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Der Pharma Chemica, 2011, 3 (4): 373-376
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Evaluation of *in vivo* antimutagenic potential of fruits extracts of *Withania coagulans*

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ABSTRACT

Medicinal plants play an important role in development of drugs. The present study is based upon the Anti-mutagenic activity of Withania coagulans, a traditionally known herb. The Anti-mutagenic activity of Withania coagulans fruit extracts was investigated on cyclophosphamide induced micronucleus formation in mouse bone marrow cells. The results confirmed that a single i.p administration of W. coagulans fruit extract at the dose of 500, 1000 and 1500 mg/kg body weight prior to 24 hours have significantly prevented the micronucleus formation in dose dependent manner in bone marrow cells of mice as compared to cyclophosphamide group.

Keywords: *Withania coagulans*, micronucleus, Bone marrow, Polychromatic Erythrocytes (P.C.E).

INTRODUCTION

Micronuclei are cytoplasmic chromatin-containing bodies that appears in the cell like a small satellite nucleus around the cell nucleus, due to chromosome fragments or entire chromosomes that are not incorporated in the main nucleus after cell division. The presence of micronuclei (MN) in cells is considered as a biomarker of damage to the DNA. The micronucleus test is an *in vivo* and *in vitro* short-time screening cytogenetic test [1, 2] is a widely used method for assessing genotoxicity of chemicals in organisms [3]. Withania is a small genus of shrubs, which are distributed in the East of the Mediterranean region and extend to South Asia. The berries of the shrub are used for milk coagulation. It is popularly known as Indian cheese maker. In Punjab, the fruits of *W. coagulans* are used as the source of coagulating enzyme for clotting the milk which is called 'paneer'. They are also used in dyspepsia, flatulent colic and other intestinal infections. In some parts of Pak-Indian sub-continent, the berries are used as a blood purifier. The twigs are chewed for cleaning of teeth and the smoke of the plant is inhaled for relief in toothache [4, 5]. *Withania coagulans* (Stocks) Dunal is used to treat nervous exhaustion,

disability, insomnia, wasting diseases, failure to thrive in children, impotence. Its fruits are used for liver complaints, asthma and biliousness. *W. coagulans* (Stocks) Dunal is used to treat nervous exhaustion, disability, insomnia, wasting diseases, failure to thrive in children, impotence. Its fruits are used for liver complaints, asthma and biliousness. Flowers of coagulans (Stocks) Dunal are used in the treatment of diabetes [6]. The root is harvested in autumn and dried for later use [7]. Some caution is advised in the use of these plants since it is toxic [8]. Antimicrobial, anti- inflammatory, antiumor, hepatoprotective, anti-hyperglycemic, cardiovascular, immuno-suppressive, free radical scavenging and central nervous system depressant activities of the plant have been reported [9]. The present study was thus done to investigate the anti-mutagenic activity of fruit extracts of *W. coagulans*.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals and reagents used were of Analytical Grade and were procured from Ranchem and CDH, India. The cyclophosphamide drug was purchased from Sigma chemical Co. U.S.A.

Animals used for the study

The study was conducted on random bred, 6-7 weeks old and 24- 28 gm body weight bead ring, male Swiss albino mice (*Mus musculus*). Animals were maintained under controlled conditions of temperature and light (Light: dark; 10 h: 14 h). They were provided standard mice feed (procured from Hindustan Levers Ltd. India) and water *ad libitum*. The study protocol is approved by the Departmental Animal Ethical Committee) (Project no:500/01/a/2001/Proj.5/27-07-09) and confirms to the guidelines set by World Health Organization, Geneva, Switzerland and Indian National Science Academy (INSA), New Delhi India.

Preparation of extracts of fruits of *W. coagulans*

The identification of fruit *Withania coagulans* (Family-Solanaceae) was done by Dr. S.S Khan, Botanist (Safia College, Bhopal) under voucher specimen no. W.C/0120/2009. The fruits were shade dried and crushed in the grinder to form powder. The powder was then after allowed to extraction with 100 % methanol in separating funnel by refluxing for 36 h at 50-60º C. Pellets of the drug were obtained and the required dose for treatment was prepared by dissolving the pellets in double distilled water at different doses.

Micronucleus Assay

For the micronucleus assay, the extract at the volume of 0.2 ml at different doses level such as 500, 1000 and 1500 mg/kg body weight was injected 24 hours before the treatment of cyclophosphamide to 6 male Swiss albino mice present in each of the three groups. Another group was of the positive control which received single i.p. injection of 50 mg/kg cyclophosphamide in 0.9 % saline. The animals were sacrificed by cervical dislocation and bone marrow cells were harvested. The slides were prepared essentially as described [2]. A total of 1000 cells were scored at the magnification of 1000 x (100 x 10 x) for each group. The results were expressed as the average number of erythrocytes cells (PCE) cells/animals (±SE) for a group of and modified [10]. The results were compared with the vehicle control group using Student's test with significance determined at $p<0.05$.

