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Research Article

ANTIGENOTOXIC POTENTIAL OF TRIFLA AGAINST POTASSIUM DICHROMATE

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Abstract

Aim of this study is to know antigenotoxic potential of *Trifla* (Triphala), an ancient Indian herbal formulation consisting of powdered dry fruit of *Terminalia chebula*, *Terminalia bellerica* and *Embelica officinalis* as 1:1:1 wt/ wt, against potassium dichromate in *Tilapia mossambica* fish model. Twenty fish in each group were exposed to tap water alone (Gr I)or containing *Trifla* in it at 100ppm (Gr II) or potassium dichromate in it at 10 ppm (Gr III) or containing both i.e. *Trifla* plus potassium dichromate at 100ppm and 10 ppm respectively Gr IV. Fish of group V were initially exposed to *Trifla* for fifteen days at 100ppm and then exposed to potassium dichromate for next 15days. Fish of group V were sacrificed on thirty first day while fish of remaining groups were sacrificed on sixteenth day. Behavior of fish was recorded and gill blood smear were made for micronuclei test.

Fish remained unaffected in tap water alone and with *trifla* but potassium dichromate altered their behavior and induced micronuclei formation in their red blood cells. Fish exposed to both *trifla* & potassium dichromates are found protected but fish pretreated with *trifla* are not protected. This observation suggested that toxicity of potassium dichromate is declined in the presence of *trifla* means some interaction might have taken place between in them. To confirm this possibility laser raman spectra of tap water containing *trifla* alone, potassium dichromate alone and their mixture were analyzed. Altered spectrum of mixture (trifla plus potassium dichromate) indicates that *trifla* has probably reduced potassium dichromate in the medium. Findings suggested that *trifla* can be tested for use in phytoremediation of environment toxicants which act via oxidative stress.

Keywords: *Trifla*, Potassium dichromate, Micronuclei, Phytoremediation, Raman spectra, Fish.

1. Introduction

Trifla (Triphala) is an ancient Indian antioxidant herbal formulation which is known to act both *in-vitro* and *in-vivo* (1,2,3). On the other hand chromium, a heavy metal pollutant is toxic, carcinogenic and mutagenic which acts

mainly via oxidative stress (4). Many plants have been tested for phytoremediation of chromium from soils (5,6) and waste waters (7). Some medicinal plants have also been tested for accumulation of heavy metals (8). Potassium dichromate induced genotoxicity is on record in fish as evident by micronuclei formulation in red blood cells (9). In the present study an attempt is made to test antioxidant potential of *trifla* against potassium dichromate, a known oxidant in fish (10) using micronucleus test which is a sensitive parameter of fish genotoxicity (11).

2. Materials and methods

Fish: Healthy fish *Tilapia mossambica* (7-8 cm long) were obtained from fisheries pond of government fisheries department Ujjain. Fish were acclimatized for fortnight before experimentation. They were fed on powdered dried shrimps once daily.

Medium of exposure: Ordinary tap water was used whose physicochemical analysis was done according to APHA (12) procedures.

Genotoxic agent: Potassium dichromate ($K_2Cr_2O_7$ LR grade) made by Central Drug House Pvt. Ltd India was used.

Test Herbal Compound:

Ready to use *trifla* was procured from local herbal shop. Its composition was powdered dried fruits of three medicinal plants *Terminalia chebula, Emblica officinalis* and *Terminalia bellerica* (1:1:1 wt/wt).

Stain: Giemsa made by SD Fine chemicals, Mumbai is used.

Experimental Design:

Five glass aquaria were filled with 20 liters of tap water. Each contained one group of fish. Twenty fishes were kept in tap water to serve as control (Gr I). Twenty fishes were exposed to tap water containing *trifla* in it at 100 ppm for 15 days (Gr II). Twenty fishes were exposed to tap water containing potassium dichromate at 10 ppm for 15 days (Gr III). Twenty fishes were simultaneously exposed to both i.e. tap water containing both i.e. *trifla* at 100 ppm and potassium dichromate at 10 ppm for 15 days (Gr IV). One group of twenty fishes was initially exposed to *trifla* (100 ppm) for 15 day and then exposed to potassium dichromate for next 15 days (Gr V).

