www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 14: 421-428 (2014) DOI: 10.4194/1303-2712-v14_2_12

Evaluation of Genotoxicity Induced by Medicinal *Plant Jatropha gossypifolia* in Freshwater Fish *Channa punctatus* (Bloch)

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Received 20 September 2013
Accepted 18 April 2014

Abstract

The genotoxic and mutagenic potential of medicinal plant *Jatropha gossypifolia*, was investigated in fresh water fish *Channa punctatus* in the present study using micronucleus assay and comet assay. Fishes were exposed at 20% and 40% of the 24 h-LC_{50} value of plant extract containing Apigenin (76.48 mg L^{-1}) and crude latex (22.33 mg L^{-1}). Both the test chemicals induced significant ($P<0.05$) genotoxic effects in exposed fishes at various exposure durations. The micronucleus induction was maximum at the highest concentration (40%) 96 h post exposure of Apigenin containing extract (0.107%) and Jatropha crude latex (0.302%) in the peripheral blood of *C. punctatus*. A similar trend was observed for the DNA damage measured in terms of % tail DNA in the erythrocytes of *C. punctatus* in Apigenin containing extract (15.20%) and Jatropha crude latex (21.17%). This study also explored the combined use of micronucleus assay and comet assay for i*n vivo* laboratory studies using fresh water fishes for screening the genotoxic potential of medicinal plants.

Keywords: Genotoxicity, *Channa punctatus*, medicinal plant, *Jatropha gossypifolia*.

Introduction

Medicinal plant species which are being used, on large scale, for treatment of particular diseases are reported to be having serious side effects. Many drugs have originated from biologically active plant chemicals, and their medicinal uses are attributed to various active chemicals found in them. Many people mistakenly assume that all medicinal herbs, being natural, are generally safe and free from undesirable side effects. However, very often, herbs may interact with medications that result in adverse effects. The use of herbal medicine is still poorly understood. Nowadays, toxicity and safety of medicinal herbs is one of the most discussed topics as herbal products have become popular worldwide. Approximately, 60-80% of the world population still relies on traditional medicines for the treatment of common illnesses (WHO, 2002; Zhang, 2004).

One such herb is *Jatropha gossypifolia*, (Euphorbiaceae) commonly known as Ratanjot, different parts of which are used worldwide in different ways. It possesses significant anticancer, hepatoprotective, pesticidal and piscicidal activity (Hartwell, 1969; Chatterjee *et al*., 1980; Singh and Singh, 2002; Panda *et al*., 2009). The stem sap prevents bleeding and itching of cuts and scratches (Labadie *et al*., 1989; Morton, 1980). The stem latex has been shown to possess coagulant activity and its mechanism of action as haemostatic agent is through precipitation of coagulant factors (Oduola*et al*., 2005). The leaves of *J. gossypifolia* are used for intermittent fevers, carbuncles, eczema, itches and sores on the tongues of babies, swollen mammae, stomach-ache, toothache, venereal disease and as blood purifier (Morton, 1981; Balee, 1994). In Southern Nigeria, the fresh leaf extract applied with crushed leaf is used by herbalists and local people to stop bleeding from skin and nose (Morton, 1981; Omoregbe *et al*., 1996). The leaf extract has been used as an anticoagulant for biochemical and haematological analyses (Oduola *et al*., 2005). Many phytoactive chemicals, triterpenoids and flavonoids (apigenin, vitexin and isovitexin) are isolated from leaves of *J. gossypifolia* (Tinto and John, 1992, Subramanian *et al*., 1971). Flavonoids are a large group of polyphenolic compounds that comprise an important class of secondary metabolites in plants.

Apigenin belongs to the flavone group of flavonoids (Hertog *et al*., 1993). It is abundantly present in common fruits and vegetables including parsley, onions, oranges, tea, chamomile, wheat sprouts and some seasonings. It possesses remarkable anti-inflammatory, antioxidant and anti-carcinogenic

properties, but at higher doses shows molluscicidal, insecticidal and piscicidal properties (Shahi and Singh, 2010). In the last few years, significant progress has been made in studying the biological effects of apigenin at cellular and molecular levels. Although, these medicinal plants are of multi-purpose use in different countries, either in isolated crude form or as active compounds, yet without established efficiency, it is unsafe to use in human health remedies (Oduola *et al*., 2005).