RESULTS

Table-1: Showing the Anti-mutagenic effect of *W. coagulans* fruit (Methanolic) extract on cyclophosphamide induced mutagenicity

S.No	Group	Treatment	MNPCE mean ±SEM	PCE/NCE mean±SEM	Suppression % of CP induced MN formation
1	Group I	Cyclophosphamide alone (50mg/kg)	2.0 ± 0.04	0.69±0.01	-
2	Group II	Plant extract alone (500mg/kg)	0.6 ± 0.02	0.7±0.03	30
3	Group III	Plant extract (500mg/kg)+ Cyclophosphamide (50mg/kg)	1.0* ± 0.03	0.5±0.08	50
4	Group IV	Plant extract 1000mg/kg)+ Cyclophosphamide (50mg/kg)	0.8 ± 0.04	0.7±0.02	40
5	Group V	Plant extract (15000mg/kg)+ Cyclophosphamide (50mg/kg)	0.5 ± 0.02	0.8*±0.01	25
6	Group VI	Vehicle alone	0.12 ± 0.04	0.43±0.08	6.0

** denotes statistical significance at <0.05 in t' test*

MMNMBN

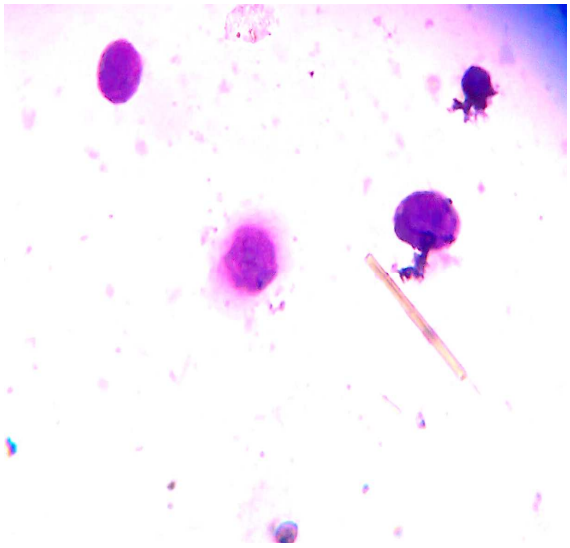

Figure-1: Micronuclei in Bone marrow

The results suggested that the fruit extracts of the plants in dose-dependent manner showed anti-mutagenic behavior when compared to standard drug, cyclophosphamide. It has been observed that with the increase in concentration of plant extract viz. 500, 1000 and 1500 mg/kg, the formation of micronucleus in bone marrow cells gets decreased thus showing the protective effect of *Withania coagulans* fruit extract (**Table 1**). It was observed that cyclophosphamide when given at a single dose of 50 mg/kg caused higher incidence of micronucleus formation in *Swiss albino* mice bone marrow cells (**Figure 1**). The dose of cyclophosphamide (50 mg/kg) caused bone marrow toxicity as evidenced by the decrease in PCE/NCE ratio [2]. For cyclophosphamide treated groups the frequency of MNPCE was 2.0 ±0.04 which was significantly higher ('t' test $p<0.05$) as compared with the experimental groups (treated with plant extracts).

DISCUSSION

The present study revealed the anti-mutagenic potential of *W. coagulans* extract in dose dependent manner. The genotoxic nature of any drug can also be determined on the basis of presence of phytoconstituents. *W. coagulans* posses withanolide which is responsible for anti-tumor activity [11, 12], flavonoids which have been shown to possess anti-mutagenic and anti-carcinogenic activity [13, 14] and lectins reported to produce structural variation of the cell envelope. The underlying mechanism behind anti-mutagenic action of *W. coagulans* is still unknown.

CONCLUSION

The present findings thus suggest that extracts of fruits of *Withania coagulans* can be utilized to formulate anti-mutagenic drugs and can replace many anticancer drugs which show mutagenic responses within the body. Further studies are however needed to isolate and characterize the active principles responsible for anti-mutagenic activity. Several steps may be implemented to protect such endangered plant species by tissue culture and germplasm storage. Further studies on this herb will provide more conclusions about its anti-genotoxic and anti-cancerous nature.

Acknowledgement
The authors are thankful to research staff of Jawaharlal Nehru Cancer Hospital & Research Centre, Bhopal (M.P), India for their contribution in the piece of study.

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