Genotoxic Effects of Synthetic Fertilizer Urea in *Anabas testudineus*

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***Abstract*: Urea, a common fertilizer used to supplement soil with nitrogen, induced 1.33%, 1.60%, and 1.83% of micronuclei and 21.6%, 24.0% and 26.0% of chromosomal abnormalities upon three different doses.The frequency of abnormalities increased with increase of doses. Polyploidy and aneuploidy were common among gross,while acentric fragments, minute fragments, chromatid breaks were more common among individual type of abnormalities.The individual type of damages were more prominent than gross type. This might be due to the formation of electrophilic radicals / ions during the metabolization of mutagens that attack the nucleophlic site of DNA leading to structural changes in chromosomes.**

***Keywords*: Urea, Genotoxicity, Micronuclei, Chromosomal abnormalities, Anabas testudineus.**

1. Introduction

The vast majority of teleost fishes are ammoniotelic excreting ammonia as the major nitrogenous end product in response to their aquatic habitat (Wood 1993; Saha and Ratha 1998).The ammonia stress on fishes includes accumulation of amino acid in different body tissues, (Levi et.al.1974; Das 1981; Dabrowska and Wlasow 1986 ;Iwata 1998;Saha 1992).Several organs such as gills,skin,liver,kidney, intestine,and gonads are extensively studied in fishes (Chatterjee and Bhattacharya 1983;Thurston et.al.1984; Ram and Sathyanesan 1986;1987a; Bhattacharya et.al 1989;Wright et.al. 1989; Banerjee and Paul 1993). Contamination of water bodies with ammonia takes place during application of the inorganic fertilizer, urea, ammonium sulphate(used for agriculture,aquaculture,)(Jhingran 1983;Ram and Sathyanesan 1987b;Sarkar 1991;Varadachari 1992) as well as ammoniotelic properties of fishes that aid also some amount of the nitrogen content in the aquatic medium.

Urea is a major nitrogen fertilizer to enrich the soil with nitrogen.Ammonia that emanates from the urea applied to agricultural fields, contribute to acid rain,while nitrates produced in soil contribute to contamination of ground water due to leaching of nitrates (http: / www.fao.org/docrep/W2598E/w2598e 0.4 htm 6/22/2005).This fertilizer has been found to be present either in residual or some metabolised /derived form among the plants grown over them, and thus get accessed to the body of the animal that feed upon these plants (Baker and Chesnin 1975;Chaurasia and Sinha 1989;Current Science,Nov 2000).As agricultural run-off they pose a serious effect that induce various histopathological (Srivastava and Sravastava 1979;Nanda et.al,2004;Ravindar kumar,2000) and cytogenetical changes (Jha,1998;Kohlpoth,1999;Dashwood, 1998;Baksi,1990)in the plants,aquatic animals,cattles and humans(Dravyam and Rajamanickam,2003,Neff,1985;Bhaskaran,1988;Singh et.al, 1998; Gupta, 2000, http :/www 3. interscience wiley,.com/cgi-bin/abstract/ABSTRACT? CRETRY=1 &SRETRY=6/15/06).

Paddy fields are inhabited by some of the air-breathing fishes where pesticides and fertilizers are used regularly in greater amount. 40-45mg N/l nitrate concentration has been reported in irrigated wells around the paddy fields.(http:/www.fao.org/docrep/W2598E/w2598e 0.4 htm,6/22/2005).As nitrogen is a major component of chromosomes (in protein and DNA) it is quite possible that residues or metabolites of urea may cause some damages in fishes.The genotoxic effects of agrochemicals has been reported in various test system (Chaurasia and Sinha,1987,1990;Chaurasia 1991),but very few reports are available in the air-breathing fishes.So, the present investigation was therefore taken up to study the hitherto almost unknown genotoxic effect of urea on chromosomal abnormalities and incidence of micronuclei in Anabas testudineus.

2. Materials and Method

Two test system viz; micronucleus test (from peripheral blood cells) and mitotic chromosomes from head kidney) were used.10-15 days acclimatized fishes were treated with freshly prepared doses of urea with three different concentration i.e Sub –lethal (SL-2.0%),half of the sub-lethal(HSL-1.0%) and quarter of the Sub-lethal (QSL-0.5%)for 7consecutive days.The animals were sacrificed after seven days of the termination of treatment.

