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RESEARCH ARTICLE

PHYTOCHEMICAL AND ANTIBACTERIAL EFFICACY OF RHIZOME AND FROND EXTRACTS OF *PITYROGRAMMA CALOMELANOS,* THE SILVERBACK FERN

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SUMMARY Comparative phytochemical analysis and antibacterial efficacy of rhizome and frond extracts of *Pityrogramma calomelanos* (L.) Link, commonly called the 'silverback fern' was carried out. Plant parts like fronds and rhizome were extracted by hot solvent extraction method with soxhlet apparatus using petroleum ether, ethyl acetate, methanol and water. Phytochemical evaluation of the extracts revealed the presence of various secondary metabolites such as alkaloids, cardioglycosides, phenolics, saponins, tannins, terpenoids, steroids, quinones, flavonoids and glycosides. Extracts were subjected to antibacterial assay using disc diffusion method, against four pathogenic bacterial strains such as *Salmonella typhimurium, Pseudomonas aeruginosa, Escherichia coli* and *Xanthomonas campestris.* The results showed that ethyl acetate and methanolic extracts were more active than that of petroleum ether and aqueous extract. Extracts of fronds exhibited significant antibacterial activity as compared to rhizome extracts against all the tested strains.

Keywords: *Pityrogramma*, Polypodiaceae, antibacterial, phytochemical.

INTRODUCTION

Pteridophytes are the primitive vascular plants that occupy the intermediate position between bryophytes and higher plants, and they constitute the major part of the world flora, next to angiosperms. The economic potential of the pteridophytes is very little evaluated. The known studies are those of Nayar & Daniel (1986) listing the medicinal ferns of India; the phytochemical studies carried out by Muraleedharannair et al. (2012) on extracts of *Adiantum caudataum, A. latifolium, A. lunulatum, Christella dentata* and *C. parasitica* reporting the presence of carbohydrates, steroids, tannins, saponins, carboxylic acid, coumarins, xanthoprotein and phenolic compounds; the quantitative analysis performed by Rajesh et al. (2014) showing high content of tannin and phenol in *Pityrogramma calomelanos* fern extract and the study of Khan et al. (2007) reporting the antibacterial properties of *Drynaria quercifolia* extracts against four gram positive (*Bacillus subtilis, B. megaterium, Staphylococcus aureus, Streptococcus haemolyticus*) and six gram negative (*Escherichia coli, Shigella dysenteriae, S. sonnei, S. flexneri, Pseudomonas aeruginosa* and *Salmonella typhimurium*) clinically isolated strains of bacteria.

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Pityrogramma calomelanos belongs to the Class Polypodiopsida, Order Polypodiales and Family Pteridaceae. The plant is commonly known as silverback fern, and this is traditionally used to treat kidney stones (Montalvo et al. 2013). The present work is aimed at analysing and evaluating the phytochemical analysis and antibacterial properties of the rhizome and frond extracts of *P. calomelanos* (L.) Link.

MATERIAL AND METHODS

Preparation of extracts

Specimens for the present study were collected from Nedumkandam, Idukki district, Kerala. The specimens were washed thoroughly, and the rhizome and frond were separated, shade dried and powdered with a blender. Extracts were prepared using soxhlet extractor. 30 g of powdered samples were extracted successively with 150 ml of petroleum ether, ethyl acetate, methanol and distilled water for 8–12 h at a temperature not exceeding the boiling point. The extracts were concentrated in vacuum using a rotary evaporator.

Qualitative phytochemical screening

Different qualitative chemical tests were performed on various extracts to detect the presence of phytoconstituents (Harborne 1998).

For testing alkaloids, 1 ml of plant extract was mixed with 1 ml of 1% HCl, warmed and filtered. 2 ml of filtrate was treated with Mayer's reagent. Turbidity or precipitation, green colour indicates the presence of alkaloid. For testing cardioglycosides, 1 ml of the extract was mixed with 2 ml of glacial acetic acid to which added a few drops of 5% ferric chloride. This was underlayered with 1 ml of concentrated sulphuric acid. Formation of a brown ring at the interface indicates the presence of cardioglycosides. To test phenolic compounds, 1 ml of extract was mixed with 2 ml of distilled water, 0.5 ml of sodium carbonate and Folin ciocalteau's reagent. Formation of a blue or green colour indicates the presence of phenol. For testing saponins, 1 ml of plant extract was dissolved in 2 ml of boiling water in a boiling tube, allowed to cool and shaken well to mix. The appearance of foam indicates the presence of saponins. To detect tannins, 2 ml of the test solution was mixed with 2 ml of ferric chloride. The formation of a blue-black or dark-green colour indicates the presence of tannins. For testing terpenoids, 1 ml of extract was mixed with 2 ml of chloroform and 1.5 ml of concentrated sulphuric acid. Formation of a reddish brown colour indicates the presence of terpenoids. For testing steroids, 1 ml of plant extract was mixed with 2 ml of chloroform and 1 ml of sulphuric acid. Formation of a reddish brown colour at the interface indicates the presence of steroids. To detect quinones, 1 ml of extract was mixed with 1 ml of concentrated sulphuric acid. Formation of red colour indicates the presence of quinones. For testing flavonoids, 3 ml of extract was mixed with 4 ml of 1N sodium hydroxide. Formation of dark yellow colour indicates the presence of flavonoids. For testing glycosides, 2 ml of extract was mixed with 3 ml of chloroform and 1 ml of 10% ammonium solution. Formation of pink colour indicates the presence of glycosides.