2.1 Sacrificing Fishes: Fish of group I, II, III & IV were sacrificed on 16^{th} day while fish of group V were sacrificed on 31^{st} day. Experiments were done thrice.

2.2 Cytological Preparation: Fish were briefly exposed to ice in polythene to immobilize them for handling. One smear was made from a drop of blood obtained by cutting one side of gill i.e. two slides were made per fish. Air dried slides for 24 hr were fixed for thirty seconds in absolute alcohol and were stained with diluted (1:20) Giemsa

stain, washed in deionized water and dried for observation under immersion oil at 1500 X.. Percentage abnormal nuclei were scored & statistically analyzed by 't'test at 5 % level of significance.

2.3 Laser Raman Spectra: Three clear i.e. filtered samples of tap water were sent to Indian Institute of Technology, one sample had only *trifla* in it, second had potassium dichromate in it and third had both i.e. *trifla* and potassium dichromate in it. Data CD provided by IIT Mumbai was processed using origin software for graphs.

3. Results and Discussion

Tap water used in the present experiments revealed usual characterstics (Table1). Behavior of fish was altered when exposed to potassium dichromate(PD) alone (Gr III) but it could be appreciably checked in the presence of *trifla* (Gr IV) but its pretreatment (Gr V) did not work. Fish were comfortable (on the basis of behavior) in controls and *trifla* exposed (Table 2) (Gr I&Gr II). Cytological observations are shown in table 3 and plate I. Tap water and *trifla* did not induce any cytological effect in the blood cells of fish. This finding gets support from a recent study which also revealed lack of cytogenotoxicity of *trifla* in *Allium cepa* model (13). Potassium dichromate induced micronuclei formation and other nuclear deformities which is an expected finding (9). *Trifla* could reduce this effect only when used with potassium dichromate (Gr IV), however, prior exposure to *trifla* could not check this genotoxic effect. These observations suggest that *trifla* did not act *in-vivo* but it has reacted with potassium dichromate in the medium and has reduced its toxicity. Analysis of Laser Raman Spectra (Graph 1) indicates that *trifla* probably reduced PD thereby lowering its toxicity. *In- vitro* antioxidant potential of *trifla* has already been reported in standard protocols (14,3). Binding affinity of the fruit of *T. chebula* with chromium is also on record (15). Results suggest that *trifla* can be tested as antigenotoxicant for phytoremediation against other environmental toxicants too.

Table-1: Physicochemical properties of tap water (Aug-Dec 2009).

S.No.	Properties	Value
01	Turbidity	90NTU
02	pH value	7.2
03	Color	Colorless
04	Total Alkalinity	90mg/l
05	Carbonates	8mg/l
06	Bicarbonates	140 mg/l
07	Hardness	162 mg/l
08	Chloride	52 mg/l
09	BOD(5days at 20^{o}C)	5 mg/l

10	COD	9 mg/l
11	Fluoride	0.49 mg/l
12	Nitrite	Nil
13	Dissolved Oxygen	5.2 mg/l
14	Calcium	120 mg/l

Table-2: Behavior of Fishes.