The micronucleus test was conducted in peripheral blood cells.A film of blood smear was prepared after mixing with few drop of anticoagulant (0.1% trisodium citrate solution)on a greese free clean slide.Preserved in methanol for 10 minutes ,stained with 0.15% Leishman's stain for 20-25 minutes and cleared in xylene for 5 minutes .3000 RBC cells were screend.A concurrent control were carried out were animals were kept in fresh water.

For studying the chromosomal abnormalities,tissue from head kidney were taken and the slides were made by the conventional Colchicine –hypotonic –acetoalcohol-flame drying –giemsa staining techinuqe.300 well spread and randomly selected metaphase plates were screened and data were analyzed by statistical procedure.A seperate common control was also carried out.

3. Results and discussion

Amidst 3000 RBCs,only 0.43% micronuclei were found in the control group while 1.33%, 1.60%and 1.83% micronuclei upon three doses of urea (SL,HSL& QSL)were observed (Table -1).A close observation of data revealed that the effect was dose –dependent (Graph -1).Most of the cells were found to have only one micronucleus of very small size (due to acentric fragment)or bigger size (due to lagging of whole chromosome)but very few cells were found to have more than one micronucleus.

Amidst 300 metaphase plates,21.6%,24.0%and 26.0% chromosomal abnormalities were found upon treatment with three doses of urea in contrast to 5.33% in the control (Table -2).The abnormalities that were found can be put in two categories – gross and individual ones.The insignificant gross changes were the stickiness, polyploidy , hypoploidy etc..The significant individual changes were mostly breaks in the chromosomes (Chromatid break, chromatid gap).Acentric fragment and minute fragment were also observed that might be due to breaks and deletion of certain part of chromosomes (telomeric or interstitial part).A quantitative estimation revealed that the abnormalities increased with the increase of the doses. Thus the effect was dose dependent (Graph-1).The individual type of damages were more prominent than the gross type because urea is synthetic in nature. While Chaurasia and Sinha 1987,1988, 1990),Chaurasia et.al. (2005)were studying on genotoxicity induced by fertilizer and silk dyeing wastes; Kumar and Sinha(1989)on doses –dependent genotoxic effects of synthetic pesticides,they observed that the individual type of damages were more frequent than the gross type.Bose and Sinha(1994),Dharmashila and Sinha (1994) and Awasthy et.al.(2000)could find that the biomutagens induced more gross type of abnormalities than individual types.This differential sensitivity might be occurred at two different levels. First ,the damages at protein level either on spindle protein or on protein packing. Second, by the production of electrophilic ions and reactive radical during the metabolization of mutagens (Klopman et.al..1985).Such electrophlic reactive radicals /ions might attack to nucleophilic site of DNA leading to structural changes in chromosomes(Awasthy et.al.1999).

The result thus shows that the synthetic fertilizer urea was mutagenic and harmful to the fishes with a regular deterioration of their population and thus affecting the economy of our country.

Incidence of micronuclei(N=3000) after urea treatment in *Anabas testudineus.*

EXPERIMENT TREATMENT (in %)	Abnormal cells NO.	%	± S.E	Micronuclei NO.	%	± S.E
Control	15	0.50	± 0.12	15	0.50	± 0.12
0.5	44	1.46	± 0.21*	44	1.46	± 0.21*
1.0	51	1.70	± 0.23*	51	1.70	± 0.23*
2.0	59	1.96	± 0 .25*	59	1.96	± 0.25*

Table-1

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Chromosomal abnormalities(N=300) after urea treatment in *Anabas testudineus*

Treatment	No. of metaphases analyzed	Abnormal metaphase				Gross Abnormality				Individual Abnormality				Total Abnormality			
		No.	%	±	S.E	No.	%	±	S.E	No.	%	±	S.E	No.	%	±	S.E
Control	300	20	6.60	±	1. 43	3	1.00	±	0.57	17	5.60	±	1.32	20	6.60	±	1.43
0.5%	300	67	22.3	±	2.40*	-				67	22.3	±	2.40*	67	22.3	±	2.40*
1.0%	300	79	26.3	±	2.54*	-				79	26.3	±	2.50*	79	26.3	±	2.54*
2.0%	300	90	30.0	±	2.64*	-				90	30.0	±	2.64*	90	30.0	±	2.64*