Several ecotoxicological characteristics of *Channa punctatus*, such as wide distribution in the freshwater environment, noninvasive, availability throughout the seasons, presence of 32 well-differentiated diploid chromosome numbers, commercial importance, ease of blood collection and acclimatization to laboratory conditions, make this species an excellent test specimen for toxicity studies (Kumar *et al*., 2010).

The micronucleus (MN) test and Comet assay used in the present study for evaluating genotoxic and mutagenic potentials of medicinal plant, *J. gossypifolia* are sensitive, rapid and widely used methods in the detection of mutagenicity and genotoxicity of chemicals and xenobiotics under field and laboratory conditions (Cavas and Ergene-Gozukara, 2005; Pandey *et al*., 2006; Talapatra *et al*., 2006; Sharma *et al*., 2007; Nagpure *et al*., 2007; Ali *et al*., 2008, 2009; Kumar *et al*., 2010; Nwani *et al*., 2011). One of the advantages of MN and Comet assays are that both can be used for the simultaneous assessment of DNA damage in many tissues from the same animal and for the comparison of their responses under identical treatment conditions. It is increasingly being used in testing of substances such as industrial chemicals, biocides, agrochemicals, food additives and pharmaceuticals for genotoxicity testing (Brendler-Schwaab *et al*., 2005).

The mutagenic and genotoxic properties of crude latex and apigenin containing extract on fishes have been analyzed in fewer studies using a variety of assays in the past. Hence, hazardous effects of this plant product are a matter of great concern due its large scale exposure to human beings worldwide. In the present study, attempts have been made to investigate the mutagenic and genotoxic effects of crude latex and apigenin containing extract from *J. gossypifolia* using MN and Comet assays in erythrocytes of *C. punctatus* exposed *in vivo*.

Materials and Methods

Test Animal

Freshwater air-breathing fish *C. punctatus* (Bloch; Family: Channidae, order: Perciformes) were caught from nearby ponds and lakes with the help of local fishermen. The specimens had an average (±SD) weight and length of 14.50±1.25g and 12.08±2.07cm, respectively. Fishes were subjected to a prophylactic treatment by bathing twice in 0.05% potassium permanganate ($KMnO_4$) for 2 min to avoid any dermal infections. The fishes were then acclimatized for two weeks under laboratory conditions in static systems. They were fed boiled eggs, minced goat liver and poultry waste materials during acclimatization. The fecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. Temperature, pH, conductivity, dissolved oxygen, chloride, total hardness, and total alkalinity were determined for water on each sampling day by standard methods (APHA, AWWA, WPCF, 2005). The measured values of pH, temperature, dissolved oxygen (DO), and hardness of water were 6.8-7.0, 26°C, 7.2 mg L^{-1} and 41 mg/$CaCO_3$, respectively.

Test Chemical

Extraction of Latex from *J. gossypifolia*

J. gossypifolia (Family Euphorbiaceae) were collected from the Botanical garden of DeenDayalUpadhyay Gorakhpur University, Gorakhpur, India and identified by Plant Taxonomist of the same University. The yellowish milky latex of *J. gossypifolia* was drained into glass tubes by cutting the stem apices. The latex was centrifuged at 1000 rpm for 20 minutes to remove the resin, this resin free latex lyophilized at –40°C and the lyophilized powder was stored for further use. The wet weight of 1 ml latex of *J. gossypifolia* was 1.04 gm and dry weight (lyophilized at –40°C) was 0.140 gm.

