



**POTENTIAL ACTIVITY OF THE SEEDS OF *BALANITES AEGYPTIACA* AGAINST
IRRITABLE BOWEL SYNDROME AND HEMORRHOID**

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Article Received on 26/11/2018

Article Revised on 16/12/2018

Article Accepted on 06/01/2019

ABSTRACT

Balanites aegyptiaca (Family: Zygophyllaceae) is scientifically proven to have extensive medicinal values. In the present research work an effort has been taken to explore its seeds kernel for the potential activity against croton oil induced hemorrhoid modal in rat and against irritable bowel syndrome induced by acetic acid in rats. The results show that anti-hemorrhoidal and anti-IBS activity of *Balanites aegyptiaca* seed kernel extract at the dose of 100 and 200 mg/kg body weight was comparable with anti-hemorrhoidal activity of standard drug treatment 20 mg/kg body weight of hydrocortisone. For IBS the intestinal movement of rat ileum given by 10% solution of seed extracts was compared with standard drug 0.01mg/ml atropine solution. The data also supplement for severity score, Recto anal coefficient and histopathological study of rectoanal tissue section of rat. Antihemorrhoidal and anti-IBS activity of seed kernel extract of *Balanites aegyptiaca* showed significant p value of, $P \leq 0.01$ and $P \leq 0.01$.

KEYWORDS: *Balanites aegyptiaca*, Irritable bowel syndrome, hemorrhoid.

1. INTRODUCTION

The interest for plant-based drugs, wellbeing items, pharmaceuticals, sustenance supplement, beautifiers are increasing in both developing and developed countries, due to the growing recognition that the natural products are nontoxic, have fewer side effects and easily available at affordable prices. The seed kernel of *Balanites aegyptiaca* (Family: Zygophyllaceae) are rough, hard and possess many types of activities like antioxidant, antimicrobial, anticancer, diuretic, hypocholesterolemic, wound-healing, antiviral, antidiabetic, hepatoprotective, mosquito larvicidal, anti-inflammatory and analgesic, antivenin, anthelmintic, cardio-protective cum antioxidant activity and antinociceptive properties. Bark, fruits, seeds, seed oil, and leaves of this plant are widely used in folk medicine. This tree is native to much of Africa and parts of the Middle East.^[1-2] In India, it is particularly found in Rajasthan, Gujarat, Madhya Pradesh, and Deccan.^[3-7] From the literature review and research work it was known that, plant *Balanites aegyptiaca* widely used as folk medicine for the treatment of hemorrhoids but there is not any scientific investigation. Hemorrhoids are swollen and inflamed veins around the anus or in the lower rectum. It needs careful attention with proper treatment. Sometimes hemorrhoids accompany Irritable bowel syndrome. Irritable bowel syndrome causes cramping, swelling, gas, looseness of the bowels, and blockage. Irritable bowel syndrome is a disorder since it can cause a few side effects like hemorrhoids. The relationships between irritable bowel syndrome and hemorrhoid are

significant. IBS is a cascade of many physiologic events that is initiated with infection, inflammation, and psychological disturbances like many stresses and eventually terminates with gut dysfunction.^[8] Keeping in view of all these findings the present study is targeted to establish the potential activity of *Balanites aegyptiaca* against hemorrhoids and irritable bowel syndrome.

2. MATERIAL AND METHODS

2.1. Drugs and chemicals

Croton oil (Devinez, Pacific computechPvt Ltd, C-Block, Sushant Lok-1, Gurugram, India), Hydrocortisone (AGIO Pharmaceutical Ltd, pune, India and Marketed by Ipca Laboratories Ltd., Mumbai, India), Acetyl Choline (Central drug house Pvt Ltd, New Delhi, India), N-hexane (Central drug house Pvt Ltd, New Delhi, India), acetic acid (RFCL Ltd., New delhi, India, Atropine (Sigma Aldrich, CH-9471, China), Isoflurane (Pariaml Healthcare Ltd., Baddi, Himanchal Pradesh, India) and all other reagents used in this study were of analytical grade.^[8,9]

2.2. Collection of Plant materials and extraction

The plant seeds were collected from local market of Agra and were preserved in the research facility, NIET Pharmacy Institute, Greater Noida. After authentication at CSIR-NISCAIR, Pusa Campus, New Delhi, the seeds of *Balanites aegyptiaca* were gathered in mass and were shade dried. The kernel parts of the seeds were separated with the help of iron mortar pestle and made it into powder form with a blender processor. The dried powder

