ASSESSMENT OF ANTIMUTAGENIC ACTIVITY OF ANNONA SQUAMOSA

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ABSTARCT:

Annona squamosa is a small, semi-deciduous tree, 3-7 m in height, with a broad, open crown or irregularly spreading branches; bark light brown with visible leaf scars and smoothish to slightly fissured into plates; inner bark light yellow and slightly bitter; twigs become brown with light brown dots. The active ingredients in Annona squamosa include glycosides, alkaloids, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, phytosterols and amino acids. The plants part was collected and authentified and dried with special care. Extraction was done by using soxhlet apparatus with 95% ethanol as the solvent. Qualitative phytochemical investigation were carried out for the confirmation of the presence of glycoside, tannins and phenolics, Flavonoids, saponins, triterpenes and carbohydrates and other phytoconstituents. The mice were divided into four groups of six animals each comprising of Normal Saline, Control group, A. squamosa Extract treated with Lower Dose (LD) (250 mg/kg b.wt) and Higher Dose (HD) 500 mg/kg b.wt). The study showed that A. squamosa extract had prevention the formation of micronucleus and chromosomal aberration.

Key Words: *Annona squamosa, micronucleus, chromosomal aberration*

INTRODUCTION:

Plants have been one of the essential sources of medicines from the start of human development. There is a developing interest in plant based medicines, health items, pharmaceuticals, nutrient supplements, beautifying agents, and so forth. As per the WHO review 80% populations living in the third world countries depend solely on conventional medication for their essential human needs[1]. According to World Health Organization (WHO) medicinal plants are the best source to obtain a variety of drugs. Therefore, medicinal plants should be investigated to better understand their properties, safety and efficiency[2].

Medicinal plants, seeing as time immemorial, have been used in almost all cultures as a source of medicine. It has been probable that about 80-85% of peoples both in developed and developing countries rely on traditional medication for their mainly health care needs and it is unspecified

that a key part of traditional remedy involves the use of plant extracts or their main chemical phytoconstituents[3].

Annona squamosa is a small, semi-deciduous tree, 3-7 m in height, with a broad, open crown or irregularly spreading branches; bark light brown with visible leaf scars and smoothish to slightly fissured into plates; inner bark light yellow and slightly bitter; twigs become brown with light brown dots. Leaves occur singly, 6-17 x 3-6 cm, lanceolate or oblong lanceolate, pale green on both surfaces. Flowers greenish-yellow, fragrant, on slender hairy stalks, produced singly or in short lateral clusters about 2.5 cm long, 2-4 flowers. The aggregate fruit formed from the numerous pistils of a flower, which are loosely united, is soft and distinct from other species of the genus. The active ingredients in Annona squamosa include glycosides, alkaloids, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, phytosterols and amino acids. The bark of custard apple tree can be used to stop diarrhea in children and adults. In addition, the plant is effective to treat diabetes. Custard apple can treat burning sensation, as it is an effective coolant. The crushed leaves of the tree are used to treat hysteria (fearful state of mind) and fainting spells. The treatment of ulcer, wound, dysentery and other ailments is also done by its concentrated leaf extract[4-6].

MATERIALS AND METHODS

COLLECTION AND EXTRACTION OF PLANT MATERIAL

The fresh plant part of *Annona squamosa* was collected from local botanical garden and market of Indore district. The dried leaves of *Annona squamosa* were grinded in mixer grinder and about 400 gm. of the powdered material was treated with. 95% ethanol using continuous hot percolation method. The solvents used were purified before use. The extracts were concentrated by vacuum distillation to reduce the volume 1/ 10.The concentrated extract was transferred to 100 ml beaker and the remaining solvent was evaporated on the water bath, then collected and placed in a desiccators to remove excessive moisture.

ANIMALS:

Adult Swiss albino mice weighing 24±2 mg were used for the experiments. All the animals were kept in polypropylene cages in the animal house at temperatures of 22±3°C. The animals were provided standard laboratory diet and water *ad libitum*. The experimental procedures were approved by institutional animal ethical committee.

ANTIMUTAGENICITY STUDIES

It was done by two methods

Experimental design:

(1)Group I - Control group received vehicle solution
(2)Group II - Cyclophosphamide induced group (50mg/kg)
(3)Group III - Treated with *Annona squamosa* extract 250 mg/kg body weight
(4)Group IV - Treated with *Annona squamosa* extract 500 mg/kg body weight

1. Micronucleous assay

The femur of mice was dissected out and the bone marrow was flushed out in Hank's balanced salt solution (HBBS).The smear was made in pre-cleaned slides, air dried and fixed in absolute methanol. The slides were stained with Maygrunwald and Giemsa stain. About 2000 cells were counted and numbers of micronucleated polychromatid erythrocytes cells were scored. Poly-chromatic erythrocytes to normochromatic erythrocytes (PCE/NCE) ratio was also calculated. The data are presented in MNPCE+SE. The statistical significance was evaluated using Student's t test.