Preparation of Ethanolic Extraction Containing Apigenin

The Apigenin was isolated from the leaf of *Jatropha gossypifolia* by the method of Subramanian *et al*. (1971). Extraction of Apigenin was completed by washing the leaves of *J. gossypifolia* properly with water and dried in incubator at 37°C. The dried leaves were then powdered. About 50 gm powdered leaf was subjected to extraction through Soxhlet apparatus with about 250-300 ml ethyl alcohol for 72 hours at 20-40°C. After extraction, a small amount of crude yellow powder was obtained. Dilute NaOH solution was added to obtaincrude powder, then filtered, added dilute HCl solution to the filtrate and again filtered. Obtained precipitate was crystallized with methanol. Apigenin extracted from leaves of *J. gossypifolia* were confirmed by UV spectra data of Dordevice and Mcakic (2000).

Determination of Sub-lethal Concentrations

Acute toxicity was conducted to determine the 24h-LC_{50} value of crude latex and Apigenin containing extract with definitive test in static system in laboratory as per standard methods of OECD guideline No 203 (OECD, 1992). The range finding

test was carried out prior to the definitive test to determine the concentrations of the crude latex and Apigenin containing extract. Toxicity experiments were performed by the method of Singh and Agarwal (1988). For the test, crude latex and Apigenin containing extract was dissolved in 10 ml of 1% gumacacia solution, filtered and added to the aquarium. A set of 10 specimens were randomly exposed to each of the four concentrations of crude latex and Apigenin containing extract viz., 10, 13, 16, 19 mg L^{-1} and 50, 55, 65, 80 mg L^{-1} respectively, obtained after range finding test. The experiment was set in triplicate to obtain the 24 h-LC_{50} value of the test chemicals for the species. The 24 h-LC_{50} value of both crudeo latex and Apigenin containing extract was determined as 22.33 mg L^{-1} and 76.48 mg L^{-1} respectively for *C. punctatus*. Toxicity data obtained from this study was estimated through POLO plus computer program version 2.0 of Robertson *et al*. (2007). The lethal concentration (LC values), upper and lower confidence limits and heterogeneity were calculated using of SPSS 10.1 computer program (SPPS Inc, Chicago, IL, USA). On the basis of LC_{50} value, 20% and 40% concentrations viz. 4.47 mg L^{-1} and 8.93 mg L^{-1} for crude latex and 15.30 mg$L^{-1}$and 30.60 mg L^{-1} for Apigenin containing extract was estimated for use in the *in vivo* experiments.

In vivo Exposure Experiment

All the fish, about age of four months were procured from same suppliers with similar relatedness and they were distributed randomly among dose/exposure duration groups. Six aquariums were used for the exposure of various concentrations of test chemicals; like first aquarium for negative control or control, second for solvent control, third and fourth aquarium for 20% and 40% of Apigenin containing extract, fifth and sixth aquarium for 20% and 40% of Jatropha crude latex in a static system. This study was conducted following the OECD guideline No 203 in the static test conditions (OECD 1992).The exposure was continued up to 96h (4 days) and blood sampling was done at the intervals of 24, 48, 72 and 96 h at the rate of five fishes (n=5) per interval from each test concentrations. The specimens maintained in tap water and those exposed to 10 ml of 1% gumacacia were considered as the negative and solvent controls, respectively. On each sampling day, the whole blood was collected and immediately processed for MN and CA. Blood was collected by incising the lateral vein using heparinized syringe and was diluted 20-fold. About 0.5 ml of diluted blood was added to an isotonic solution (10 ml) for further dilution (Tiano *et al*., 2000) in dark and dim light to prevent the occurrence of additional DNA damage. The physico-chemical properties of test water, namely temperature, pH, dissolved oxygen, conductivity and total hardness were analyzed by standard methods (APHA, AWWA and WPCF, 2005).

Micronucleus (MN) Assay

Peripheral blood samples obtained from the caudal vein were smeared on clean, grease free frosted glass slides. Slides were fixed in methanol for 10 min and left to air-dry at room temperature and finally stained with 6% Giemsa in Sorenson's buffer (pH 6.9) for 20 min. After dehydration through graded alcohol and clearing in xylene, slides were mounted in DPX (distyrene, plasticizer and xylene). From each slide, 2000 erythrocyte cells were scored under light microscope (LeitzWetzlar Germany, Type 307 – 083.103, oil immersion lens, 100/1.25). The criteria used for the identification of MN were; their smaller size, one-third of the main nucleus, same color, intensity and no attachment with the main nucleus.