(500 gm) was undergone steam distillation with n-hexane up for 1 hour at the temperature of 45⁰C. The extract of *Balanites aegyptiaca* was separated with the help of vacuum filtration and collected into a container.^[9] After estimating its yields the n-hexane extract of the kernel parts of the seeds of *Balanites aegyptiaca* was used for quantitative estimation of phytoconstituents and assessment of its pharmacological potential against hemorrhoids and irritable bowel syndrome.

2.3. Animals

Wistar albino rats weighing between 200-250 gm of either sex were utilized for the present study to induce hemorrhoids and irritable bowel syndrome. They were obtained from Central Animal House of NIET Pharmacy Institute, Greater Noida. The animals were housed in standard polypropylene confines at 25 ± 2⁰C with 12 hrs light and day cycle. Every one of the animals was free access to standard rodent feed, and palatable water. The animals were given a period of seven days for acclimatization to laboratory conditions. The protocol of the present study using experimental animals with protocol number IAEC/NIET/2017/01/01 was approved by Institutional Animals Ethics Committee (1845/PO/Re/S/16/CPCSEA).^[10]

2.3. Induction of Hemorrhoid and antihemorrhoid Activity

Hemorrhoids were incited in rats by controlled rectal administration of croton oil (20 mg/kg body weight). Hydrocortisone referred to the antihemorrhoidal agent was taken as standard drug (20 mg/kg body weight). Propylene glycol was used as a vehicle to for the rectal preparation of the extract and the standard medication in appropriate concentration. The rats haphazardly separated into four groups of six animals. Hemorrhoids were induced to all the groups, except normal control group, by applying croton oil preparation. Twenty-four hours after induction, all the animals were subjected to treatment as assigned to the groups once daily for five days. The normal and positive control groups of animals were treated with only propylene glycol. The standard group of animals was treated with Hydrocortisone. The test groups of animals were treated with the extract of the seeds of *Balanites aegyptiaca* at the doses of 100 mg/kg and 200 mg/kg respectively. On the fifth day, 1 hour after the drug administration, all the animals were euthanized by exsanguination under deep isoflurane anaesthesia, also, rectoanal tissues (20 mm long) were separated. They were assessed for the severity score, weighed, and settled in 10% unbiased supported formalin solution for histological examination. The histological study was performed following the staining method with hematoxylin and eosin. The RAC was figured utilizing the recipe.^[10]

$$\text{Recto anal coefficient (RAC)} = \frac{\text{Weight of recto anal tissue (mg)}}{\text{Body weight (g)}}$$

2.4. Induction of Irritable bowel syndrome and activity against irritable bowel syndrome

The irritable bowel syndrome was induced in rat by intracolonic instillation of acetic acid (4%, 1 ml/rat) aqueous solution slowly for 30 s by a plastic catheter on anesthetizing with ether. Then, 1 ml phosphate buffered saline (PBS) was instilled to dilute the acetic acid and flush the colon. Twenty-four hours after induction, the animal was fasted overnight. They were euthanized by exsanguination under deep isoflurane anaesthesia and ileum was collected. The ileum was mounted in the organ bath and leave for acclimatization as per standard procedure. Acetylcholine was used to increase contractility of ileum of IBS induced rat at increasing concentrations of acetylcholine (1, 2, 4, 6, 8, 16, 32, and 64 µg) from the stock solution (10 µg/ml) till a cumulative response. The responses were recorded via a frontal writing lever on kymograph paper. For relaxation the tissue was allowed to wash with fresh tyrode solution to prevent the accumulation of the treatment and its metabolic end products. Atropine was considered as the standard preparation to counteract the contractile effect of acetyl choline. at the dose of 0.01 mg/ ml. The effect of the extract on the contractile responses of acetyl choline by repeating the same concentration responses of acetyl choline with the n-hexane extract of the kernel parts of the seeds of *Balanites aegyptiaca* (0.1 mg). In the same manner another dose response of acetyl choline was observed with atropine (0.01 mg). The responses were repeated three times and mean percentage inhibition calculated. All the responses achieved on organ bath, were assessed for the concentration graph curve and percentage response.^[11,12]

$$\text{Percentage Response} = \frac{\text{Concentration of unknown}}{\text{Higher height of response}} \times 100$$

2.5. Statistical analysis method (SEM)

The results from each group were calculated as mean ± standard error of mean (SEM). The results were estimated by one-way analysis of variance (ANOVA) software followed by Dunnet-test. Probability (*p*) values less than 0.01 and 0.001 were considered significant.