2. Chromosomal aberration

Animals were killed by cervical dislocation and bone marrow was used for analyzing chromosome abnormalities. For this proximal ends of the femurs were shortened with scissors to visualize the bone marrow canal. 0.5 mL of phosphate buffered saline (PBS) was aspirated into a disposable syringe and the needle was inserted into the bone marrow canal. The bone marrow was flushed out into a centrifuge tube containing PBS and mixed thoroughly. Tubes were centrifuged at 1000 rpm for 8 min. The cell button was suspended in a hypotonic solution of 0.075M KCl, The femur was excised and the bone marrow was extracted in 0.56% KCl. The harvested cells were incubated at 37°C for 20 min and then centrifuged for 10 min at 1000 rpm. Cells were fixed in Carney's fixative and burst opened on a fresh slide to liberate the chromosomes. The slides were stained with 5% Giemsa solution for 15 min and then put in xylene and mounted with DPX. A total of 100 well spread metaphase plates were scored for chromosomal aberrations at a magnification of 1000× (100×10) for each group. Different types of chromosomal aberrations such as chromatid breaks, Ring and association, etc. were scored and expressed as percentage chromosomal aberrations.[7-8]

RESULT AND DISCUSSION

Micronucleus assay of *Annona Squamosa*

The micronucleus study showed that the single application of the *Annona Squamosa* leaves extract at the dose of 250 and 500mg/kg body wt. prior to the administration of cyclophosphamide have significantly prevented the micronucleus formation in dose dependent manner. The PCE/NCE ratio of *Annona Squamosa* also not suppressed as compared to control group.

TABLE: 01 - Effect of *Annona Squamosa* leaves extract on MN formation in mouse bone marrow cell

GROUP	MNPCE+SE	PCE/NCE RATIO
Normal	0.3±0.5	0.06±0.03
Cyclophasphomide (CP)	4. 10±0.3	3.60±0.5
A. Squamosa + CP (250mg/kg)	2.30±0.5*	1.45±0.02
A. Squamosa + CP (500mg/kg)	1.55±0.4*	1.15±0.03

Values are expressed as Mean ± SEM of 3 mice in each group

*P<0.001 comparison to CP group.

Numbers of micronuclei prevention was found increased with the increase in the concentration of *A. Squamosa* extract (250mg/kg- 2.3±0.5, 500mg/kg- 1.55±0.4).

Chromosomal aberration of ***Annona Squamosa***

The Chromosomal aberration showed that the single application of *Annona Squamosa* extract at the dose of 250 and 500mg/kg body wt. prior to the administration of cyclophosphamide have significantly prevented the structural changes in chromosomes in dose dependent manner.

TABLE 02: Effect of *A. Squamosa* extract on prevention of chromosomal aberration

S.N.	Treatment	Chromosomal Aberration (%)
1	Normal	3.5 ± 1.7
2.	Cyclophosphmide (50 mg / kg)	66.7 ± 3.2
3.	*A. Squamosa* extract + CP(250 mg/kg +50)	25.6 ± 3.2*
4	*A. Squamosa* extract + CP (500 mg/kg +50)	18.5 ± 2.4*

Values are expressed as Mean ± SEM of 3 mice in each group

*P<0.001 comparison to CP group

In case of chromosomal aberration test, there was a significant elevation of protection in chromosomal aberration in group with Cyclophosphamide plus *A. Squamosa* extract as compared to cyclophasphomide group with the increase in the dose of extract (250mg/kg- 25.6%, 500mg/kg- 18.5%).

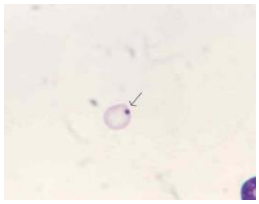

Fig 1: Micronucleus formation

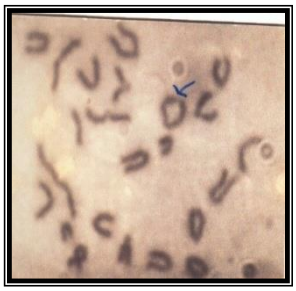

Fig 2: Chromosomes (Ring formation)

CONCLUSION:

In the present study we have tested the anticancer property of *Annona Squamosa* plants using experimentally induced cancer in animal models and tried to elucidate possible mechanism of action. Numbers of micronuclei prevention are found increase with the increase in the concentration of *Annona Squamosa* extract as compared to control group (Cyclophosphamide only). In case of chromosomal aberration test, there was a significant elevation of protection in chromosomal aberration in group with Cyclophosphamide with *Annona Squamosa* extract with the increase in the dose of extract. Thus these studies provide a scientific support to the selected medicinal plants which claims its use in tradition medicine. This result supports using the plants *Annona Squamosa* in folk medicine to treat cancer.

CONFLICT OF INTEREST

There is no conflict of interests regarding publication of this paper.

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