Test for antibacterial activity

The agar disc diffusion method was used to evaluate the antibacterial activity (Murray et al.

1957). The antibacterial activity of different extracts was tested on pathogenic bacteria such as *Salmonella typhimurium* (NCIM 2501)*, Pseudomonas aeruginosa* (NCIM 5210)*, Escherichia coli* (NCIM 5846) and *Xanthomonas campestris* (NCIM 5028), obtained from the CSIR National Chemical Laboratory, Pune. Standard antibiotic discs such as amoxyclav (30 mcg) and methicillin (5 mcg) were used as the positive control. Solvents like petroleum ether, ethyl acetate, methanol and distilled water were used as the negative control. The test for antibacterial activity was carried out by measuring the diameter of inhibition zone (in mm). The experiment was repeated thrice and the results recorded were the mean of three replicates.

OBSERVATIONS

Qualitative phytochemical analysis

Preliminary phytochemical analysis carried out in different extracts of *P. calomelanos* indicated the presence of various secondary metabolites. Extracts of rhizome and fronds showed the existence of phytoconstituents such as alkaloids, cardioglycosides, phenols, saponins, tannins, terpenoids, steroids, quinones, flavonoids and glycosides (Table 1). Ethyl acetate and methanol extracts yielded more secondary metabolites than petroleum ether and aqueous extracts. Most of the phytoconstituents showed strongly positive results in frond extracts.

Antibacterial evaluation

The different solvent extracts of *P. calomelanos* parts were found to be effective against all the bacterial strains tested. Ethyl acetate and methanolic extracts of fronds showed significant antibacterial activity aganist *S. typhimurium, P. aeruginosa, E. coli* and *X. campestris* (Table 2).

TABLE 1: Phytochemical analysis of *P. calomelanos*.

Phytoconstituents	Rhizome				Frond			
	PE	EA	M	A	PE	EA	M	A
Alkaloid	++	++	–	–	++	++	–	–
Cardio glycoside	–	–	–	+	++	++	–	+
Phenol	–	++	–	++	–	++	++	++
Saponin	+	++	–	–	+	++	–	–
Tannin	–	–	–	–	++	++	–	–
Terpenoid	–	–	+	–	+	++	–	+
Steroid	–	–	–	–	–	–	–	++
Quinone	–	–	–	–	–	–	–	+
Flavonoid	–	–	–	–	++	++	+++	–
Glycoside	–	++	–	++	–	++	–	–

(PE, Petroleum ether; EA, Ethyl acetate; M, Methanol; A, Water).

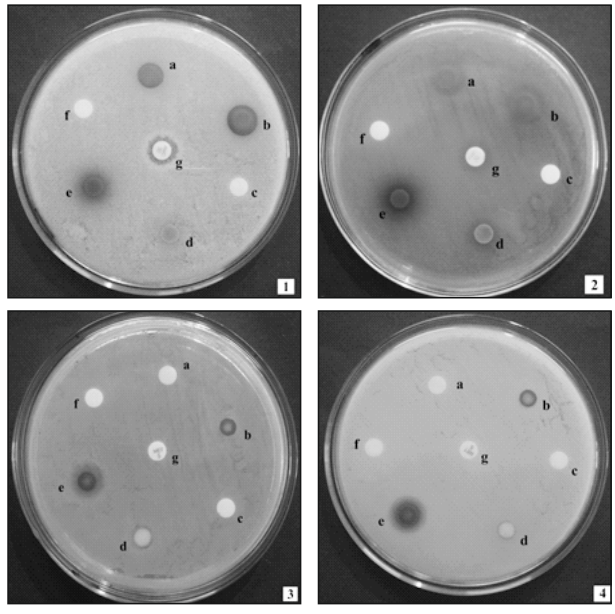
(**+++**, Strongly positive; **++**, Moderately positive; **+**, Positive; **-**, Negative).