3. RESULTS AND DISCUSSION

3.1. Phytochemical investigation

With a yield value of 30% w/w, the n-hexane extract of the kernel parts of the seeds of *Balanites aegyptiaca* were undergone phytochemical screening and exhibited the presence of proteins, alkaloids, saponins, tannins and phenolic compounds.

3.2. Anti-hemorrhoid activity

In the experiment, application of croton oil to rectoanal portion of rats induced significant alterations in RAC, severity score, and histopathological findings. The congestion, vasodilation or hemorrhage was not evident. In the group of treatment with 100 mg/kg extract, it was observed that the histologically approximately one third of the lamina propria appeared normal. Other half of the lamina propria was disrupted. Adjacent to the disrupted

part extensive area of mononuclear inflammatory cell infiltrate was seen in muscularis mucosae and sub mucosa. Heterophils were the predominant cell type. Focal area of tissue necrosis was seen. Focal area of hemorrhage and mild congestion of vessel was also evident. In the group of treatment with 200 mg/kg extract, it was observed that the histologically approximately three fourth part of the lamina was appeared normal. Other half of the lamina propria was disrupted. Adjacent to the disrupted part extensive area of mononuclear inflammatory cell infiltrate was seen in muscularis mucosa and submucosa. Heterophils were the predominant cell type. Focal area of tissue necrosis was seen. Mild hemorrhagic foci were seen. The congestion and vasodilatation was not evident. Whereas in the standard group it was observed that the lamina propria appeared normal. Diffuse area of mononuclear inflammatory cell infiltrate was seen in muscularis mucosa and sub mucosa comprising primarily of heterophils. Histopathological examination revealed that

there was a significant difference in RAC, severity score in normal control, positive control and treated groups. Normal group animals showed normal cytoarchitecture of the rectoanal region. The RAC of normal control and positive control group was found to be 0.822 ± 0.017 and 1.48 ± 0.03 , respectively. The RAC of the groups treated with extract at 100 mg/kg and 200 mg/kg and hydrocortisone was found to be 0.98 ± 0.03 and 0.98 ± 0.03 , 1.18 ± 0.04 and 1.11 ± 0.03 ($p < 0.01$), respectively. Interestingly, treatment with *Balanites aegyptiaca* extract at 100mg/kg, ir and at 200mg/kg, ir showed significant dose dependent antihemorrhoid activity with $p < 0.01$. The extracts showed significant reduction in vasodilation (1.71 ± 0.05 and 1.94 ± 0.03 ; $p < 0.01$) which is well comparable to that of the standard group (1.51 ± 0.07 ; $p < 0.01$). The extracts (100 mg/kg) showed negligible recovery of necrosis, whereas at 200mg/kg dose it showed significant recovery of necrosis (1.92 ± 0.05 ; $p < 0.01$).

Group	Severity Score	Rectoanal coefficient	Inflammation	Congestion	Vasodilation	Necrosis
Normal (Vehicle only)	0.0 ± 0.0	0.822 ± 0.017	0.42 ± 0.26	0.0 ± 0.0	0.35 ± 0.09	0.0 ± 0.0
Positive control (Vehicle + Inducer)	1.45 ± 0.07	1.48 ± 0.03	3.20 ± 0.03	3.01 ± 0.01	2.98 ± 0.05	3.99 ± 0.05
Standard (Hydrocortisone)	0.43 ± 0.04	$0.98 \pm 0.03^*$	$0.93 \pm 0.03^{**}$	$2.01 \pm 0.02^*$	$1.51 \pm 0.07^{**}$	$1.49 \pm 0.03^*$
Test-I (Seeds extract; 100 mg/kg)	0.68 ± 0.04	$1.18 \pm 0.04^*$	$1.97 \pm 0.07^*$	2.39 ± 0.07	$1.71 \pm 0.05^*$	3.74 ± 0.03
Test-II (Seeds extract; 200 mg/kg)	0.48 ± 0.03	$1.11 \pm 0.03^*$	$1.86 \pm 0.09^*$	$2.08 \pm 0.03^*$	$1.94 \pm 0.03^*$	$1.92 \pm 0.05^*$