Alkaline Single Cell Gel Electrophoresis (SCGE)

The alkaline single cell gel electrophoresis or comet assay (CA) was performed as a three-layer procedure (Singh *et al*., 1988) with slight modifications (Klaude *et al*., 1996). The blood was suspended in chilled phosphate buffered saline (PBS). Viability of the erythrocytes was evaluated by the trypan blue exclusion test method (Anderson *et al*., 1994) and tissue samples showing cell viability exceeding 84% were further processed for comet assay. In brief, about 15µl of cell suspension (approx 20,000 cells) was mixed with 85 µl of 0.5% low melting point agarose (LMPA) and layered on one end of a frosted glass slide, coated with a layer of 200 µl of 1% normal agarose. It was covered with a third layer of 100 µl LMPA. After solidification of the gel, the slides were immersed in lysing solution (2.5 MNaCl, 100 mM Na_2-EDTA, 10 MmTris, pH 10, with 10% DMSO and 1% triton X- 100, added fresh) overnight at 4ºC. After lysis, the slides were placed side by side in a horizontal electrophoresis unit containing fresh cold alkaline electrophoresis buffer (300 mM NaOH, 1 mM Na_2-EDTA and 0.2% DMSO, pH 13.5) and left in the solution for 20 min at 4ºC for DNA unwinding and conversion of alkali-labile sites to single-strand breaks. Electrophoresis was performed using the same alkaline electrophoresis buffer for 20 min at 15 V (0.8 V/cm) and 300 mA at 4ºC. The slides were then washed three times for 5 min with neutralization buffer (0.4 MTris, pH 7.5) to remove the excess alkali. Theprocessed slides were further stained with 75 µl ethidium bromide (20 µg ml^{-1}). Two slides per specimen were prepared and 25 cells per slide (250 cells per concentration) were scored randomly and analyzed using an image analysis system (Komet – 5.5 Kinetic Imaging, UK) attached to florescent microscope (Leica) equipped with appropriate filters. The parameter selected for quantification of DNA damage was percent tail DNA (%tail DNA = 100-%head DNA) as determined by the software.

Statistical Analysis

One-way analysis of variance was employed to compare the mean differences in % tail DNA between concentrations within exposure duration and chemical compound, between durations within concentration and chemical compound and between chemical compounds within concentration and exposure duration. The percentage of MN was compared between concentrations, exposure durations and chemical compounds using Mann-Whitney test. The *P* values less than 0.05 were considered statistically significant.

Results

Physicochemical Parameters of the Test Water

The water temperature varied from 22.90 to 27.60ºC and the pH ranged from 7.2 to 7.8. The dissolved oxygen concentration ranged from 6.5 to 7.9 mg L^{-1}, conductivity values ranged from 245 to 307 µM cm^{-1} while total hardness varied from 167-188 mg L^{-1} during the experimental period.

24 h-LC_{50} Values

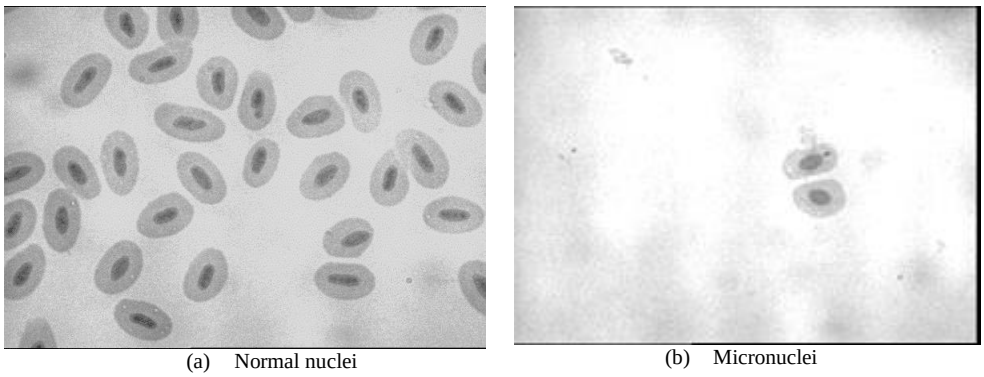
In acute toxicity bioassay, the LC_{50} values (with 95% confidence limits) of different concentration of crude latex (Table 1) were found to be 22.33 (18.46-46.14), 16.63 (14.63-21.43), 12.61 (10.64-14.11) and 10.49 mg L^{-1} (7.70-11.98) for 24, 48, 72 and 96 h respectively and the LC_{50} values of different concentration of Apigenin containing extract were found to be 76.48 (67.50-117.45), 68.45 (61.36-85.38), 58.51 (49.07-66.29) and 52.90 mg L^{-1} (41.45-58.49) for 24, 48, 72 and 96 h respectively in *C. punctatus*. The present study revealed that toxicity of crude latex and apigenin containing extract powder from *J. gossypifolia* was time and dose dependent in the fresh water fish *C. punctatus*.

