshila kumari[2]

[1]Research scholar, University Department of Zoology, TMBU, Bhagalpure-812007, Bihar, India
[2]Professor, University Department of Zoology, TMBU, Bhagalpure-812007, Bihar, India

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***Corresponding author:**
Sabista Anjum

ABSTRACT

Urea fertilizer are wide use fertilizer in worldwide due to highly presence of Nitrogen and reduce plant parts damageExtensive uses of Urea fertilizer by farmer in paddy cum fish also exposed to breathing fish, *Channapuntata.* Present work was designed to evaluate genotoxicity of Urea fertilizer on *Channapuntata* with two different concentrations of 1.585g/l and 2g/l for duration of 96 hrs. One group takes as control group, which were untreated group. For evaluation two parameter were used that is Chromosomal abnormality (CA) and Micronucleus (MN)Here we conclude higher significant in both parameter, In CA (1.585g/l) 25.3% and (2g/l) 33% respectively as compare to control(20.6%) .In MN (1.585g/l) 0.33% and (2g/l) 0.37% respectively as compare to control 0.12%. There are cytological abnormality were absorbed in these evaluation. Various abnormalities evaluated were – hypoploidy, hyperploidy, clumpiness, stickiness, and chromatic break, chromatic gap, and ring.

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INTRODUCTION

India now has the most people, yet its farmland is tiny compared to other countries. Farmers have worked hard to meet the rising demand for grain to feed the world.Fertilizer use was ramped up to nourish crops and boost output. Natural and synthetic compounds used to increase crop yields are together known as fertilizer. While both organic and inorganic fertilizers have their uses, in India most farmers rely on inorganic NPK fertilizers because of the precision and economy of their manufacture. Urea is the preferred fertilizer among farmers since nitrogen fertilizers are the most effective. India has overtaken China as Urea's second largest consumer. Widely use fertilizer in agriculture land worldwide are Urea ($(NH_2)_2CO$) (http://aostat.fao.org) because it has nitrogen, highly soluble in water and reduce plant damage but excess use of inorganic nitrogen become explosive (Glibert, 2006) and increase pollution (Palanichamy, 1985; Rani, 1997). Extensive practice of paddy cum fish culture to rear breathing fish (Channa, Claris, Anabas species etc.) where urea are found in greater amount. Urea gets decomposed into cyanate and ammonium ion, $(NH_2)_2CO \rightarrow CNo^- + NH_4^+$ and ionized ammonia toxoid aquatic environment (https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1095-8649.2005.00930.x.), concentration of unionized ammonia also show toxicity (5), accumulation of ammonia in fresh water and agriculture land can damage water body by increasing acidification, eutrophication and toxicities in aquatic organisms (Bonciu, 2018; Baker, 1991) and can disturb the food web of aquatic ecosystem by forming more algae bloom ,which cause production of hydrosulphate (Kremser, 2002). As fishes are good source of protein for human diet and this may cause hypoxia, anoxia (Chislock, 2013). These compound may damage spleen , kidney , liver , gill , other tissue of fish and may also cause breathing problem (Benli, 2008; Dolomatov, 2011). NH_4^+ which can be used as NH_3, No_2/ No_3^- or N_2o and N_2 by volatilization, nitrification and denitrification respectively (Swensen, 1997). Highest amount of nitrate present in urea (Anjana, 2007) that accumulation of No_3 in soil which form nitrosamines which are carcinogenic substance (15). 40 – 45mg nitrate (N/1 concentration) has been reported near paddy fields (http;/www.fao.org/docrep/W2598E/w2598e 0.4htm,6/22/2005). NEERI give a report after survey of 17 state that level of No_3^- increase by 27% , which has crossed WHO drinking water standard, WHO standard 50mg/l (WHO). Solapure and Bareilly areaes reported with mean value in range of 7 and 47mg/ and concentration of nitrate is more than 50mg/l (Hossain, 2020; Mazhar, 2020; Mukanga, 2016) regular intake can cause methemoglobinaemia, thyroid cancer and also effect aquatic bodied can damage fishes (WHO, 2023), (Tokazhanov, 2020) also cause disruption of endocrine system (Khalil, 2016). As chromosome formation have nitrogen as a major component which increasing possibility to cause impairment in DNA (Khalil, 2016) and cause DNA damage (Helen *et al*., 2020) by chromosomal abnormalities binuclear, multinuclear (Bonciu, 2018) polyploidy, aneuploidy, acentric fragment , minute fragments, chromatic break (Kumari, 2019).

