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ANTIMUTAGENIC ACTIVITY OF *SYZYGIUM AROMATICUM* EXTRACT IN MICE USING BONE MARROW MICRONUCLEUS TEST

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ABSTRACT

Syzygium aromaticum is commonly known as clove, it is the dried bud of the tree. Cloves are of high medicinal value they are widely used in ayurveda, chinese medicine, western herbalism and also as a food ingredient. The present study was taken up to evaluate antimutagenic activity of ethyl acetate extract of clove with reference to the frequency of polychromatic erythrocytes (PCE) in the bone marrow of mice, administered intraperitoneally (i.p) using bone marrow micro nucleus test. Mice were given with ethyl acetate extract doses of 500,750 mg/kg/day intraperitoneal. In addition, Mitomycin C (MMC) given to the animals at the dose of 4mg/kg by single intraperitoneal injection. Smear specimens were prepared from bone marrows and stained with May-Grunwald's followed by Giemsa stain then the frequencies of micro nucleated PCEs and the ratio of total number of (PCE/NCE) were calculated. Ethyl acetate extract of *Syzygium aromaticum* (750mg/kg) showed significant antimutagenic activity.

KEYWORDS: ***Syzygium aromaticum,*** **antimutagenic activity, Mitomycin C and bone marrow micronucleus test**

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INTRODUCTION

Mutagenic and potentially carcinogenic agents are omnipresent in the human environment and it seems to be impossible to eliminate all of them. Moreover, several well-known mutagenic risk factors are closely connected with a modern lifestyle and their entire eradication appears to be very burdensome, even unattainable [1]. There exists a need to reduce genotoxic effects of mutagenic and carcinogenic factors by the regular intake of antimutagenic agents. For this reason the antimutagenic agents should be taken commonly and continuously.[2] Naturally occurring substances of plant origin and dietary components that have been widely studied for their antimutagenic activity. Extensive work has been carried out for demonstrating the antimutagenetic potential of some commonly consumed spices and vegetables[3,5].*Syzygium aromaticum* commonly known as clove is the dried bud of a clove tree. This tree is classified under the family of Myrtaceae, a dicotyledonous plant[4]. Clove is rich in minerals such as calcium, hydrochloric acid, iron, phosphorus, sodium, potassium, and vitamin A and vitamin C. Several constituents of clove has been identified, mainly eugenol, eugenyl acetate,beta-caryophyllene, 2-heptanone, acetyleugenol, alpha-humulene, methyl salicylate, isoeugenol, methyleugenol (Yang *et al.,* 2003). Cloves are used in medicine ayurveda, studies have reported its Antimutagenic[6], anti-inflammatory[7], antioxidant[8], antiulcerogenic[9,10] antithrombotic [11]and antiparasitic[12]. Keeping in mind the great medicinal value and high content of several active agents of *Syzygium aromaticum* present investigation was planned to study its antimutagenetic activity *in vivo* in mice using bone marrow micronucleus test.

MATERIALS AND METHODS

Extract preparation

2 g of cloves was weighed and crushed to make powder and it is mixed with 10 ml ethyl acetate kept it aside for overnight. Then it was filtered followed by centrifugation (8000 rpm for 20 min) to remove the debris. And the supernatant was collected and it was subjected to steam distillation.

Animals

A total of 25 Male Swiss albino mice obtained from the animal facility, Sugen Life Sciences (Tirupati, India) were used for the study. All mice were certified for good health at the time of receipt. Age of the animals at the start of the treatment was approximately 8- 12 weeks. In the experimental room, 12 h of artificial fluorescent lighting and 12 h darkness were maintained, light hours being 6 to 18 h. Mice were allowed to acclimate to the experimental room conditions for a period of five days prior to randomization and treatment. During the acclimatization period, the mice were observed for clinical signs.

Preparation of Mitomycin C solution

Mitomycin C solution was dissolved in distilled water and administered intraperitoneally at a dose of 3mg/kg and 4 mg/kg according to the body weight of the animal. The animals were divided into 5 groups. Each group consisted of six animals. The first group of animals were considered as positive controls received only distilled water, Second group as negative control treated with mitomycin C 3mg/kg ,third group also negative control treated with mitomycin C 4mg/kg. This standard was dosed once daily for 2 days intraperitoneally to the respective group of animals according to their body weights. Fourth group treated with mitomycin C intraperitoneally and simultaneously with ethyl acetate extract of clove 500mg/kg b.w. and 5th group treated with mitomycin C intraperitoneally and simultaneously with ethyl acetate extract of clove 750 mg/kg b.w.