TABLE 2: Antibacterial evaluation of *P. calomelanos.*

Bacterial Strains	Zone of inhibition (mm) : Values = Mean ± S. D													
	Rhizome				Fronds				Negative control				Positive control	
	PE	EA	M	A	PE	EA	M	A	PE	EA	M	A	Methicillin (5 mcg)	Amoxyclav (30 mcg)
S.typhimurium	0	0	9.6 ± 0.52	0	0	11 ± 0	11.34 ± 1	7 ± 0	0	0	0	0	0	9.33 ± 0.57
P.aeruginosa	0	0	10.66 ± 0.57	0	0	11.16 ± 0.28	13.67 ± 0.56	0	0	0	0	0	0	10 ± 0
E.coli	0	0	12 ± 1	0	0	10 ± 0	12 ± 0	0	0	0	0	0	0	9.5 ± 0.57
X.campestris	0	0	9.67 ± 0.57	0	0	12.7 ± 0.58	10.66 ± 0.57	7 ± 0.5	0	0	0	0	0	11 ± 1

(S.D, Standard deviation; PE, Petroleum ether; EA, Ethyl acetate; M, Methanol; A, Water)

Aqueous extract of fronds were sensitive only to *S. typhimurium* and *X. campestris* whereas, petroleum ether extracts were not sensitive. Only the methanolic extract of rhizome showed notable inhibitory effect against the tested strains. Petroleum ether, ethyl acetate and aqueous extracts of rhizome did not show any inhibitory action. While comparing the effect of rhizome and fronds, the extracts of fronds showed considerable inhibition on bacterial growth. The respective pure solvents that were taken as negative control showed no inhibitory action. The antibiotic amoxyclav (30 mcg) showed zone of inhibition against the bacterial strains tested whereas methicillin (5 mcg) did not show any response (Figs 1–4).



Figs 1–4: Antibacterial activity of *P. calomelanos.* 1. Against *S. typhimurium* 2. Against *P. aeruginosa* (a, Methanol extract of rhizome; b, Methanol extract of fronds; c, Methanol control; d, Aquoeous extract of rhizome; e, Aquoeous extract of fronds; f, Water; g, amoxyclav) 3. Against *E. coli* 4. Against *X. campestris* (a, Petroleum ether extract of rhizome; b, Petroleum ether extract of fronds; c, Petroleum ether control; d, Ethyl acetate extract of rhizome; e, Ethyl acetate extract of fronds; f, Ethyl acetate control; g, methicillin).

DISCUSSION

Plants produce secondary metabolites not simply to adapt to their environment, but also to exert resistance themselves against several environmental stresses and also for the process of co-evolution with various interacting organisms (Berenbaum et al.1995).The secondary metabolites produced against the various adverse environmental conditions are, flavonoids, alkaloids, polyphenols, terpenoids, quinones, steroids, polysaccharides etc. (Swain 1977). The present study also revealed the presence of various phytoconstituents in the silverback fern. The attribute of antimicrobial activity may be due to the presence of one or more bioactive compounds such as alkaloids, steroids, saponins etc. (Balandrin & Klocke 1988). Raimana et al. (2009) have enunciated that many fern species belonging to *Drynaria, Lemmaphyllum, Microgramma, Microsorum, Phyllitis, Phymatosorus, Polymatosorus, Polypodium, Pyrrosia* of Polypodiaceae were widely used for the treatment of several ailments such as rheumatoid arthritis, liver disease etc.

Earlier studies reported that the aqueous decoction of *P. calomelanos* at a concentration of 37.5 mg/ml repressed the growth of *P. aeruginosa, Staphylococcus aureus* and *S. saprophyticus* at a range of 2–20% relative to the antibiotic streptomycin, which inhibited the growth at a range of 100% in a concentration of 5 mg/ml. *P. aeruginosa* plays a great role in biofilm formation on kidney stones which lead to urinary infections. This justifies the use of *P. calomelanos* as an herbal medicine in the treatment of urinary infections (Montalvo et al. 2013). Similarly, the present study is suggestive that the extracts of *P. calomelanos* are sensitive to *P. aeruginosa* and *E. coli* which cause urinary tract infections.

The present findings have indicated the antibacterial activity of the ethyl acetate and methanolic extracts of *P. calomelanos* fronds as very effective against all the four bacterial strains tested. Petroleum ether extracts had no effect whereas, aqueous extracts of the fronds showed least inhibitory effect. The extract of rhizome in methanol was sensitive only to the pathogenic strains, though the extracts in petroleum ether, ethyl acetate and water were insensitive. While comparing the effect of rhizome and frond, the extracts of fronds showed considerable inhibition on bacterial growth. The present study substantiates the earlier findings that ferns should be further screened for bioactive constituents. *P. calomelanos* having several phytochemicals possesses notable antibacterial efficacy.

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RESEARCH ARTICLE

KARYOTYPIC STUDIES ON *CYPRINUS CARPIO* AND *CYPRINUS CARPIO HAEMATOPTERUS* FROM HIMACHAL PRADESH, INDIA

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SUMMARY Karyotypic studies on two fresh water fishes, *Cyprinus carpio* and *C. carpio haematopterus* from Himachal Pradesh were carried out. The diploid chromosome number was 2n = 100 in both these fish species. The karyotype of *C. carpio* consisted of 22 metacentric, 20 submetacentric, 10 subtelocentric and 48 telocentric chromosomes whereas karyotype of *C. carpio haematopterus* revealed 14 metacentric, 8 submetacentric, 8 subtelocentric and 70 telocentric chromosomes. Length of chromosomes, the total length of haploid complements, arm ratio of the complements and centromeric indexes were determined for both fish species. Idiograms of both species showed gradual decrease in the size of chromosome pairs.

