THE ANTI-ALLERGIC ACTIVITY OF *BUCHANANIA LANZAN* SPRENG. LEAVES ON MAST CELL MEDIATED ALLERGIC MODELS.

**Running title:** Shevale: *Buchanania lanzan* spreng. On mast cell mediated allergic models.

V. N. Shevale, V. M. Chandrashekhar, I. S. Muchandi, V. G. Saudagar.

Department of Pharmacology, BVVS’S HSK College of Pharmacy Bagalkot - 587101

**Abstract:**

**Objectives:** The purpose of this study was to assess the anti-allergic action of Methanol extract of plant Buchanania lanzan Spreng. leaves on mast cell intermediated allergic models.

**Materials and methods:** The methanol extracts of plant Buchanania lanzan spreng. Leaves protecting effect is checked in pruritus and anaphylaxis models of the acute phase of allergic reactions induced by compound 48/80. The mast cell degranulation and histamine release from blood induced by compound 48/80 is determined.

**Results:** The methanol extract of Buchanania lanzan Spreng. Leaves produced noteworthy inhibition of pruritus and anaphylaxis induced by compound 48/80 and Dose-dependent noteworthy Stabilization of mast cell membrane is also observed in compound 48/80 induced mast cell activation. It is observed that the dose-dependant decrease in the release of histamine.

**Conclusion:** These results show the methanol extract of plant Buchanania lanzan Spreng. Leaves by inhibition of histamine release from mast cells have efficient anti-allergic property.

**Keywords:** Compound 48/80, Anti-allergic, Mast cells, Buchanania lanzan, Antihistaminic.

**Corresponding author:**

Vijaykumar Shevale,

Department of Pharmacology,

SGM College of Pharmacy, Mahagaon, Gadhinglaj,

Dist: Kolhapur, Maharashtra-416503.

Tel.: +91 8867956766.

E-mail address: vijayshevale5@gmail.com.

Please cite this article in press as Vijaykumar Shevale et al., *The anti-allergic activity of buchananiana lanzan spreng. Leaves on mast cell mediated allergic models*, Indo Am. J. P. Sci, 2018; 05(02).
INTRODUCTION:
The exposure of human or animal with a variety of agents (allergens) causes a hypersensitivity reaction by reacting with an immune system.[1] In conditions such as asthma, food allergies, hay fever and drug-induced allergies are the type I allergy (Immediate hypersensitivity). Which is an immunoglobulin E (IgE) mediated immune response. The worldwide population of patients with these state is increasing.[2] The essential constituent of all organs and tissue, mast cells are important mediators for hypersensitivity and anaphylaxis reactions. The histamine discharge causes anaphylaxis in response to cross-linking of antigens and IgE antibodies bound to FcεRI on mast cells. The degranulation process of mast cells by their activation results from the release of allergic mediators such as histamine, as well as other inflammatory and pro-inflammatory mediators such as prostaglandins, leukotrienes, proteases and chemotactic cytokines.[3] The compound 48/80 stimulate a mast cell degranulation. Hence, it is a suitable agent to understand the process of anaphylaxis.[4] The inflow of calcium ions from the extracellular atmosphere into the cytoplasm by ion-gating process leads histamine release. It is studied that this mechanism may be inhibited by a variety of anti-allergic compounds.[5]

The Buchanania lanzan Spreng. is an extensively used traditional medicinal plant for the cure of different diseases.[6] Preliminary phytochemical tests of the different leaf extracts of Buchanania lanzan shows the presence of glycosides, flavonoids and phenolic compound.[7] The bark of Buchanania lanzan Spreng. is used to treat disease related to abdomen, bronchitis and cough. The plant parts like roots, leaves, fruits, seeds and gum are used for the treatment of a blood disorder, ulcers, fever, burning sensation of body, dysentery, diarrhea, asthma and snakebite.[8,9] The Buchanania lanzan spreng. was assessed for the Antimicrobial, Anti-ulcer, Analgesic and anti-inflammatory activity.[10,11,12] In this study, we checked the anti-allergic effect of Buchanania lanzan spreng. on mast cell intermedated allergic rat models.

