ASSESSMENT OF PHYTOCHEMICAL INVESTIGATION AND ANTIMUTAGENIC ACTIVITY OF AVERRHOA BILIMBI

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ABSTARCT:

Averrhoa bilimbi belongs to the family Oxalidaceae is a common plants found in the southern part of India. Their fruits were edible and it is consumed by the public. Traditionally various parts of these plants especially fruits and leaves were widely used by the ethnic communities in the treatment of various disorders. Averrhoa fruits are good source of minerals such as potassium, calcium, phosphorous and iron. Averrhoa fruits are low in calorie, sodium and lipids. 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 Nomal Saline, Control group, A. Bilimbi 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. bilimbi extract had prevention the formation of micronucleus and chromosomal aberration.

Key Words: Averrhoa bilimbi, phytochemical investigation, micronucleus, chromosomal aberration

INTRODUCTION:

Medicinal plants played a significant function in Indian culture since Rig-Veda (5600BC). Herbal medication is the ancient type of healthcare known to human being. Herbs had been used by all cultures all over history. It was an essential part of the expansion of modern civilization. Ancient man experiential and appreciated the great diversity of plants available to him. Medicinal plants have been curing various disorders in humans from the time.[1]

Immemorial and are considered to be intermittently associated as integral part of the Indian traditional medicinal system. 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].

Averrhoa bilimbi (common name: Bilimbi) is a medicinal plant belonging to the family *Oxalidaceae.* The genus *Averrhoa* was named after an Arab Philosopher, physician and Islamic Jurist Ibn Rushd often known as Averroes.

Scientific Classification:

- Kingdom: Plantae – Plants
- Subkingdom: Tracheobionta – Vascular plants
- Superdivision: Spermatophyta – Seed plants
- Division: Magnoliophyta – Flowering plants
- Class: Magnoliopsida – Dicotyledons
- Subclass: Rosidae
- Order: Geraniales
- Family: Oxalidaceae – Wood-Sorrel family
- Genus: Averrhoa Adans – averrhoa
- Species: *A. bilimbi* L. – bilimbi.

The fruit extracts contain flavonoids, saponins and triterpenoid. The chemical constituents of A. bilimbi include Amino acids, citric acid, cyanidin–3–O–h–D–glucoside, phenolics, potassium ion, Vitamin A and sugars. *A. bilimbi* has been used in the traditional remedy for the management of a variety of ailments. Infusions and decoctions of the plants are used as an antibacterial, astringent, antiscorbutic, in the treatment of fever, inflammation of the rectum, and diabetes. The paste of leaves is used in the treatment of itches, boils, and skin eruptions, bites of poisonous creatures, rheumatism, cough, cold, mumps, and syphilis. Grated fruits, with a little salt added, are useful on the face for the treatment of pimples. Fruit juice is employed in the treatment of scurvy, bilious colic, whooping cough, hypertension, cancer, obesity and diabetes[3,4].

MATERIALS AND METHODS

COLLECTION AND EXTRACTION OF PLANT MATERIAL

The fresh plant part of *Averrhoa bilimbi*, *was* collected from local botanical garden and market of Indore district. The entire specimen was rinsed with distilled water for the removal of traces of dust and soil present in the plant part.

The fresh fruits of *Averrhoa bilimbi* was grinded with the help of mechanical grinder and approximately 400gm. of the powdered drug was treated with 95% ethanol using continuous hot percolation method. The extracts were concentrated to decrease 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[5].

PHYTOCHEMICAL SCREENING:

In order to Evaluation of chemical constituents present in the plant part extract , ethanolic extracts of plant sample were subjected separately to phytochemical screening according to the methods for identification of Carbohydrates, Glycosides, Alkaloids, Tannin, Sterol, Saponin Wax, Protein and amino acid, Resin and Phenol[6].