Keywords: Fresh water fish, chromosomes, karyotype, idiogram, *Cyprinu*s.

INTRODUCTION

Fresh water fishes constitute an important group for biogeographic studies. It is the most speciose group among all the vertebrates. Fish chromosome data has great significance concerning systematic, aquaculture and mutagenesis (Al-sabti 1991). Cytogenetic studies are authentic and advanced tools for the characteristic fish species. As this group is the originator of all the vertebrates, the karyotypic data of these organisms are of great significance in understanding evolutionary pathways of vertebrates.

Fishes also have substantial economic importance besides being an important source of proteins in human diet. They are exploited for recreation through angling (Raat 1985, Arlinghaus 2004) and also have many ecological functions comprising primary production, biogeochemical cycling, pollutant remediation etc. Fish species also act as an important indicator of ecological health and the health of a water body, which is shown by the abundance and health of the fish of that water body (Hamzah 2007).

There are many natural as well as man-made water resources like lakes, ponds, wells, rivers and rivulets in Himachal Pradesh which support a variety of fish life. Ninety-seven species of fresh water fishes, belonging to 51 genera, 18 families and 6 orders have been reported from Himachal Pradesh (Sharma 2010). Parmar & Gautam (2018) reported the chromosomes of three species of *Labeo* from Himachal Pradesh. There is no other karyological work reported on the fishes of Himachal Pradesh. Keeping in view the increasing importance of karyotypic studies on fishes in general and the lack of data on fish karyotypes in Himachal Pradesh in particular, it is

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desirable to investigate the chromosomes of fish fauna from Himachal Pradesh. Thus, in the present study, the chromosomes of two fish species namely, *C. carpio* and *C. carpio haematopterus* were investigated.

MATERIALS AND METHODS

The fresh water fishes were collected in living condition with the help of local fishermen from different fresh water bodies of Himachal Pradesh. *C. carpio* was collected from Deoli, Bilaspur (Altitude 1890 m above sea level, 31.37160 (ºN) Latitude and 76.80956 (ºE) Longitude) and *C. carpio haematopterus* was collected from Alsu, Mandi (Altitude 1798 m above sea level 31.41542 (ºN) Latitude and 76.84568 (ºE) Longitude). Collected fishes were then brought to the working place and were kept in well aerated water before conducting chromosome analysis. For chromosomal studies, an air-dried technique of Thorgaard & Disney (1990) with some modifications was used and the karyotypes were prepared. Classification of chromosomes was done according to the method of Levan et al. (1964). The procedure followed for preparation of chromosomal slides is as follows:

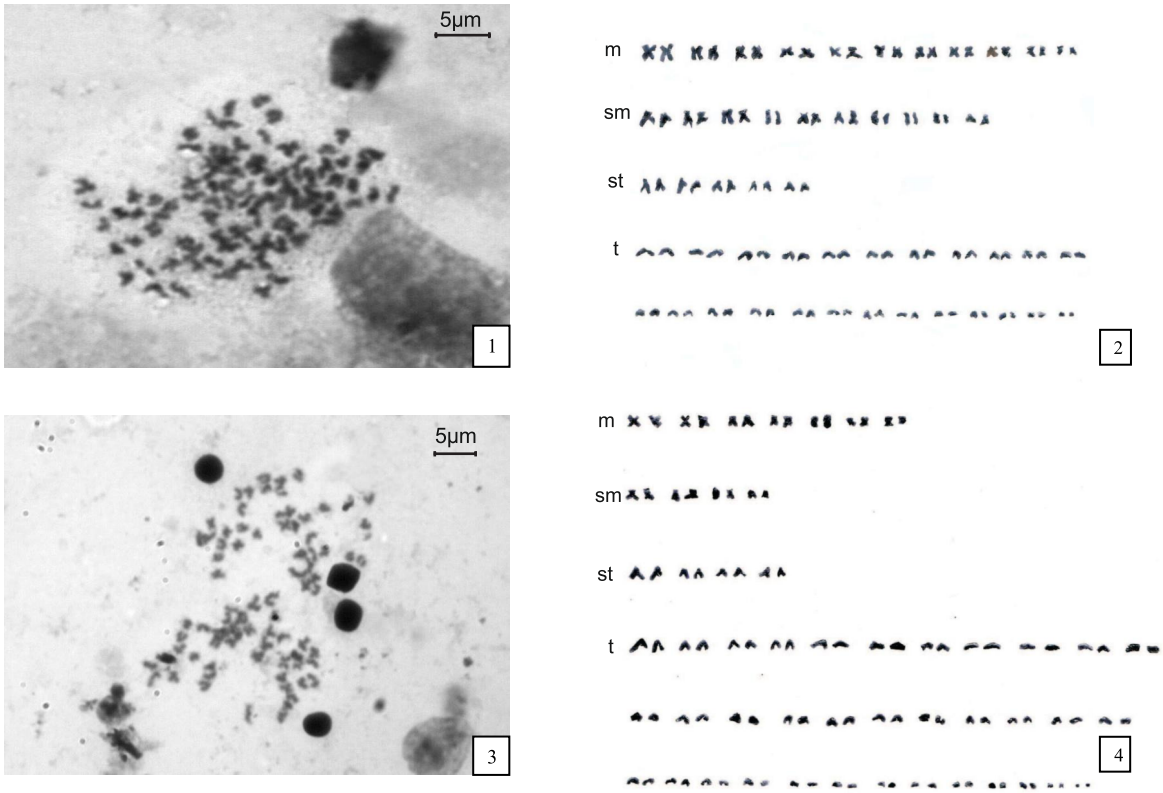
Pretreatment was done by giving an intramuscular injection of 0.05% colchicine at the rate of 1 ml/100 g body weight to each fish. The injected fishes were left in well aerated water for 2 to 3 h. After 3 h of pretreatment, the colchicine injected fishes were dissected from the ventral side to remove the anterior kidneys. The tissues were further minced into smaller pieces and were placed in hypotonic solution of 0.56% KCl for 30 to 40 min. After that, the hypotonic solution was discarded and the tissues were fixed in freshly made Carnoy's fixative (3:1 methanol: glacial acetic acid) for at least 30 min. Two or three washings were given with the fresh fixative, each lasting for about 30 min. Tissues were taken from the fixative and centrifuged at 1000 to 1500 rpm for 10 min. Supernatants were discarded and the pellets were suspended in small amount of fresh fixative. The suspension was dropped on clean, dry and chilled slides using the splash method from 30 to 60 cm height and were air-dried for at least 24 h. Staining was done in 2% Giemsa solution for 30 to 40 min. After 2 or 3 d, air dried slides were dipped in xylene and finally mounted in D.P.X. The permanent slides were then thoroughly scanned under binocular research microscope and photomicrographs were taken. Well spread and non-overlapping metaphasic plates were selected for chromosomal measurements. The photomicrographic prints were prepared and each individual chromosome was cut out from the photomicrographs. Pairing of homologous chromosomes was done on the basis of their centromeric positions, arm lengths and gross morphology. The chromosomes were then arranged in decreasing order of their lengths to prepare the karyotype of each species.

OBSERVATIONS

In the present study, chromosomes of two fishes namely, *C. carpio* and *C. carpio haematopterus* have been studied.

C. carpio

Its body is wide, elongated and torpedo-shaped with two pairs of barbles at the angles of mouth, shorter ones on the upper lip. In the present study, 2n = 100 was observed in this species (Fig. 1). The karyotypic composition was 22 metacentric, 20 submetacentric, 10 subtelocentric and 48 telocentric chromosomes (Fig. 2). Karyotype was prepared based on the size and position of centromere. The length of chromosomes ranged from 0.38 µm to 2.3 µm. The total length of haploid complement was 73.94 µm. Arm ratio of



Figs 1–4: 1, 2. *C. carpio.* 1. Somatic metaphase. 2. Karyotype. 3, 4. *C. carpio haematopterus.* 3. Somatic metaphase. 4. Karyotype.

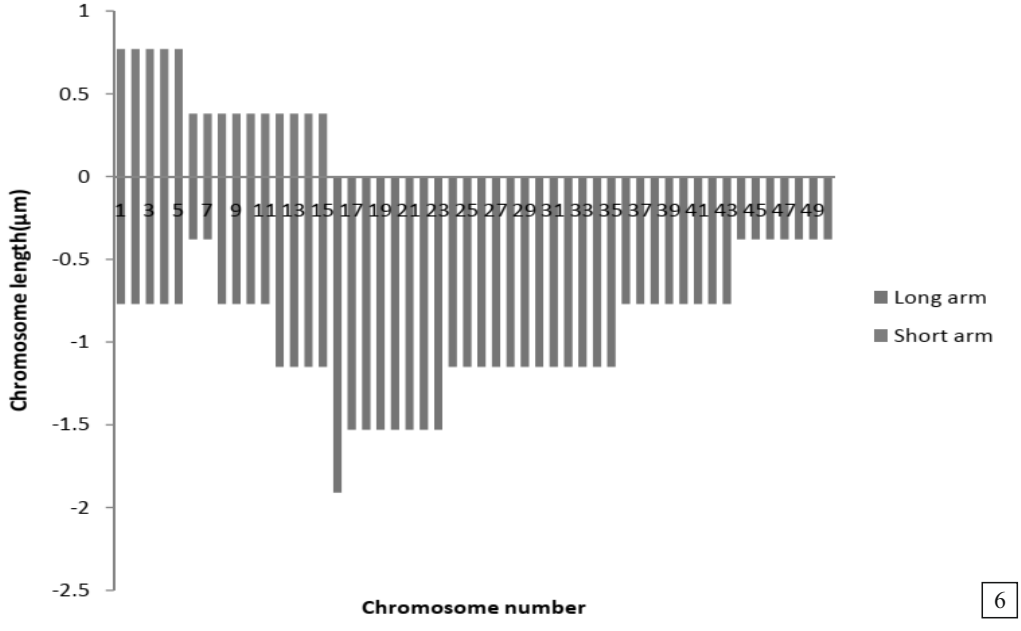
the complement ranged between 1–∞ and the centromeric index ranged between 0–50. Idiogram was constructed on the basis of absolute lengths and centromeric positions of the chromosomes arranged in decreasing order of their lengths (Fig.5). The karyotype formula is 22m + 20sm + 10st + 48t.