Induction of Micronuclei

There was a significant induction of MN in the erythrocytes of exposed fish group at different concentrations of the test chemicals as compared their respective control groups (Figure 1a, Figure 1b). The highest concentrations of both the test chemicals show significant induction of MN than their respective

Table 1. Lethal concentrations of Jatropha crude latex and apigenin containing extract (mg L^{-1}) (95% confidence intervals) depending on exposure time for *C. punctatus* (n=10 in three replications)

Tested materials	Lethal concentrations	Exposure time (h)			
		24	48	72	96
Jatropha crude latex	LC_{10}	11.28 (6.46-13.26)	8.278 (4.029-10.364)	6.87 (3.52-8.79)	5.66 (2.25-7.70)
	LC_{50}	22.33 (18.46-46.14)	16.63 (14.63-21.43)	12.61 (10.64-14.11)	10.49 (7.70-11.98)
	LC_{90}	44.20 (28.68-95.55)	33.43 (24.36-96.68)	23.14 (19.12-38.13)	19.435 (16.55-29.65)
Apigenin containing extract	LC_{10}	49.80 (28.14-57.34)	45.55 (26.27-52.95)	38.34 (16.14-46.84)	36.67 (15.73-44.79)
	LC_{50}	76.48 (67.50-117.59)	68.45 (61.36-85.38)	58.51 (49.07-66.29)	52.90 (41.45-58.49)
	LC_{90}	117.45 (90.31-432.27)	102.86 (83.47-236.36)	89.29 (74.88-186.85)	76.30 (66.94-124.65)



(a) Normal nuclei (b) Micronuclei

Figure 1. (a) Micronuclei formation in the erythrocytes of *C. punctatus*in control, (b) Micronuclei formation in the erythrocytes of *C. punctatus*after exposure to crude latex and apigenine containing extract.

solvent control groups (Table 2). The significant the highest MN frequency was observed in 40% concentration of both the chemicalsfor 96 h and significant lowest MN frequency was observed at 20% concentration of both the test chemicals after 24 h post exposure with compare their respective control groups. No significant variation in MN frequency was observed between the test concentrations of both the chemicals. The MN frequency increases 0.0776 to 0.107% with increasing concentration of Apigenin containing extract and exposure durations while, after exposure of Jatropha crude latex, it rises 0.107 to 0.302% with increasing concentration and exposure durations. Between test chemicals, Jatropha crude latex exhibited more MN induction than Apigenin containing extract at various concentrations and exposure durations.

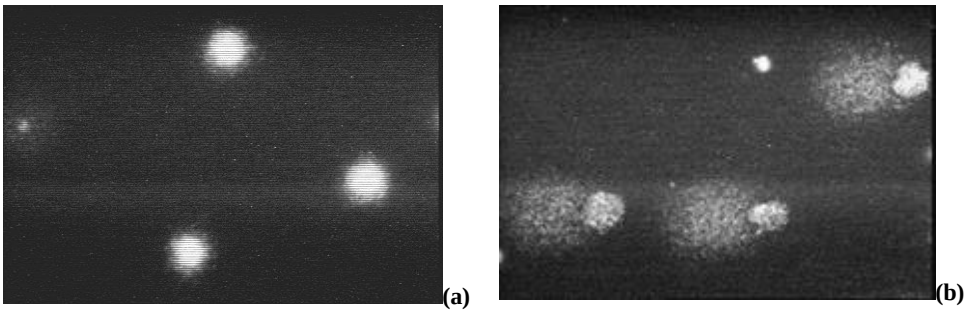
DNA Damage

The DNA damage measured as % tail DNA in the erythrocytes of the control and treatment groups indicated that the fish exposed to different concentrations of Apigenin containing extract and