MATERIALS AND METHODS:
Chemicals and instruments
The Compound 48/80, Griess reagent, Toluidine blue were procured from the Sigma Chemical Co. (St. Louis, MO, USA). The Disodium Cromoglycate (DSCG), RPMI-1640 medium (AT028) and o-Phthalaldehyde were obtained from Hi-Media Lab. Pvt. Ltd, Mumbai, India. The Refrigerated centrifuge (MPW-350R) from MPW Med. instrument, Warszawa, Poland and UV-Spectrophotometer (UV-1601) Shimadzu, Japan, Fluorimeter (Model No. DX-300), Pune, India. All other chemicals and reagents used were of analytical grade.

Plant material and Preparation of plant extract
In the present study, the leaves of Buchanania lanzan Spreng. were collected from the Khanapur, Belgaum Dist., Karnataka, India. The sample of prepared herbarium was recognized and authenticated in Department of Botany, Basaveshwar Science College, Bagalkot, Karnataka. A voucher specimen (B.sc./Bot./55/2013) was deposited in the same college. All leaves are dried at room temperature until they were free from moisture. Then, the coarse powder of leaves was prepared using pulverizer. After this, to get uniform powder the coarse powder was passed through the sieve (# 44). The powdered leaves were subjected to consecutive extraction with methanol (60-65oC). After the residue taking out, a solvent was distilled out and with the help of rotatory flash evaporator, extra solvent with residue was removed to get concentrate, then dried in a freeze dryer (Mini Lyotrap, LTE Scientific Ltd., Great Britain) and stored in a sealed container in refrigeration. The obtained Methanol extract of Buchanania lanzan (MEB) then used for the anti-allergic activity.

Animals
Male Wistar Albino rats (200-250g) and Swiss Albino mice (20-25g) were acquired from the Central animal house of H.S.K. College of Pharmacy & Research Centre, Bagalkot. The animals were housed in standard atmospheric conditions (temperature 25±1oC, comparative humidity 50-55%) for 12hrs dark and 12hrs night cycle respectively. The standard laboratory feed (Pranava Agro Industries LTD, Sangli, Maharashtra) were given to animals and water ad libitum. The study was started after getting a clearance certificate from the Institutional Animal Ethical Committee, as per the CPCSEA guidelines (F. No. HSK College of Pharmacy, Bagalkot/IAEC, Clear/2012-13/1-12).

Acute toxicity study
The female Swiss albino mice (25-30g) were used and acute toxicity was studied according to the OECD guidelines-425. The limit dose of 5000mg/kg (p.o.) was administered and they were observed for behaviour and other signs of toxicity such as twitches, respiratory changes, righting reflex and motor coordination for 4hrs and monitored up to 14 days. No mortality has occurred with higher limit dose.
**Anti-pruritic activity**

The subcutaneous injection of Compound 48/80 at a dose of 3mg/kg was given to the back side surface of the neck of Swiss Albino mice to induce scratching behavior (SB). The DSCG at a dose of 10mg/kg as standard drug and a methanol extract of *Buchanania lanzan* Spreng. was given at doses of 100, 200 and 400mg/kg orally, 1hr before administration of compound 48/80. Then, after administration of compound 48/80, the occurrences of scratching behavior (SB) on the entire body and the site of the injected region were counted for 20min.[15]

Scratching (%) = No. of SB in control group – No. of SB in treated group × 100

No. of SB in control group

**Anti-anaphylaxis activity**

The mice were given compound 48/80 at a dose of 8mg/kg by an intraperitoneal injection to induce anaphylaxis. A mast cell stabilization compound DSCG as a standard drug (10mg/kg) and methanol extract of *Buchanania lanzan* Spreng. is given at doses of 100, 200 and 400mg/kg orally, 1 hr before to administration of compound 48/80 (n = 10). Mortality was screened for 1 hr after induction of anaphylactic shock.[13,14]