C. carpio haematopterus

It is an improved strain of *C. carpio.* Its body is cylindrical and belly is smaller than the existing stock of common carp. The somatic complement of this species revealed a diploid chromosome number of 100 with karyotypic composition of 14 metacentric, 8 submetacentric, 8 subtelocentric and 70 telocentric chromosomes (Figs 3, 4). The lengths of chromosomes ranged from 0.38 µm to 1.54 µm. The total length of haploid complement is 55.18 µm. Arm ratio of the complement ranged from 1–∞ and the centromeric index ranged from 0–50. Idiogram of this species showed gradual decrease in the size of chromosome pairs (Fig. 6). The karyotype formula is 14m + 8sm + 8st +70t.

DISCUSSION

C. carpio usually called common carp, is a widespread fresh water fish with a tetraploid origin (Anjum 2005). The diploid chromosome number of 2n = 100 reported here for this species.



Figs 5 & 6: Idiograms. 5. *C. carpio.* 6. *C. carpio haematopterus.*

The same chromosome number had been reported earlier by many workers (Raicu et al. 1972, Denton 1973, Zan & Song 1980, Blaxhall 1983, Labat et al. 1983, Rab et al. 1989, Lakra & Rishi 1991, Anjum & Jankum 1994, Anjum 1995, Salih & Majeed 2012). In certain cases, deviation from this diploid chromosome number, pertaining to the presence of two or more micro-chromosomes, had also been reported by Sola et al. (1986) and Brzuska (1988).

Karyotypic configuration also differed in different cases. In the present investigation, the karyotypic configuration comprised of 22 metacentric, 20 submetacentric, 10 subtelocentric and 48 telocentric chromosomes, However, Anjum (2005) recorded 16 metacentric, 34 submetacentric and 50 subtelocentric /acrocentric chromosomes in the same fish species. In the present study, karyotype and idiogram showed gradual decrease in size of the chromosomes.

Two non-homologous submetacentric chromosomes of different sizes bearing Nucleolar Organizer Regions (NORs) on their upper shorter arms had been detected by different workers by using silver staining (Ruifang et al. 1985, Mayr et al.1986, Sola et al. 1986, Anjum 1995, Anjum & Jankun 1998, Anjum 2005). Salih & Majeed (2012) reported 22 metacentric, 32 submetacentric, 12 subtelocentric and 34 acrocentric chromosomes in the same species.

C. carpio haematopterus commonly known as Amur common carp, is an improved strain of the common carp having faster growth rate (27% faster than existing stock of common carp), delayed sexual maturity and less susceptibility for diseases. It was introduced to Himachal Pradesh at fish farm Alsu (Mandi) in 2006 from Hesserghatta Seed Farm (Bengaluru) of Fisheries Research and Information Centre. In this species, 2n has been found to be 100, which is similar to the other native common carps. Same diploid chromosome number had been reported by Zan et al. (1986) and Rab et al. (1989). However, there is difference in the morphology of reported karyotypes. In the present study, 14 metacentric, 8 submetacentric, 8 subtelocentric and 70 telocentric chromosomes were reported. Earlier, Zan et al. (1986) reported 20 metacentric, 30 submetacentric and 50 subtelocentric/ acrocentric chromosomes in the same species. Rab et al. (1989) reported the same diploid chromosome number with karyotypic composition of 28 metacentric, 38 submetacentric, 22 subtelocentric and 12 acrocentric chromosomes in this fish and claimed that 2n = 100 in this species can be divided into eight well defined categories in all male, female and young unsexed individuals. In the present study, karyotype was grouped into four different categories and idiogram was showing gradual decrease in the size of chromosomes.

Zan et al. (1986) also reported a heteromorphic pair of large submetacentric chromosomes, which was earlier considered as XX/XY sex chromosomes, but no such heteromorphic pair has been reported by Rab et al. (1989). In the present study also, no such heteromorphic pair was reported. Rab et al. (1989) stated that the morphology of karyotype mainly depends on the quality of chromosome preparations that results into different degree of spiralization and this should be taken into consideration while comparing the data from different workers.

