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# **Research Article**

## ASSESSMENT OF ANTI BACTERIAL AND ANTIMUTAGENIC ACTIVITY OF BALANITES AEGYPTIACA

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#### ABSTRACT

*Balanites aegyptiaca* belongs to the family Zygophyllaceae. It is a common plants found in the several state of India. It is multibranched, spiny shrub or tree up to 10 m tall. Crown sphere-shaped, in one or several distinct masses. It contains saponin, furanocoumarin, and flavonoid namely quercetin 3-glucoside, quercetin-3-rutinoside; 3-glucoside, 3-rutinoside, 3- 7-diglucoside and 3-rhamnogalactoside of isorhamnetin Balanitoside. Extraction was done by using soxhlet apparatus with 95% ethanol as the solvent. 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 of chromosomal aberration.

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## **INTRODUCTION**

Nature has been a wealth and very good quality source of medicinal agents for thousands of years, and a remarkable number of current drugs have been derivative from the natural sources, many of these isolations were based on the uses of the agents in traditional medicine. Herbal medication is defined as a branch of science in which treat formulations which is based on the herbal plants. Which are used to alleviate diseases. It is also known as botanical remedy<sup>1</sup>. According to World Health Organization (WHO), 65-80% of the global population use plant products for their primary health care. The investigations on therapeutic applications of plants have led to the discovery of several clinically applicable drugs<sup>2</sup>.

Herbal medicines are an essential and growing part of the international pharmacopeia. Knowledge of their medicinal properties is growing as a result of research and testing, which will make them an increasingly safe alternative or a preferred option to allopathic medicine. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources<sup>3</sup>. *Balanites aegyptiaca* is multibranched, spiny shrub or tree up to 10 m tall. Crown sphere-shaped, in one or several distinct masses. Leaves with two separate leaflets; leaflets obovate, asymmetric, 2.5 to 6 cm long, bright green, leathery<sup>4</sup>. Trunk short and often branching

from near the base. ark dark brown to grey, deeply fissured. Branches armed with stout yellow or green thorns up to 8 cm long. Fruit is a rather long, narrow drupe, 2.5 to 7 cm long, 1.5 to 4 cm in diameter. Young fruits are green and tormentose, turning yellow and glabrous when mature. It contains saponin, furanocoumarin, and flavonoid namely quercetin 3-glucoside, quercetin-3-rutinoside; 3-glucoside, 3-rutinoside, 3- 7-diglucoside and 3-rhamnogalactoside.<sup>5-7</sup>.

## **MATERIALS AND METHODS**

#### **Collection and Extraction of Plant Material**

The plant part of *Balanites aegyptiaca was* collected from local botanical garden of Indore district. Seeds of *B. aegyptiaca* were 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 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

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in the animal house at temperatures of  $22\pm3$  °C. The animals were provided standard laboratory diet and water *ad libitum*. The experimental procedures were approved by institutional animal ethical committee.

#### Antibacterial Screening

#### Antibacterial Sensitivity test by Disk Diffusion Susceptibility Testing (Kirby-Bauer Method)

*Culture Media:* The medium used for the activation of the microorganisms was nutrient broth. The nutrient agar media was used for the antibacterial test.

*Microorganisms used:* The test organisms (*Pseudomonas aureogenosa, Staphylococcus aureus, Staphylococcus epidermis, Shigella flexineri, Bacillus substilis & E.Coli.*)

**Concentration:** Four different concentrations of *Balanites aegyptiaca* extract were prepared (100, 75, 50, and 25%). 100% = 1 g crude extract in 1 ml of freshly prepared double distilled water. Afterward, serial dilution was prepared: 75% = 75 mg in 1 ml, 50% = 50 mg in 1 ml, and 25% = 25 mg in 1 m.<sup>8</sup>

#### Antimutagenicity Studies

#### **Experimental Design**

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

#### **Chromosomal** Aberration

After the treatment, the animals were treated for 90 min with colchicine (0.4ml, 0.05%) through intraperitoneal injection and were sacrificed by cervical dislocation. Bone marrow from the femur bone was collected into hypotonic solution (0.056 M % KCl) and incubated at 37 °C for 25 min and fixed in fixative solution (Methanol:Acetic Acid; 3:1). The permanent slides were prepared by the drop method that included the chilled blank slides and these slides were gently heated on a spirit lamp to fix cells permanently on the slides. The prepared slides were stained with Geimsa stain; the slides were dipped into the Geimsa stain (10%) for 20 min. then washed in PBS (three times) for 5 min.<sup>9,10</sup>

## **RESULT AND DISCUSSION**

#### Antibacterial Activity

 Table 1 Effect of Balanites aegyptiaca extract on inhibition of different bacteria

S.No	Microorganism	Concentration (Zone of inhibition in mm)			
		25% conc.	50%c onc.	75%c onc.	100% conc.
1.	Bacillus subtilis	15	17	21	24
2	Shigella flexineri	13	16	18	22
3.	Staphylococcus epidermis	12	14	17	20
4.	Staphylococcus aureus	16	18	20	21
5.	E. coli	15	16	18	22
6.	Pseudomonas aureogenosa	10	13	15	18

The above observations suggest that different concentration (25%, 50 %, 75 % & 100 %) were having good antibacterial activity against *Streptococcus aureus, Streptococcus epidermis, Psudomonas aeriginosa, Shigella flexineri, Escherichia coli* and *Bacillus subtilis.* Thus the extract is showing varying activity against the entire microorganism.



Fig 1 Effect of Balanites aegyptiaca extract on inhibition of different bacteria

- 1. Bacillus Subtilis
- 2. Shigella flexinie
- 3. Staphylococcus epidermis
- 4. Staphyloccus arusse
- 5. E.coli
- 6. Pseudomonas Aroginoso

#### Antimutagenicity Studies

#### Chromosomal aberration of Balanites aegyptiaca

Evaluation of chromosomal aberration was conducted at two dose levels that is 250 mg/kg body weight and 500mg/kg body wt. prior to the administration of cyclophosphamide have significantly prevented the structural changes in chromosomes in dose dependent manner. All the data statistically calculated using student t-test. In this assay chromosomal gap and break, fragmentation and ring chromosomes were taken as a parameter to score the % of cells with aberration.

 
 Table 2 Effect of Balanites aegyptiaca extract on prevention of chromosomal aberration

S.N.	Treatment	Chromosomal Aberration (%)
1	Normal	$3.5 \pm 1.7$
2.	Cyclophosphmide (50 mg / kg)	$62.5 \pm 4.2$
3.	<b>B.</b> aegyptiaca extract + CP(250 mg/kg +50)	$20.7 \pm 2.8*$
4	<i>B. aegyptiaca</i> extract + CP (500 mg/kg +50)	15.8 ± 2.2*

Values are expressed as Mean  $\pm$  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 *B. aegyptiaca* extract as compared to cyclophasphomide group with the increase in the dose of extract (250mg/kg- 20.7%, 500mg/kg- 15.8%).



(Normal)



(Fragment)



Fig 2 Chromosomal Abbrevation

## CONCLUSION

From the very beginning the plants have been recognized as the most imperative source of the medicine. The different phytochemicals derived from the different parts of plant provide the potential bioactive agent for various disease treatment strategies. The protective effect of *B. aegyptiaca* seeds extract was seen against the mutation induced by cyclophosphamide. The tests were performed on mice bone chromosomal aberration assay. The results find statistically significant. Therefore, from the present study, it can be concluded that *B. aegyptiaca* seeds extract possesses antibacterial and antimutagenic property.

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**Conflict of Interests** The authors declare no conflict of interests.

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