Table 2. Induction of MNi in Blood of exposed to different concentrations of Apigenin containing extract and Jatropha crude latex

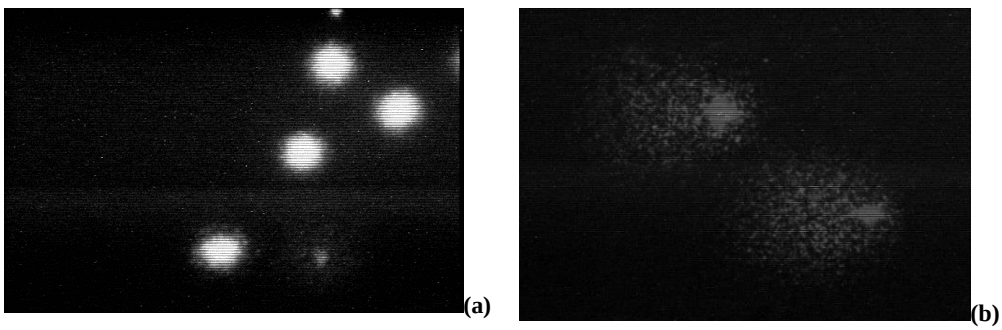
Tested materials	Concentration	% MN frequencies (mean ±SE)			
		24h	48h	72h	96h
Apigenin containing extract	Control	0.0098 ± 0.009^{a1A}	0.0096 ± 0.009^{a1A}	0.0196 ± 0.019^{a1A}	0.0196 ± 0.019^{a1A}
	Solvent control	0.0194 ± 0.011^{a1A}	0.0196 ± 0.012^{ab1A}	0.0296 ± 0.019^{ab1A}	0.0294 ± 0.019^{ab1A}
	20%	0.0776 ± 0.012^{b1A}	0.078 ± 0.012^{b1A}	0.0886 ± 0.019^{b1A}	0.0886 ± 0.018^{b1A}
	40%	0.0878 ± 0.009^{b1A}	0.0972 ± 0.015^{bc1A}	0.1078 ± 0.018^{b1A}	0.107 ± 0.018^{b1A}
Jatropha crude latex	Control	0.0239 ± 0.018^{a1A}	0.0248 ± 0.015^{a1A}	0.0159 ± 0.009^{a1A}	0.0158 ± 0.009^{a1A}
	Solvent control	0.0358 ± 0.009^{a1A}	0.0368 ± 0.011^{a1A}	0.0378 ± 0.011^{a1A}	0.0468 ± 0.019^{a1A}
	20%	0.107 ± 0.19^{ab1A}	0.145 ± 0.021^{b12B}	0.185 ± 0.019^{b12B}	0.224 ± 0.024^{b2B}
	40%	0.164 ± 0.024^{b1B}	0.233 ± 0.018^{c12B}	0.282 ± 0.027^{b2B}	0.302 ± 0.037^{b2B}

The values with different alphabet (lowercase) superscript differ significantly (P<0.05) between concentrations within sampling time. Values with numeric superscript indicate significant (P<0.05) difference between sampling times and within concentration. Value with different uppercase alphabet superscript differ significantly (P<0.05) between chemical compounds within duration and concentration.



(a) **(b)**

Figure 2. (a) Control erythrocytes, (b) DNA damaged in blood erythrocytes of *C. punctatus* when treated with Apigenin containing extract.