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RESEARCH ARTICLE

GENOTOXIC EFFECT OF DISTILLERY EFFLUENT ON MITOTIC CHROMOSOMES OF MICE (*MUS MUSCULUS*)

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SUMMARY Young weaning Swiss albino mice were orally administered different concentrations of 1%, 2% and 4% distillery effluent at 1ml/d for 7 consecutive days. Among 300 abnormal metaphase cells, the total frequency of abnormalities of 18/6, 24/8, and 32 /10.66 in 1%,2% and 4% of effluent respectively were found. It shows that the effect of effluent is dose-dependent. C-mitosis, stickiness and clumping were common among gross type, while acentric fragment, minute fragment, chromatid break and chromatid gaps were more common among the individual type of abnormalities. The individual type of damage was prominent than that of the gross type. Effluents probably cause an increase in the formation of base analogue to act as a mutagen that induces chromosomal abnormalities.

Keywords: Mice, distillery effluent, chromosome anomalies, bone marrow.

INTRODUCTION

Anthropogenic activities bring contamination and subsequent pollution to our ecosystems. Industrialization is believed to cause most of the pollution problems in the natural ecosystem by the release of hazardous wastes into the environment. Industrial untreated effluents are the major causes of water pollution that poses a serious threat to plants, aquaculture, cattle, and human beings. These contain many toxic substances and make water unsuitable for consumption.

Alcohol is a basic material for several industries (chemical, pharmaceutical, cosmetics, beverage and food). The number of alcohol distilleries is increasing worldwide. India is one of the largest producers of bioethanol in the world. Factories discharge a large quantity of untreated effluents which contain many toxic substances thus posing solemn damage to the life of plants, cattle, human beings etc. They make water unsuitable for consumption. Cytogenetic toxicity by these pollutants is one such hazard. However, previously, the genoto-xicity of industrial effluent (Moore et al. 2003, Shipra & Sharma 2002), chemical (Rangaswamy & Shanthamurty 1980), steel (Mahapatra et al.1986, Thakur & Roy 1986), and distillery (Manivannan et al. 2004) has been investigated.

North Bihar has a large number of sugar-based factories. Unprocessed effluents from these factories are discharged directly into ponds, rivers, and their tributaries and cause water pollution. Therefore, in the present study, an attempt has been made to evaluate the cytogenetic toxicity of distillery effluent on mitotic chromosomes in bone marrow cells of mice. The main objective of this work was to present a comprehensive account of the genotoxic effect of distilleries.

MATERIAL AND METHODS

The distillery effluent was collected from the main outlet of the factory. The collected effluent is considered as of 100% concentration. By dilution with distilled water, 1, 2, and 4% concentrations of the effluents were prepared. Six to eight wks old laboratory bred Swiss albino mice *Mus musculus* (Scdri, 2n = 40), seed colony obtained from Central Drug Research Institute, Lucknow (India) were used.

Mice were orally administered each concentration of effluent at the rate of 1 ml/d for 7 consecutive ds. The mice of the control group were given only 1 ml of distilled water per d. The animals were sacrificed immediately after the completion of such treatment. The extraction of marrow and preparation of slides were made by colchicine-hypotonic-aceto-alcohol-flame drying Giemsa staining technique (Preston et al. 1987). About 300 well spread metaphase plates at the rate of 20 plates/animal from 15 animals of each group were screened randomly. Data were analyzed by the standard statistical procedure.

OBSERVATIONS

The details of chromosomal abnormalities in bone marrow cells of mice treated with different concentrations of distillery effluent are given in (Table 1).

The abnormalities found can be put into two categories–gross and individual ones. The insignificant gross changes were stickiness, polyploidy, hypopolidy, etc. (Figs 1–6). The significant ones were mostly, breaks in the chromosomes. Acentric fragment and minute fragment (Figs 1–6) were also observed that might be due to breaks and deletion of a certain part of the chromosome (telomeric or interstitial part). A quantitative estimation revealed that the abnormalities were increased with the increase of the doses. Thus, the effect was dose-dependent (Fig. 7). The individual type of damages was more prominent than the gross type (Fig. 8).