Table-2

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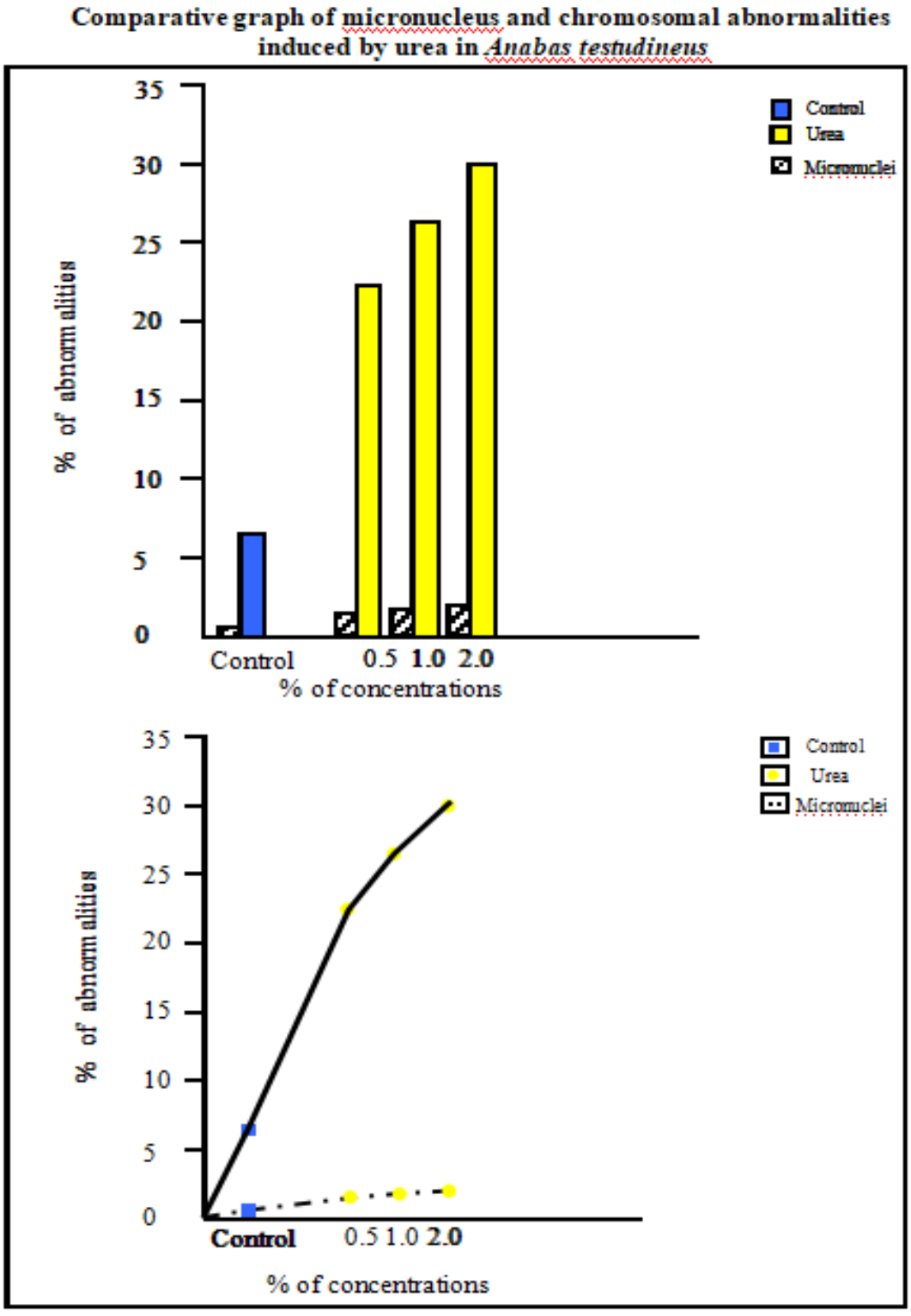
Comparative graph of micronucleus and chromosomal abnormalities induced by urea in Anabas testudineus
35
30
25
20
15
10
5
0
% of abnormalities
Control
Urea
Micronuclei
Control
0.5
1.0
2.0
% of concentrations
35
30
25
20
15
10
5
0
% of abnormalities
Control
Urea
Micronuclei
Control
0.5 1.0 2.0
% of concentrations

GRAPH-1

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