Groups	**Attraction towards food A**	**Swimming B**	**Equilibrium C**	**Opercular activity D**	**Response towards external stimulus E**
I Control in tap water	Quick	Normal	Normal	45/min.	Quick
II *Triphala*(100ppm)exposed	Little Late	Normal	Normal	44/min.	Quick
III Potassium Dichromate(10ppm) exposed	Late	Fast, Erratic	Loss, which Progressively increased	High 58/min.	Dull
IV *Triphala*(100ppm)plus Potassium Dichromate(10ppm) exposed	Little Late	Normal	Normal	Normal 43/min.	Quick
V *Triphala*(100ppm)pre exposed then exposed to potassium dichromate(10ppm) for next 15 days	Late	Initially normal but later on fast & erratic	Loss, which Progressively increased	High 56/min.	Dull

In column A, quick=within 01minute, Little late=after 05minutes, Late=after 20 minutes. In column E, quick =within 02seconds, Dull=within 10 seconds

Table-3: Nuclear abnormalities (5000 cells/ group, mean ±SEM,n=10).

Groups.	**Treatments**	**Percentage of normal nuclei**	**Percentage of Total abnormal nuclei (MNC,BNC,DN,DI,BbN)**
I	**Control exposed to tap water**	**100%**	**0.00%**
II	**Exposed to *Triphala* in tap water at 100 ppm**	**100%**	**0.00%**
III	**Exposed to Potassium Dichromate at 10ppm in tap water**	**80.87 +_ 2.10[a] %**	**19.13+_0.87[a] %**
IV	**Potassium Dichromate (10 ppm)plus *Triphala* (100ppm) in tap water**	**91.71+_1.76[a,b] %**	**8.29+_0.76 [ab] %**
V	***Triphala* pretreated, then exposed to potassium dichromate**	**80.36+_1.36 [a] %**	**19.55+_0.89[a] %**

MNC=micronuclei; BNC=binucleate; DN=damaged nuclei, DI=disintegrating nuclei; BbN=blebed nuclei. a=significant (p<2.09) when all groups compared with controls, and b=significant when group III compared with Gr IV or V.

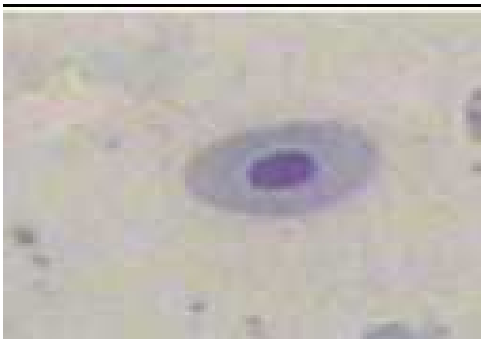


Fig.-1: Control showing normal nucleus of erythrocyte.

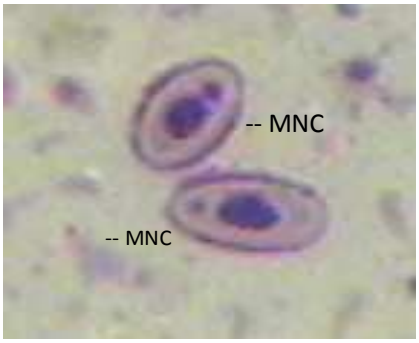


Fig.-2: Showing micronuclei (MNC).

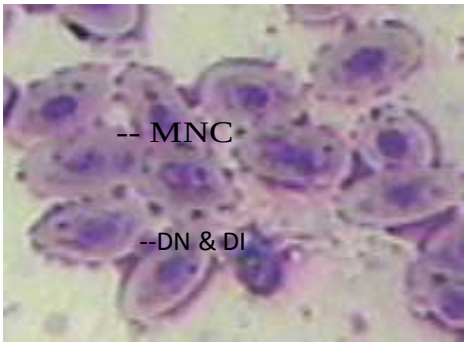


Fig.-3: Showing micronuclei (MNC), damaged) & disintegrating nuclei (DN –DI)

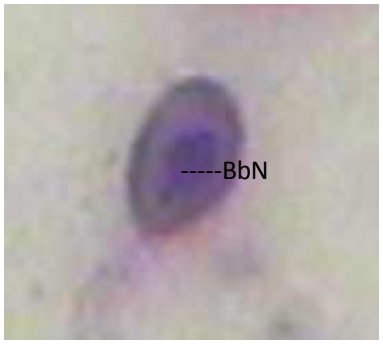


Fig.-4: Showing blebed nucleus (BbN).

