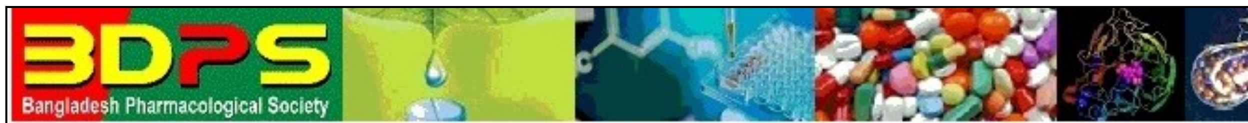

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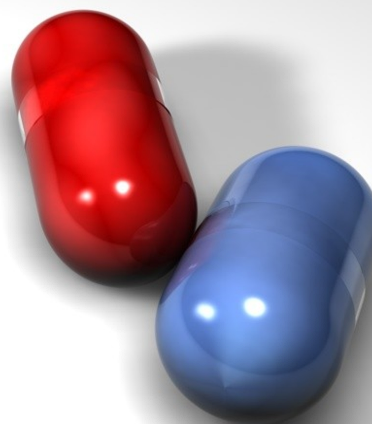

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Letter to the Editor

In vitro antimutagenic activity of *Ipomoea staphylina*

Sir,

The study of antimutagenic activity gives information which discovers the possibilities for combating the genotoxic risk of mutagens. Genotoxicity includes DNA damage, gene mutation, chromosomal aberration, and formation of micronucleus (Swift, Golsteyn, 2014). Genotoxicity commonly occurs with anti-cancer drugs which causes a high level of DNA damage that activates cell cycle checkpoints leading to cell cycle arrest or cell death (Helleday et al., 2008). Several studies on medicinal plants have revealed antimutagenic and antigenotoxic properties mainly due to antioxidants that scavenge reactive oxygen species (ROS) (Zani et al., 1993; González-Avila et al., 2003; Park et al., 2004).

Ipomoea staphylina leaves extract possesses mainly anti-inflammatory (Firdous and Koneri, 2013), antiulcer (Banerjee and Firdous, 2015), anti-diabetic (Firdous and Singh, 2016), hepatoprotective, nephroprotective (Bag and Mumtaz, 2013), cytotoxic (Padmashree et al., 2018a) and antibacterial (Padmashree et al., 2018b) activities. There is no report on the antimutagenic activity of this plant. Hence, an attempt was taken to evaluate the antimutagenic property of the hydroalcoholic extract of *I. staphylina* leaves.

The leaves were collected from the forest area in Karnataka and taxonomically authenticated by Dr. K Karthigeyan of Central National Herbarium, Botanical Garden, Howrah. A voucher specimen was conserved with a reference No. SMF-01.

The dried leaves were powdered and defatted with petroleum ether (bp 60-80°C) for 72 hours and then extracted with a mixture of ethanol : distilled water (7:3) to get a yield of 11.6% w/w. The dried extract was then stored at 4°C.

Ames test was performed using two nonvirulent strains of *Salmonella typhi* viz., TA 1535 and TA 1538 (Maron and Ames, 1983). Sodium azide and 2-nitrofluorene were used as standard mutagens at a concentration of 50 µg/mL. To determine the mutagenic effect of *I. staphylina* extract, 5 mg of the extract per plate was given along with both strains of *S. Typhi* differently. Besides, three different concentrations of the extract viz., 5, 10, and 20 mg/plate along with standard mutagen were exposed to both strains of *S. typhi* differently. After 48 hours of incubation number of bacterial colonies was counted. The mutagenicity of sodium azide and 2-nitrofluorene in absence of the extract was by the formula given below;

$$\%Inhibition = [1-T/M]\times 100$$

where T = number of revertants per plate in the presence of mutagen and extract and M = number of revertants per plate in the presence of mutagen only

Allium cepa chromosomal aberration test (Ping et al., 2012) was carried out using onions (20-25 g) which were grown in reverse osmosis water for 3-4 days until the length of the roots reaches up to 1-1.5 cm. Then onions were exposed in different concentrations (250 and 500 µg/mL) of extract and cyclophosphamide (50 µg/mL) which was considered as a positive control. To determine the antimutagenic property of extract, a few onions were exposed to cyclophosphamide (50 µg/mL) in the presence of extract (250 and 500 µg/mL). After 24 hours (at least one mitotic cycle) of chemical exposure roots of deferent onions were collected in glass vials containing fixative solution [methanol : glacial acetic acid (3:1)] and stored overnight at 4°C. On the next day, roots were taken into clear glass vials containing a mixture of 2% aceto-orcein and 1N HCl in a ratio of 5:1 and heated gently for 2-5 sec and kept for 1-1.5 hours. Then, a small drop of 45% acetic acid was added in a clear microscopic glass slide and root tip (about 1-2 mm) was placed onto the acetic acid and left for few sec for washing the excess stain. A coverslip was mounted on it in such a way that no air bubble was formed and the root tip was squashed a little with a gentle tap of thumb or a match stick. At last the open interface of coverslip and slide was sealed with dibutylphthalate polystyrene xylene to prevent the drying of cells. Three slides were made and analyzed for each treatment group for the determination of mitotic index and various chromosomal abnormalities. In mitotic index about 1,000 cells were counted and the mitotic index was calculated as given below;