(a) **(b)**

Figure 3. (a) Control erythrocytes, (b) DNA damaged in blood erythrocytes of *C. punctatus* when treated with of *J. gossypifolia* latex.

crude latex, exhibited significantly higher DNA damage ($P<0.05$) in their blood cell erythrocytes as compared to the control as shown in Figure 2, Figure 3 respectively. The lowest DNA damage was observed at 24 h and there was a gradual non-linear increase in the DNA damage in all concentration of both tested chemicals with progression of the experiment and the highest DNA damage was observed on 96 h for all treatment groups. The DNA damage were found to be dose and time dependent in crude latex while in Apigenin extract exposed groups, it was to be time dependent. The crude latex exhibited comparatively higher DNA damage than the Apigenin containing extract at all concentrations and time durations in the exposed fishes. The highest level of DNA damage was reported at 40% of the LC_{50} of crude latex (21.17%) for 96 h followed by the damage induced by 40% of the LC_{50} of Apigenin containing extract (15.20%) for 96 h (Table 3).

Discussion

Fishes are often used as sentinel organisms for ecotoxicological studies because they play numerous roles in the trophic web, accumulate toxic substances and respond to low concentration of mutagens (Cavas and Ergene-Gozukara, 2005). Some medicinal plants that are toxic to fish enter through food chain to human beings, making it unsafe and questionable for medicinal use. In the present study, fish blood is used as it comprises 97% erythrocytes, thus ensuring great homogeneity of cells for Comet assay studies. The LC_{50} values at 24 h of both plant extracts containing crude latex and Apigenin containing extract was determined as 22.334 mg L^{-1} and 38.24 mg L^{-1} respectively, which indicated that both are very toxic to *C. punctatus*, in a static system. It is clear from the present study, that crude latex as well as Apigenin containing extract significantly increases the frequency of MN and DNA damage after exposure to sub lethal doses in a time and dose-dependent manner.

There is very less data available on the genotoxic effects of plant products in fish. However, some reports are available on genotoxic effect of synthetic pesticides viz. Profenofos, which is commonly used in India for pest control in mango, banana, cotton, and pineapple agriculture (Das *et al*., 2006; Reddy and Rao, 2008; Kavitha and Rao, 2009). Pandey *et al*., 2011 reported that organophosphate pesticide profenofos induces acute toxicity (96-h LC_{50} 2.675 µg L^{-1}) to freshwater fish, *C. punctatus* (Bloch), in a static bioassay. Ali *et al*. (2009) estimated the LC_{50}-96 h of Chlorpyrifos (organophosphate pesticide) in a semi-static system in the fish *C. punctatus* and significant effects in the induction of micronuclei and DNA damage were observed. Nwani *et al*. (2010) conducted studies on the mutagenic and genotoxic effect of carbosulfan and found the 96 h LC_{50} of carbosulfan as 0.268 mg L^{-1} in fresh water fish *C. punctatus*. Pandey *et al*., 2005 conducted acute toxicity tests to determine the lethal toxicity of an organophosphorus pesticide, malathion, to air-breathing teleost, *C. punctatus* (Bloch). Singh and Agrawal, 1990 reported that Apigenin containing extract and crude latex of *J. gossypifolia* have significant anti-acetylcholinesterase activities. The site of action of both tested chemicals is neuro-enzyme acetylcholinesterase (AChE) activity, which is same as for certain organophosphates. The mechanism which causes the genotoxic effect in case of organophospahate pesticides cannot be ruled out in case of the medicinal plant *J. gossypifolia*.

The use of herbal products derived from medicinal plants for therapeutic purposes was initially considered having no side effects and non-toxic. However, the results of the present study reveal that some medicinal plants products may be genotoxic and mutagenic making it unsafe for further consumption. Finally, when applied in higher doses, leaf extract of *J. gossypifolia* shows genotoxic activity. Investigating with crude extracts is appropriate because traditional medicinal herbs are generally used as crude extracts. Conversely, working with crude extracts also means working with complex mixtures of biologically active compounds which can be genotoxic; others can be antigenotoxic. These plant products are likely to contain toxic chemical ingredients which have adverse effects on consumers. The present study clearly reveals the existence of toxic ingredients in the crude latex as well as extract containing Apigenin of