Table 1- Severity score: NAD: 0 (no abnormality detected); minimal: 1 (very small amount of changes $\leq 10\%$); mild: 2 (lesion is easily identified but limited severity 11–25%); moderate: 3 (lesion is predominant 26–75%); severe: 4 (the degree of changes is 76–100%

or great enough in intensity or extent to expect significant tissue or organ dysfunction). Results are represented as mean \pm S.E.M. * $p \leq 0.01$ and ** $p \leq 0.001$ is considered versus positive control.

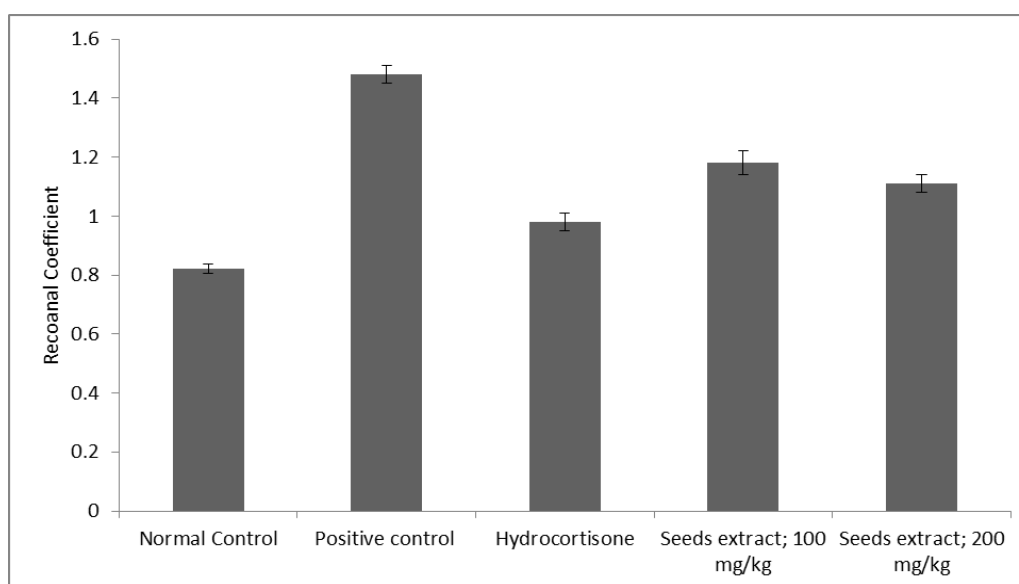


Figure 5: Effect of the n-hexane extract of the kernel parts of the seeds of *Balanites aegyptiaca* on rectoanal coefficient in rat model of croton oil-induced hemorrhoids. All the values are expressed as mean \pm SEM.

3.3. Activity against irritable bowel syndrome

In the experiment, application of acetic acid was used to induce the irritable bowel syndrome in rat and after

plotting the graph using organ bath at different concentrations of the agonist, the percentage response was calculated by using the dose and height of graph.

The responses of the agonist with the extract and atropine were also calculated for their percentage response. It was observed that the extracts found to counteract the contractile effect of acetyl choline. The

relaxant effect of the extracts were observed to be less than that of the competitive antagonist of acetyl choline; atropine.

