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GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: <https://www.gsconlinepress.com/journals/gscbps>



(RESEARCH ARTICLE)



Dietary protection by *Tapinanthus globiferus* (Mistletoe) leaf extract against prontosil-induced clastogenicity in mice *in vivo*

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Publication history: Received on 15 March 2020; revised on 25 March 2020; accepted on 28 March 20

Article DOI: <https://doi.org/10.30574/gscbps.2020.10.3.0067>

Abstract

Mistletoe (*Tapinanthus globiferus*) as freshly prepared leaf extract was administered at variable concentration (25, 50 and 100 mg/kg) per body weight as dietary supplement to laboratory albino mice via oral gavage for two weeks consecutively. The mice were also simultaneously administered 1.5 mg/kg pesticide precursor (Prontosil). 24 hours after the last day, the mice were sacrificed and chromosome preparations were made from the bone marrow cells. The end-points studied were polychromatic erythrocytes and damage cells. The results indicated that the clastogenicity in the mice fed with *Tapinanthus globiferus* leaf extract reduced significantly ($P < 0.05$) compared to those fed with prontosil only. The marked reduction of clastogenicity by the extract may be attributed to the interaction with polyphenolic and phytochemical components of the extract.

Keywords: *Tapinanthus globiferus*; Polychromatic erythrocytes; Prontosil; Clastogenicity.

1. Introduction

Dietary intervention has been found to be an effective means of reducing cytotoxic effect of chronic exposure to subtoxic doses of heavy metals and toxic allied chemicals mostly used on farmland to control pests [1]. Besides, National Toxicology Program (NTP) has reported the high propensity of chemical's ability to induce chromosomal damage because it has been shown that most, if not all cancers is characterized by chromosomal changes [2]. Large amount of these chemicals are clastogens and were tested on their ability to cause damage with newly developed short and long term tests which accounts for mutagenicity, clastogenicity and carcinogenicity [3]. Myriad of studies on phytomedicines have been reported that phenolic compounds protect against oxidative stress [4]. Some of these medicinal plants have been investigated for their antioxidative properties and used for treatment of various diseases [5].

Most of the bioactive metabolites from these plants especially flavonoids demonstrated potent antioxidant activity *in vitro* and *in vivo* [6]. Many synthetic antioxidants and metal chelator components have also exhibited toxic or mutagenic effect coupled with suppression of body immunity which has shifted attention towards naturally occurring antioxidants [7]. Just like commonly employed medicinal plants, *Tapinanthus globiferus* is grown specifically for the essential oil in its leaves and stems where thymol, eugenol, citral, geraniol and linalool have been extracted [8, 9].

This study is sought to investigate the anticlastogenic potential of *Tapinanthus globiferus* leaf extract against genotoxic effect of prontosil commonly used to control pests on farmland.

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2. Material and methods

2.1. Extract Preparation

50 g of powdered *Tapinanthus globiferus* was extracted with 500 ml distilled water via maceration for 48 hours using method described by [10, 11]. The mixture was decanted and filtered using sterile Whatman paper No 1. The filtrate measured up 425 ml and evaporated to dryness with a freeze dryer to obtain 8.5% yield.

2.2. Experimental Animals

The *in vivo* experiment was conducted with 36 laboratory bred Swiss albino mice with average weight of 30 g, housed in stainless cages with temperature maintained at 25 ± 2 °C and 12 hours alternating day/night cycle in accordance with NIH guidelines No 423 (2001) for the care and use of laboratory animals.

2.3. Experimental Protocol

Animals were divided into four groups A, B, C and D with five mice in each group; mice in group A serve as control and were treated with distilled water only. Group B received 1.5 mg/kg prontosil while group C mice were simultaneously fed with 25, 50 and 100 mg/kg extract concentrations and 1.5 mg/kg prontosil at ratio 1:1. Mice in group D were administered the 25, 50 and 100 mg/kg extract doses only. The schedule of animal treatment was divided into three sets where Set I fed with 25 mg/kg, Set II fed with 50 mg/kg and Set III fed with 100 mg/kg extract doses respectively. The pesticide concentration was made equivalent to one - tenth of the LD₅₀ while highest extract dose corresponds to exact concentration used for treating specific diseases.

2.4. Bone Marrow Chromosome Preparation

Animals were sacrificed 24 hours after the last treatment, 1 hour prior to sacrifice, each animal was injected with 0.04% colchicine (1 ml/100 g b.w Sigma, U.S.A). Femurs of the mice were removed and bone marrow cells were flushed out in 75 mM KCl-hypotonic solution, incubated for 20 minutes at 37 °C and fixed in methanol-glacial acetic acid (3:1). Chromosome preparations were made following the standard procedure of air-drying and then stained in 7% Giemsa solution [12]. The slides were then coded, mounted on microscope coupled with chromosome tally counter and scored blind.