ANIMAL STUDY

Heads of adult snakes (Channapuntata) were used as a. Bloch were measured at a length of 23.1 3.0 cm and weighed up at 100 5.0 g at a Bhagalpur market. C. puntata is an easily accessible, nutritious, and air-breathing fish. Rearing fishes in an aerated aquarium for 10–15 days to acclimatize to room temperature and provide a nutritious diet and water, water of aquarium would be change every day (Channapuntata have chromosome number 2n= 32) (Dhar and Chatterjee,1984). These adaptation procedures were performed so that

FERTILIZER

Urea (($(NH_2)_2CO$),synthesize fertilizer having in crystal with white color ,It is combination of ammonia and carbonate group .For plant growth and reduce plants part damages N- nitrogen are used by further broken down of ammonia

EXPOSURE

Two batches of fertilizer urea, each of a different weight 2g/l and 1.585g/l , were diluted in 20 liters of water. Eight fish were exposed in these several concentrations of urea for 96 hours to determine the genotoxicity of C. punctatus. Three groups of fish were tested for 96 hours each. Group one served as the "control" in this experiment, which were untreated. The second group received a 2g/l reduction in fertilizer dose. The third group received a lower concentration of fertilizer (1.585g/l).table -1

Treatment

Table 1 missing

SN.NO.	TREATMENT	
1	CONTROL	No treatment ,normal diet
2	UREA	i)2g/l ii)1.585 g/l

Slide preparation: After the procedure was finished, the animal was unconscious, and a slide of a kidney cell's mitosis was prepared .Chromosome arrest at the metaphase stage was achieved by injecting 0.04% colchicine intramuscularly at 1ml/100g body weight in fish for 2 hours. A hypotonic solution of 0.56 percent KCl has been prepared. Apical part of kidney was removed and subsequently smashed in hypotonic solution. After being incubated at room temperature for 20 minutes, the cell suspension was transferred into a 15 ml centrifuge tube.After adding freshly prepared fixative (methanol:acetic,3:1 v/v) to a cell suspension and gently mixing the mixture, centrifuging the cells at 1000 rpm for 10 minutes, and discarding the resulting cell suspension, the process was repeated twice more using fixative (methanol:acetic,2:1 v/v).

Using a flame drying process, the slides were then stained with 6% Geimsa. About 300 cells were chosen and scored for aberration from each group, and aberrations were counted for statistical analysis after being inspected at a magnification of 100x under immersion oil. Blood was taken from the fish's heart chamber for the micronucleus test by making an unconscious incision at the anterior dorsal region and puncturing the heart .The micronucleated erythrocytes method (Al-Sabti, 1990) was used to directly generate a smear on clean, dry slides from a small sample of blood.After the slides were dried, they were stained for 30 minutes with 6% Geimsa. A 100x microscope was used to examine each slide .A frequency was determined by assigning scores to cells the frequency was calculated (Raisuddin, 2004). Micronuclei were defined as discrete nuclei of the same color as the surrounding cytoplasm but with a diameter of 1/20-1/50 of a normal nucleus.

STATISTICAL ANALYSIS: The data were expressed as (mean±S.E) and t- test ($p<0.05$) was used for evaluation of data

RESULT AND DISCUSSION

In this study we use two test of genotoxic effect of urea .Urea show significant increase in frequencies of MN and CA Here, we classify chromosomal abnormalities into two broad categories: structural and mitotic.There has been a dramatic rise in the incidence of all kinds of abnormalities, including but not limited to hypoploidy, hyperploidy, clumpiness, stickiness, and chromatic break, chromatic gap ring, and so on. Possible chromosomal loss or breakage resulted in the absorption of an acentric fragment. Statistical tests show both doses, higher doses (2g/l)and lower doses(1.585g/l) result in a greater occurrence of abnormalitiesshows,significant 33% and 25.3% which are higher than control group (20.6%).