$$[(\text{number of dividing cells}/\text{total number of cells}) \times 100]$$

The Ames test was performed without metabolic activation (addition of S9 mix) and the numbers of revertant colonies per plate were counted (Table I). In this study, sodium azide and 2-nitrofluorene (50 µg/plate) were been able to cause gene mutation in

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Table I

Effect of *I. staphylina* on TA 1535 and TA 1538 along with mutagens

Treatment	Treatment	Number of colonies (mean ± SD)	%Inhibition
TA 1535 control		27.7 ± 1.0	-
TA 1535 (sodium azide 5 µg/plate)		776.3 ± 13.9	-
TA 1535 (HEIS 5 mg/plate)		26.3 ± 0.9	-
TA 1535 (sodium azide 5 µg/plate)	Extract (5 mg/plate)	803.3 ± 12.5	-3.5
TA 1535 (sodium azide 5 µg/plate)	Extract (10 mg/plate)	788.7 ± 16.4	-1.6
TA 1535 (sodium azide 5 µg/plate)	Extract (20 mg/plate)	792.7 ± 10.2	-2.1
TA 1538 control		10.3 ± 0.7	-
TA 1538 (2-nitrofluorene 5 µg/plate)		461.0 ± 7.4	-
TA 1538	Extract (5 mg/plate)	12.3 ± 0.5	-
TA 1538 (2-nitrofluorene 5 µg/plate)	Extract (5 mg/plate)	298.7 ± 11.5	35.2
TA 1538 (2-nitrofluorene 5 µg/plate)	Extract (10 mg/plate)	180.5 ± 8.7	60.8
TA 1538 (2-nitrofluorene 5 µg/plate)	Extract (20 mg/plate)	93.3 ± 4.6	79.8

Data are mean ± SD; Experiment was performed in triplicate

Table II

Effect of *I. staphylina* on mitotic index in presence of mutagen in onion

Treatment	Dividing cells (mean)				Non-dividing cells (mean)	Mitotic index (mean ± SD)
	Prophase	Metaphase	Anaphase	Telophase	Interphase	
Water (control)	35	13	7	20	925	8.1 ± 1.0
Extract (250 µg/mL)	30	13	6	21	930	7.5 ± 0.8
Extract (500 µg/mL)	16	6	2	7	969	3.2 ± 0.4
Cyclophosphamide (50 µg/mL)	7	3	2	2	986	1.4 ± 0.3
Cyclophosphamide (50 µg/mL) plus extract (250 µg/mL)	10	5	3	3	979	2.1 ± 0.5
Cyclophosphamide (50 µg/mL) plus extract (500 µg/mL)	8	4	2	2	984	1.6 ± 0.3

Experiment was performed in triplicate

histidine operon of *Salmonella* strains TA 1535, and TA 1538 respectively, that was confirmed by colony counter. The number of colonies in the extract (5 mg/plate) treated group was almost same to that of control groups of both the strains. From this study, it was clear that extract does not possess any mutagenic nature. When extract was given along with mutagen (sodium azide), the decrease in the number of colonies was not found in TA 1535. But extract in TA 1538 was found effective to reduce the number of revertant colonies in the presence of 2-nitrofluorene. The extract produced 79.8% protection at the dose of 20 mg per plate. Thus, *I. staphylina* possesses antimutagenic activity against 2-nitrofluorene induced base-pair mutation.

Besides, the extract (500 µg/mL) causes a considerable decrease in the mitotic index when compared to the control group (Table II). Cyclophosphamide (50 µg/mL) produced extensive chromosomal abnormalities. When the extract (250 µg/mL) was combined with cyclophosphamide (50 µg/mL), a slight decrease in chromosomal abnormalities was observed which was indicated by an increase in mitotic index. Spindle fiber disruption occurs due to plant alkaloids (Fasola and Egunyomi, 2005), and *I. staphylina* in the preliminary phytochemical study showed the presence of alkaloids.

In conclusion, antimutagenic potential of *I. staphylina* is dose-dependent.

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