Table 3. Mean ± SE % tail DNA in erythrocytes of *C. punctatus* exposed to different concentrations of Apigenin containing extract and *Jatropha gossypifolia* crude latex

	Concentrations	24h	48h	72h	96h
Apigenin containing extract	Control	$4.01(\pm0.28)^{a1A}$	$4.09(\pm0.22)^{a1A}$	$4.18(\pm0.31)^{a1A}$	$4.13(\pm0.26)^{a1A}$
	Solvent control	$4.95(\pm0.28)^{a1A}$	$4.88(\pm0.22)^{a1A}$	$5.05(\pm0.34)^{a1A}$	$4.97(\pm0.29)^{a1A}$
	20%	$6.81(\pm0.55)^{b1A}$	$8.72(\pm0.67)^{b1A}$	$10.93(\pm0.68)^{b2A}$	$13.04(\pm0.80)^{b3A}$
	40%	$8.49(\pm0.69)^{b1A}$	$9.94(\pm0.71)^{b1A}$	$12.44(\pm0.80)^{b2A}$	$15.20(\pm0.84)^{b3A}$
Jatropha crude latex	Control	$4.11(\pm0.29)^{a1A}$	$4.05(\pm0.31)^{a1A}$	$4.15(\pm0.37)^{a1A}$	$4.20(\pm0.34)^{a1A}$
	Solvent control	$5.11(\pm0.30)^{a1A}$	$5.23(\pm0.38)^{a1A}$	$5.16(\pm0.31)^{a1A}$	$5.22(\pm0.39)^{a1A}$
	20%	$9.93(\pm0.69)^{b1B}$	$13.08(\pm0.77)^{b2B}$	$16.31(\pm0.93)^{b3B}$	$18.96(\pm1.05)^{b3B}$
	40%	$13.73(\pm0.83)^{c1B}$	$16.35(\pm0.92)^{c2B}$	$19.55(\pm1.20)^{c3B}$	$21.17(\pm1.24)^{b3B}$

Value with different lowercase alphabet superscript differ significantly ($P<0.05$) between concentrations within duration and chemical compound. Value with different numeric superscript differ significantly ($P<0.05$) between durations within concentration and chemical compound. Value with different uppercase alphabet superscript differ significantly ($P<0.05$) between chemical compounds within duration and concentration.

J. gossypifolia. The results of this study suggest that, although *J. gossypifolia* has beneficial effects as a medicinal herb, it can cause serious problems and damage on cells when used improperly. As accepted globally, herbal formulations have curative effects but in view of the presence of certain toxic chemicals, it is likely advisable to identify and eliminate such toxic substances making them safe for medicinal use.

The mutagenic and genotoxic potentials of the herbal formulation of *J. gossypifolia* in *C. punctatus* reveal a serious apprehension about the impending dangers of crude latex and Apigenin containing extract to aquatic organisms as well as human beings. The genotoxicity of the crude latex compared to the Apigenin containing extract of the plant suggests that the higher toxicity of the latex is due to the presence of toxic components. The nature of these toxic components responsible for the higher toxicity of latex is further needed to be studied. The results further suggest that during the extraction of the crude, toxic components have been eliminated from the crude, rendering the extract less toxic as compared to the latex of the plant. The latex may therefore, be unsafe for medicinal use. The extract, however, is further required to be chemically analyzed for the identification of any left over toxic components and its subsequent elimination so that the extract could be made non-toxic for herbal medications. The genotoxicity of the pure form of the Apigenin containing extract is also required to be assessed for toxicity studies.

Declaration of Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors are thankful to the Director, National Bureau of Fish Genetic Resources, Lucknow, India for providing support, suggestions, materials and laboratory facility for conducting this study.The corresponding author Dr. Pratibha Singh is also thankful to University Grants Commission, New Delhi, for the award of the Dr. D.S. Kothari Post-Doctoral Fellowship Scheme (No. F.4-2/2006 (BSR)/ 13-214/2008 (BSR), dated 1st October 2009-30th September 2012) for financial assistance.

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