Collection of Bone marrow cells

The mice were sacrificed by cervical dislocation on the third day after initial dosing. Both the femoral bones were excised and bone marrow

cells were aspirated using 1.5 ml of fetal calf serum. The samples were transferred to prelabeled tubes. The cells were centrifuged at 2000rpm for 10 m. The collected supernatant was smeared on to a clean glass slide. The slides were stained using Geimsa and Maygrunwald stains for clear differentiation of polychromatic erythrocytes and normochromatic erythrocytes during scoring.

Scoring of slides

Two slides were prepared from each animal. More than 1000 polychromatic erythrocytes (PCE) were scored from each slide with corresponding normochromatic erythrocytes (NCE) for the calculation of P/N ratio. The number of micronucleated polychromatic erythrocytes was scored separately to evaluate the clastogenic activity of mitomycin C.

RESULTS

The present study shows antimutagenic activity of clove extract was evaluated by mitomycin C induced mutagenesis. Table 1 show the mean body weights of mice on Day 0 (before dosage starts), Day 1 (after the dosage) and the day of sacrifice. (Fig.1) The number of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) are calculated individually. Positive control groups (mitomycin-C 3mg/kg and 4mg/kg) represent significant reduction in number of PCE. A high increase in number of PCE was identified in the group treated with Clove extract 750mg/kg with mitomycin-C 4mg/kg (Table 2) and fig.3a and Fig.3b The ratio of polychromatic to normochromatic erythrocytes (P/N) was calculated together with appropriate group mean values and standard deviations. P/N ratio represents the frequency of PCE/NCE ratio. Positive control groups (Mitomycin-C 3mg/kg and 4mg/kg) produces a significant decrease in P/N ratio. Where as in extract with MMC treated groups decrease in P/N ratio was inhibited by Clove extract. The results are tabulated in Table.3 and Fig.4.And the results reveal that the ethyl acetate clove extract decreases the Mitomycin C induced formation of micronuclei in PCE and NCE. Clove extracts of 500mg/kg and 750 mg/kg shows antimutagenic activity against MMC induced mutations. These results suggest that clove has pharmacological importance for the prevention of mutations.

Table 1
Mean Body Weight

Group	Dose Levels	Body weight (g) Mean ± SD		
		Day 0	Day 1	Day of sacrifice
Control (DW)	0.0mg/kg	31.1±5.90	31.6±6.13	31.8±5.63
Mitomycin-C	3mg/kg	32.9±5.88	31.5±5.18	30.2±6.29
Mitomycin-C	4mg/kg	30.6±3.56	30.5±3.81	29.9±3.97
Clove extract and Mitomycin-C	500mg/kg + 3mg/kg	35.6±3.79	34.8±4.40	33.9±4.02
Clove extract and Mitomycin-C	750mg/kg+ 4mg/kg	36.4±5.40	35.9±4.05	35.8±3.90

Table 2
Individual PCE and NCE Data

Animal No	Group	PCE	NCE
1	Control	112	88
2		121	79
3		117	83
4		120	80
5		115	85
6	Mitomycin-C (3mg/kg)	82	118
7		81	119
8		79	121
9		83	117
10		85	115
11	Mitomycin-C (4mg/kg)	78	122
12		81	119
13		72	128
14		79	121
15		76	124
16	Clove Extract (500mg/kg) + Mitomycin-C (3mg/kg)	125	75
17		123	77
18		131	69
19		108	92
20		117	83
21	Clove Extract (750mg/kg) + Mitomycin-C (4mg/kg)	131	69
22		126	74
23		135	65
24		121	79
25		127	73

Table 3
Mean P/N Ratio

Group	Dose Levels	P/N ratio Mean ± SD (n = 5)
Control (DW)	0.0mg/kg	1.41 ± 0.106
Mitomycin-C	3mg/kg	0.69 ± 0.029
Mitomycin-C	4mg/kg	0.63 ± 0.053
Clove extract and Mitomycin-C	500mg/kg+3mg/kg	1.54 ± 0.271
Clove extract and Mitomycin-C	750mg/kg+4mg/kg	1.58 ± 0.607