PLATE - I

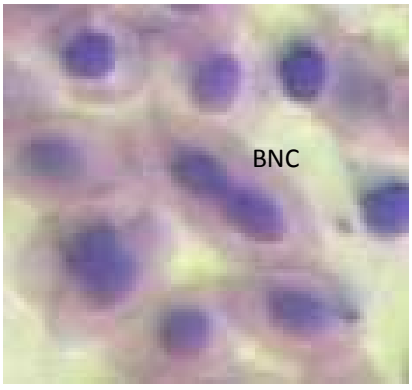
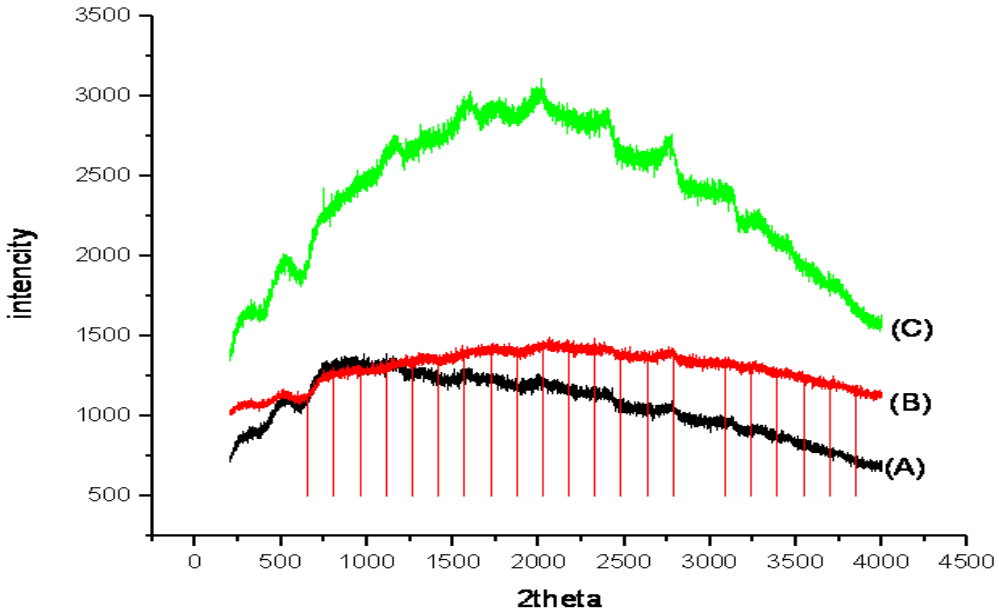


Fig.-5: Showing binucleate nucleus (BNC)

Explanation of Figures (1500X)

* Normal nuclei like Fig.-1 are seen in control & triphala treated groups (I & II).

* Micronuclei (Fig.-2), damaged & disintegrating nuclei(Fig.-3),blebled nucleus (Fig.-4) & binucleated (Fig.-5) condition are seen only after potassium dichromate exposure group (III). Frequency of these aberrations was significantly reduced in the presence of triphala in group (IV) but not in gr V.



Graph-1: Raman spectra of Potassium dichromate (A), Triphala (C) and Triphala potassium plus dichromate (B) in the range 0-5000 cm^{-1} . Strong band appeared in the spectrum of triphla in the range 1000- 2000 cm^{-1} can be assigned to benzene ring modes. Bands around 3000cm^{-1} can be assigned to C-H stretching modes of benzene ring. Bands appeared around 35cm-1 can be assigned to –OH stretching modes. Change in the band positions and intensity of these bands suggests oxidation of triphala with dichromate. These observations suggest the probable reduction of dichromate and oxidation of triphala in the reacting mixture thus considerably reducing the toxic nature of potassium dichromate.

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