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 *Averrhoa Bilimbi* extract 250 mg/kg body weight

(4)Group IV - Treated with *Averrhoa Bilimbi* extract 500 mg/kg body weight

1. Micronucleous assay

To collect bone marrow cells, both femurs were removed and bones were freed from muscles. The proximal ends of the femurs were carefully shortened with scissors until a small opening to the marrow canal became visible. Approximately 5mL PBS with 100µL serum (FCS) were aspirated into a disposable syringe and the needle was inserted a few millimeters into the bone marrow canal. Bone marrow was flushed into a centrifuge tube and mixed gently. Tubes were centrifuged at 1000 rpm for 10 min. The cell button was

Collected and smears were made. The air dried smears were fixed in methanol for 5 to 10min. and then stained using May-Grunwald Giemsa (undiluted) for 3min, followed by diluted May Grunwald in distilled water (1:1) for 2min and finally with diluted Giemsa (1:6 distilled water) for 10min. The slides were rinsed in distilled water, air dried and mounted in DPX. The slides were screened for 2000 polychromatic erythrocytes and corresponding normochromatic erythrocytes and also for the presence of micronuclei.

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]

RESULT AND DISCUSSION

The phytochemical analysis of the fruit extract showed the presence of carbohydrates, flavonoids, phenols, glycosides and amino acids (Table 1)

Table No. 1: Preliminary phytochemical screening of ethanolic extract of *Averrhoa Bilimbi*

S. No.	Constituents	Tests	*Averrhoa Bilimbi*
1.	Carbohydrate	Molish's test	++
2.	Glycoside	Borntrager's test	++
		Legal's test	++
3.	Fixed oil & fats	Spot test	+
		Soap formation test	+
4.	Proteins & amino acids	Million's test	+
		Ninhydrin test	++
		Biuret test	++
5.	Mucilage & Gum	Ppt with 90% alcohol	+
6.	Tannins	$FeCl_3$ test	-
		Lead acetate test	-
7.	Steroids	Salkowiski test	-
		Libermann burchard test	+
8.	Alkaloids	Dragendroff's test	+
		Mayer's test	+
		Wagner's test	+
		Hager's test	-
9.	Triterpenoids	Salkowiski test	+
10.	Flavonoids	Libermann burchard test	++
		Alkaline reagent test	++
		Shinoda's test	+
11.	Wax	Ppt. with alcoholic KOH	+

++ Strongly present, + -Present and – Absent

ANTIMUTAGENICITY STUDIES

Micronucleus assay of *Averrhoa Bilimbi*

The micronucleus study showed that the single application of the *Averrhoa Bilimbi* fruit 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 *Averrhoa Bilimbi* also not suppressed as compared to control group.

TABLE: 2 - Effect of *Averrhoa Bilimbi* seeds 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. 26±0.7	3.78±0.46
A. bilimbi + CP (250mg/kg)	2.30±0.6*	1.35±0.03
A. bilimbi + CP (500mg/kg)	1.25±0.48*	1.05±0.02

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 *Averrhoa Bilimbi* extract (250mg/kg- 2.30±0.6, 500mg/kg- 1.25±0.48).

Chromosomal aberration of ***Averrhoa Bilimbi***

The Chromosomal aberration showed that the single application of the *Averrhoa Bilimbi* 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 3: Effect of *Averrhoa Bilimbi* extract on prevention of chromosomal aberration

S.N.	Treatment	Chromosomal Aberration (%)
1	Normal	3.5 ± 1.7
2.	Cyclophosphmide (50 mg / kg)	52.9 ± 3.8
3.	*A. Bilimbi* extract + CP(250 mg/kg +50)	18.7 ± 2.5*
4	*A. Bilimbi* extract + CP (500 mg/kg +50)	15.5 ± 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. Bilimbi* extract as compared to cyclophasphomide group with the increase in the dose of extract (250mg/kg- 18.7%, 500mg/kg- 15.5%).

CONCLUSION

Physical properties and dimensions of mature Averrhoa fruits are ideal as green vegetable for human consumption. The parts of plants were taken up for the study by us to screen and give a report on the possible pharmacognostical, phytochemical and pharmacological studies. Numbers of micronuclei prevention are found increase with the increase in the concentration of *Averrhoa Bilimbi,* 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 *Averrhoa Bilimbi,* extract with the increase in the dose of extract.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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