Figure.1
Syzygium aromaticum (clove)

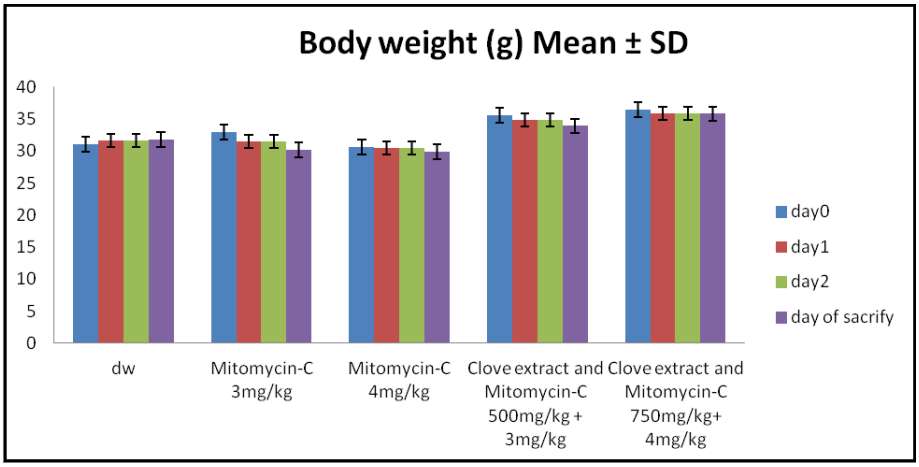

Figure.2
Mean Body Weight

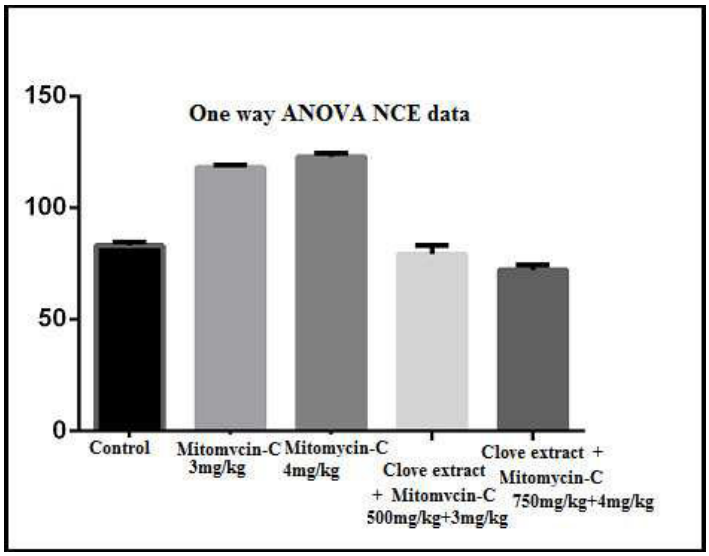

Figure. 3.(a)
Individual NCE Data

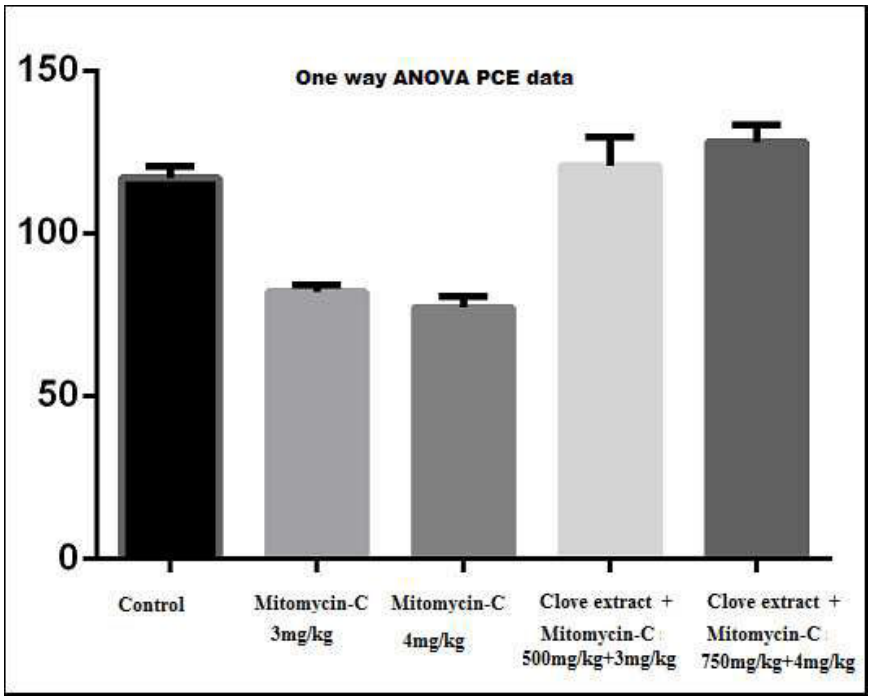

Figure.3. (b)
Individual PCE Data

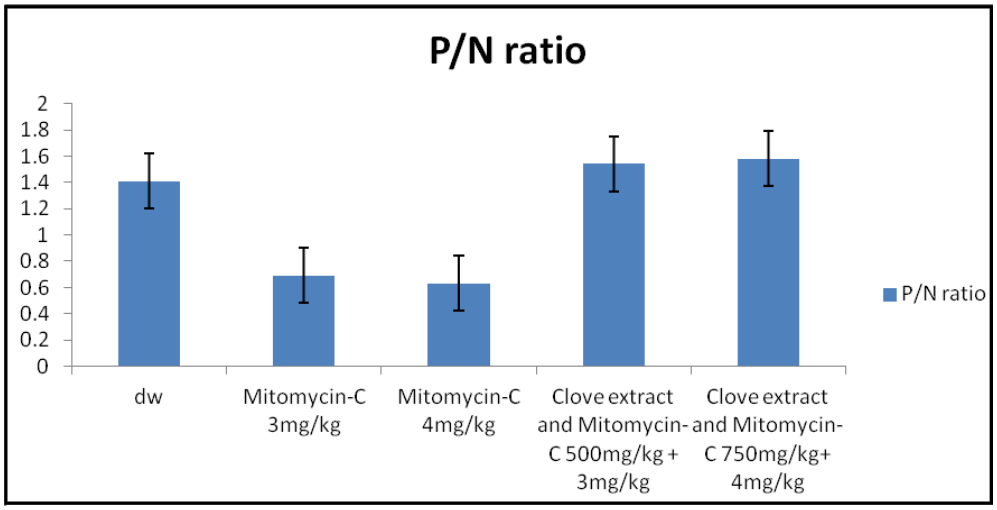

Figure. 4
Mean P/N Ratio

DISCUSSION

The bone marrow micronucleus test developed by Heddle and Schimid [13, 14] is a short term assay widely used for genotoxicity tests.[15]This study was performed to assess the potential of the test material to produce damage to chromosomes. This test presents some advantages compared to others kinds of assays, in which we may mainly mention the low cost and the reliability. In addition, this assay utilizes mammalians, which present capacity of metabolization similar to humans that hardly can be reproduced in totality in "*in vitro*" assays [16]. The intraperitoneal via (i.p.) was applied

because this procedure maximizes the exposure of the bone marrow to chemical mutagens [17]. The recommended methodology has been published in OECD Test Guideline 475 [18]. The advantage of the micronucleus test for mutagenicity screening has been well established in several systems i.e. ovary, bone marrow, epithelial tissues, peripheral blood, liver, exfoliated buccal cells and fetus cells of several laboratory animals or human [19,20,21,22,23]. Intraperitoneal administration of mitomycin C shows significant rise in NCE indicating the chromosomal damage in mice bone marrow cells. These fragmented chromosomes were condensed to form micronuclei which are not included in the main nucleus [24]. Administration of ethyl acetate clove extract alone does not produce any significant variations in percentage of PCE and NCE indicates it is devoid of any genotoxicity. When normal proliferation of the bone marrow cells is affected by a toxic agent, the number of immature erythrocytes (PCE) is prejudiced in relation to mature erythrocytes (NCE). Thus, the PCE/NCE ratio may decrease [16]. Our results indicated that (Table 3), negative controls shows reduced P/N ratio where extract groups shows increased P/N ratio. However, the antimutagenic effect was strongly observed for doses tested (Ethyl acetate clove extract 500mg/kg + MMC 3mg and 750 mg/kg + MMC 4mg).

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