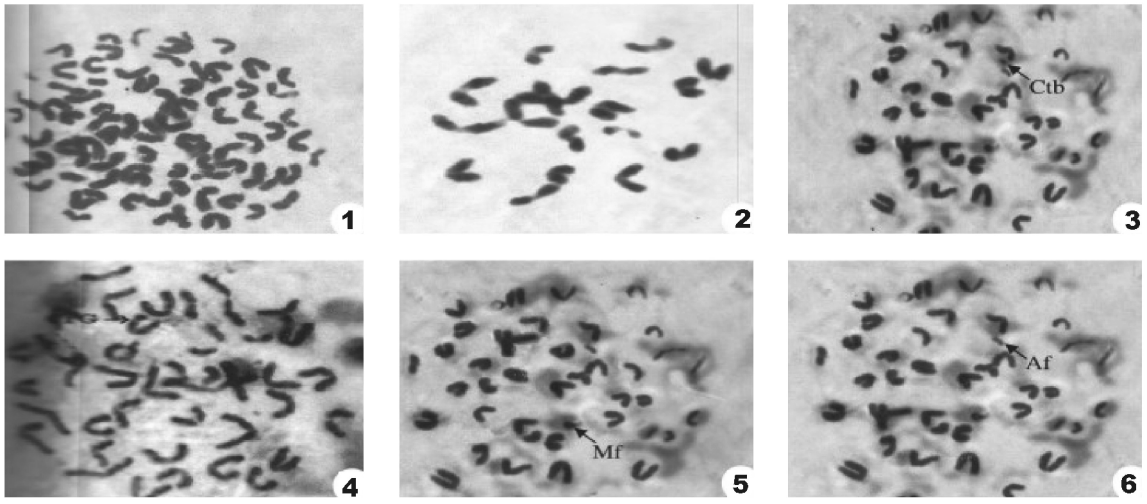
DISCUSSION

Distilleries are among the most polluting industries because of ethanol fermentation resulting in the discharge of large quantities of high-strength liquid effluents with high concentrations of organic matter and nitrogen compounds, low pH, high temperature, dark brown colour, and high salinity. It shows that the effect of effluent on mice is dose-dependent. The induction of gross and individual types of abnormalities revealed that these effluents might cause the damage at two different levels, first

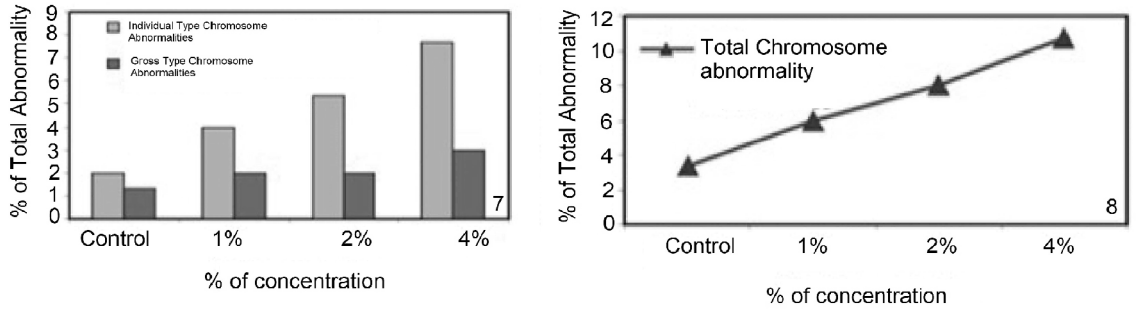
TABLE 1: Frequency of chromosomal abnormalities in distillery effluent treated bone marrow cells in mice.

Effluent	Abnormal metaphase		Individual abnormalities		Gross abnormalities		Total abnormalities	
	No.	(%) S.E.	No.	(%) S.E.	No.	(%) S.E.	No.	(%) S.E.
Control	10	3.33 ± 1.03	6	2.00 ± 0.80	4	1.33 ± 0.66	10	3.33 ± 1.03
1%	14	4.66 ± 1.21	12	4.00 ± 1.13	6	2.00 ± 0.80	18	6.00 ± 1.37
2%	19	6.33 ± 1.40	16	5.33 ± 1.29*	8	2.00 ± 0.92	24	8.00 ± 1.56*
4%	24	8.00 ± 1.56	23	7.66 ± 1.53*	9	3.00 ± 0.98	32	10.66 ± 1.78*

* Indicates significant difference at 5% level with corresponding values in the control.



Figs 1–6: Metaphases showing chromosomal abnormalitiesin mice. 1. Polyploidy 2. Hypoploidy. 3. Chromatid break (arrow). 4. Chromatid gap. 5. Arrow points to minute fragment. 6. Arrow indicates acentric fragment.



Figs 7 & 8: 7. Comparison of individual and gross types of chromosomal abnormalities in mice. 8. Dose-depending effect of distillery effluent on chromosomes.

interfere with the functioning or assemblage at the spindle apparatus leading to gross type of changes. Previously, Chaurasia & Sinha (1988, 1990) and Chaurasia et al. (2005) studied genotoxicity induced by fertilizer and silk dyeing wastes and Kumar & Sinha (1989) studied dose-dependent genotoxic effect of synthetic pesticides. They observed that the individual type of damages was more frequent than the gross type. Bose & Sinha (1994), Kumari & Sinha (1994) and Awasthy et al. (2000) found the bio-mutagens induced more gross type of abnormalities than individual types. Production of electrophilic ions and reactive radicals during the metabolism of effluents might be interacting with the nucleophilic sites in DNA leading to breaks and other related damages in the latter (Klopman et al. 1985).

The present study revealed that the individual type of damage was more prominent than the gross type. This might be due to the formation of electrophilic radicals/ions during the metabolization of mutagens. Effluents probably cause increased formation of base analogue to act as a mutagen that induces chromosomal abnormalities.

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