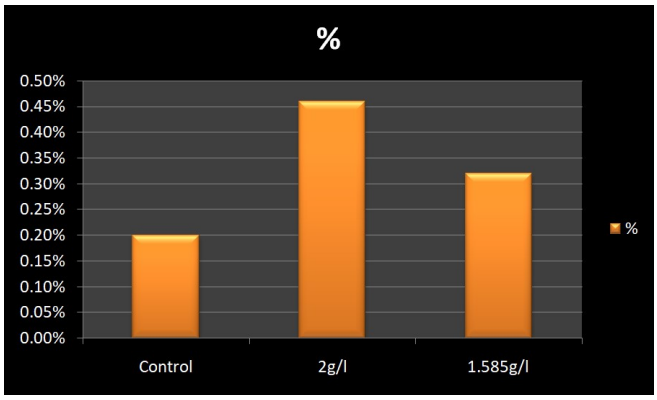
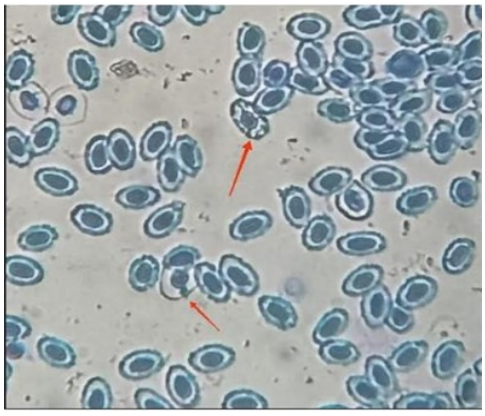
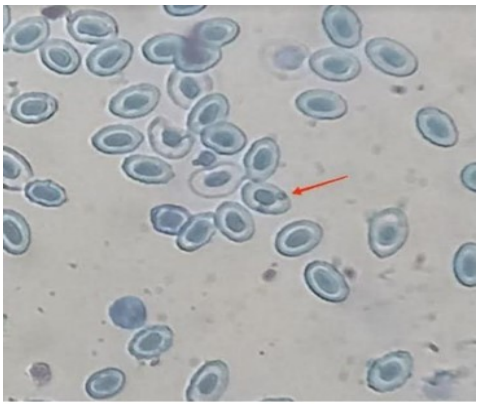


Figure 1. Graph showing the percwntage of three experimental varient

In MN test we got 0.33% in 2g/l and 1.585g/l respectively as compare to control (0.12%).



(a)



(b)

Fig. 2. Micronucleus in treated group (a) higher doses -2g/l (b) lower doses – 1.585g/l

Table 2.

Exp. variation	Total no. nucleus	Total no. of micronucleus	% ± S.E
Control	24728	30	0.12%±0.0004
2g/l	21801	81	0.37%±0.0005
1.585 g/l	14290	48	0.33%±0.004

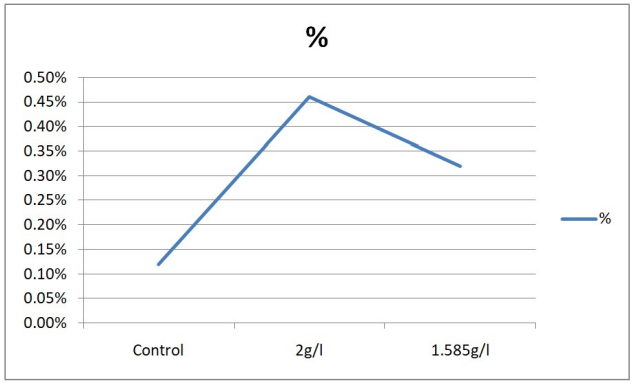


Fig. 3. Line graph shows Micronucleus % of three variant group

When comparing the treated group to the control group statistically, there is a clear and substantial increase in both groups. Both parameter shows increase in frequency, which indicate chromosomal damage, inchromosal number and structure.

CONCLUSION

As can be seen from the foregoing, the synthesis of urea has a genotoxic and mutagenic effect, altering the number and structure of chromosomes and causing nuclear disruption. My hope is that by publishing this, I will help raise awareness about the dangers of urea and encourage governments to adopt policies that encourage the use of organic fertilizers as an alternative.

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