2.5. Statistical Analysis

The data from the groups were pooled and analyzed statistically using one-way analysis of variance [13]. This was followed by Duncan's multiple range tests in order to compare significance of difference among different experimental animals.

3. Results

3.1. Set I

Table 1 Total polychromatic erythrocytes [PCEs] following treatment with 25 mg/ml *Tapinanthus globiferus* leaf extract [T.G] and 0.5 mg/kg prontosil

Group	Treatment (mg/kg)	PCEs (%)	Mean±S.D
A	Distilled water only	0	0.01±0.03
B	1.5 mg/kg prontosil only	25	0.17±0.10
C	1.5 mg/kg prontosil + 25 mg/kg T.G extract	6	0.03±0.21
D	25 mg/kg T.G extract only	1	0.01±0.11

3.2. Set II

Table 2 Total polychromatic erythrocytes [PCEs] following treatment with 50mg/ml *Tapinanthus globiferus* leaf extract [T.G] and 0.5 mg/kg prontosil

Group	Treatment (mg/kg)	PCEs (%)	Mean±S.D
A	Distilled water only	0	0.01±0.03
B	1.5 mg/kg prontosil only	24	0.17±0.12
C	1.5 mg/kg prontosil + 50 mg/kg T.G extract	10	0.13±0.31
D	50 mg/kg T.G extract only	2	0.01±0.11

3.3. Set III

Table 3 Total polychromatic erythrocytes [PCEs] following treatment with 100 mg/ml *Tapinanthus globiferus* leaf extract [T.G] and 0.5 mg/kg prontosil

Group	Treatment (mg/kg)	PCEs (%)	Mean±S.D
A	Distilled water only	0	0.01±0.03
B	1.5 mg/kg prontosil only	25	0.17±0.10
C	1.5 mg/kg prontosil + 100 mg/kg T.G extract	12	0.15±0.19
D	100 mg/kg T.G extract only	1	0.01±0.11

4. Discussion

Results from the three sets of experiment (Tables 1, 2 and 3) show the frequency of polychromatic erythrocytes which is an indication of chromosomal aberration induced in bone marrow cells of the mice following oral administration of the pesticides and the plant extract. Comparatively, the frequency of polychromatic erythrocytes in groups A, C and D was lower than group B animals fed with the clastogen (prontosil) alone. This further justifies the clastogenic potential of the pesticides as being capable to induce chromosomal damage in mammalian cells [2]. The frequency of polychromatic erythrocytes from damage cells was significantly ($P < 0.05$) reduced in mice administered with the pesticide and the extract together. This is similar to the effect observed in mice fed with garlic extract and sodium arsenite simultaneously for 30-days [14]. However, the frequency of polychromatic erythrocytes in group D animals fed with the extract only was insignificant when compared to the control animals. This suggests that *Tapinanthus globiferus* is not genotoxic to mammalian cells *in vivo*. The significant reduction of the clastogenic effects of the pesticides by crude *Tapinanthus globiferus* leaf extract in group C animals in this study could be attributed to the interaction of the inherent phenolic and phytochemicals of the extract with the chemical pesticides, thereby, exhibiting their anticlastogenic potential.

5. Conclusion

The anticlastogenic potential of *Tapinanthus globiferus* leaf extract against genotoxic effect of prontosil commonly used to control pests on farmland has been successful investigated in the present work. Hence, the anticlastogenic effects demonstrated by this extract in this study suggests that *Tapinanthus globiferus* (mistletoe) plant is a viable protective dietary supplement against chemical toxicants capable of inducing chromosome aberrations and other related genetic birth defects.

Compliance with ethical standards

Acknowledgments

The authors appreciate everyone who contributed to success of this work.

Disclosure of conflict of interest

There is no conflict of interest with publishing the present data of the study.

Statement of ethical approval

The approval for the use of mice for the present work was obtained from appropriate channel.

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How to cite this article

Tugbobo OS, Abegunde SM, Awonegan PA, Akinyede KA and Oyewusi HA. (2020). Dietary protection by *Tapinanthus globiferus* (Mistletoe) leaf extract against prontosil-induced clastogenicity in mice *in vivo*. *GSC Biological and Pharmaceutical Sciences*, 10(3), 160-163.