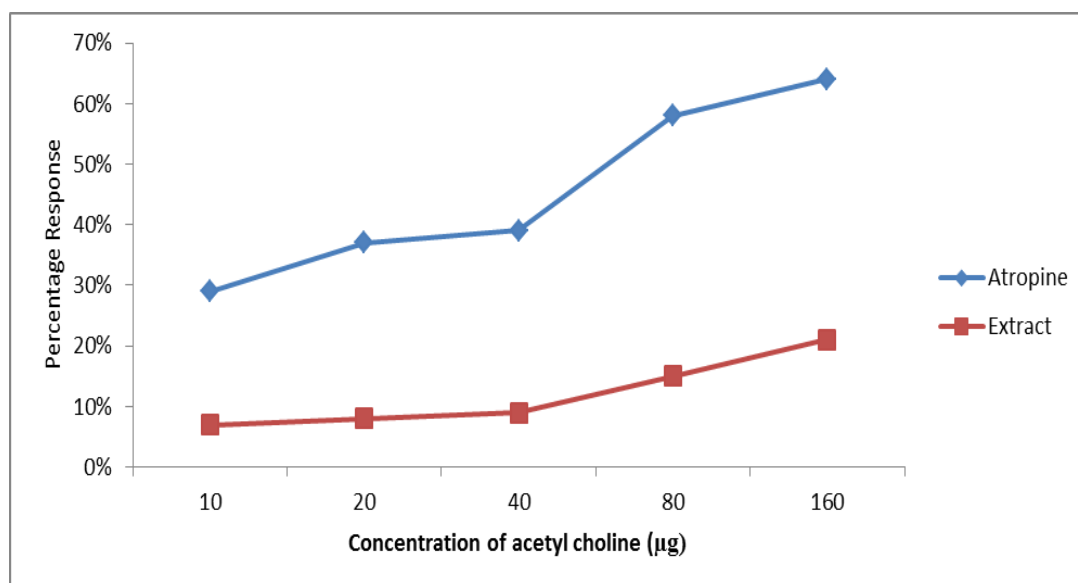


Fig. 2: Effect of the n-hexane extract of the kernel parts of the seeds of *Balanites aegyptiaca* (0.1 mg) and atropine (0.01 mg) on the concentration response curve of acetyl choline.

4. CONCLUSION

Hemorrhoid is characterized by a severe vasodilatation at the rectoanal region, which leads to inflammation of the surrounding tissues. In the present study, croton oil has been used as inducer/phlogistic agent to induce experimental hemorrhoids. Croton oil causes inflammation due to the release of soluble factors involving inflammatory lipid metabolites (prostaglandins, leukotrienes, and lipoxins), kinins (bradykinins and chemokines), nitric oxide, and cytokines (TNF- α and IL-6). The plant *Balanites aegyptiaca* seed are widely used as folk medicine for the treatment of hemorrhoids.^[13,14]

Over the investigation it was analyzed that, the kernel of the seed of *Balanites aegyptiaca* possesses significant anti-hemorrhoid activity against croton oil induced hemorrhoid in rats. And it was also showing affinity on receptor for treatment of IBS, we know hemorrhoids are correlated with IBS. Thus, this plant was used as Anti-Hemorrhoid drug in treatment of hemorrhoids and IBS. However, further study can be performed to evaluate a long term study with its adverse and beneficial effects of *Balanites aegyptiaca* seed. The seed extract of *Balanites aegyptiaca*, showed a dose dependently effect on severity score, Ractoanal coefficient and histopathological observation (inflammatory cells, vasodilation, necrosis, congestion). Irritable bowel syndrome is a disorder since it can cause a few side effects like hemorrhoids. The relationships between irritable bowel syndrome and hemorrhoid are significant. The present study also observed that the extract of the kernels of the seeds of *Balanites aegyptiaca* was also

found to have potential activity against irritable bowel syndrome. The earlier reports have established the role of flavonoids and saponins in oxidative stress, inflammation and hemorrhoid in both clinical and pre-clinical settings. It is well known fact that flavonoids reduce the concentration of PGE2e and PGE2a and other inflammatory mediators. Moreover, it is also well documented that flavonoids increases the vascular tone and reduces the vascular fragility and resistance.^[15] The preliminary phytochemical analysis revealed the presence of different phytoconstituents such as alkaloids, flavonoids and saponins which may impart their anti-hemorrhoidal activity by acting as cytoprotective agents. In view of aforementioned facts, the observed potential activity of the n-hexane extract of *Balanites aegyptiaca* can be attributed to presence of alkaloids, flavonoids and saponins in the kernel parts of the seeds of *Balanites aegyptiaca*.

Thus the study provides the evidence that the extract of the kernels of the seeds of *Balanites aegyptiaca* possesses potential activity against hemorrhoid and irritable bowel syndrome, which are related to the prevention of inflammation, reduction in vasodilation, necrosis and congestion. However further investigation will be beneficial to isolate the exact active principles and mechanism responsible for its anti-hemorrhoidal and activity against irritable bowel syndrome.

5. REFERENCES

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