Mortality (%) = Number of dead mice × 100

Total number of experimental mice

**Mast cell stabilizing activity**

The methanol extract of *Buchanania lanzan* Spreng. at doses of 100, 200 and 400 mg/kg and a DSCG at a dose of 10mg/kg as a standard drug was administered to rats every day 5days prior to the collection of mast cells. The anesthetic ether is used to produce anesthesia in rats and after anesthesia, blood samples were collected by cardiac puncture and these samples were incubated with compound 48/80 (1µg/ml) and histamine present in cells was released due to destruction of the cells structure with perchloric acid and centrifugation at 400xg for 5min at 4oC. The amount of histamine was determined by the o-phthalaldehyde spectrofluorimetric method.[17]

**Statistical analysis**

All the data established as mean ± SEM. The one-way analysis of variance (ANOVA) followed by multiple comparisons Tukey test was used to determine the significance of the difference between the means of control and treated animals for different parameters. The (P<0.05) is considered as significant.

**RESULTS:**

**Anti-pruritis activity**

As compared to control group the percentage of scratching behavior was reduced in mice treated with standard drug (DSCG) and methanol extract of *Buchanania lanzan* Spreng. The standard group of animals showed 37.71% of protection and the 100, 200 and 400mg/kg treated groups of animals showed 16.81%, 22.12% and 59.42% of protection respectively from scratching occurrences. The results are summarized in Table 2 and Fig. 1.

<table>
<thead>
<tr>
<th>Table 1: Effect of <em>Buchanania lanzan</em> extract on anaphylaxis reaction in Swiss albino mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment / conc. (mg/kg)</strong></td>
</tr>
<tr>
<td>Normal (saline 1ml/kg; p.o.)</td>
</tr>
<tr>
<td>Control (comp. 48/80)</td>
</tr>
<tr>
<td>Standard (DSCG)</td>
</tr>
<tr>
<td>MEB 100</td>
</tr>
<tr>
<td>MEB 200</td>
</tr>
<tr>
<td>MEB 400</td>
</tr>
</tbody>
</table>

All the values are expressed as a mean ± SEM, n=10, MEB: Methanol extract of *Buchanania lanzan* DSCG=Disodium Cromoglycate.
Table 2: Effect of *Buchanania lanzan* extract on compound 48/80 induced allergic reactions.

<table>
<thead>
<tr>
<th>Treatment / conc. (mg/kg)</th>
<th>Number of Scratching Behaviors</th>
<th>Activated mast cells</th>
<th>Blood Histamine Content (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (saline 1ml/kg; p.o.)</td>
<td>3.833±0.4773</td>
<td>16.50±2.901</td>
<td>0.05025±0.00888</td>
</tr>
<tr>
<td>Control (comp. 48/80)</td>
<td>40.67±1.961a</td>
<td>68.25±2.689a</td>
<td>0.1525±0.0165a</td>
</tr>
<tr>
<td>Standard ( DSCG)</td>
<td>25.33±1.229***</td>
<td>36.75±1.750***</td>
<td>0.06425±0.00540***</td>
</tr>
<tr>
<td>MEB 100</td>
<td>33.83±1.447ns</td>
<td>51.50±3.571*</td>
<td>0.0375±0.02094***</td>
</tr>
<tr>
<td>MEB 200</td>
<td>31.67±2.754*</td>
<td>41.25±3.637***</td>
<td>0.0350±0.01168***</td>
</tr>
<tr>
<td>MEB 400</td>
<td>16.50±2.125***</td>
<td>17.75±3.351***</td>
<td>0.02817±0.0050***</td>
</tr>
</tbody>
</table>

All the values are expressed as a mean ± SEM, n=4, \(p<0.05\) was considered as significant. \(ap<0.001\) as compared with normal group (Student’s \(t\)-test). \(*p<0.05, **p<0.01, ***p<0.001\) as a comparison to control group (One Way Analysis of Variance (ANOVA) followed by multiple comparison Tukey test). MEB= Methanol extract of *Buchanania lanzan*, DSCG=Disodium Cromoglycate.

**Fig. 1:** Effect of methanol extract of *Buchanania lanzan* Spreng. on pruritis in Swiss albino mice.

All the values are expressed as a mean ± SEM, n=4, \(p<0.05\) was considered as significant. \(ap<0.001\) as compared with normal group (Student’s \(t\)-test). \(*p<0.05, **p<0.01, ***p<0.001\) as a comparison to control group (One Way Analysis of Variance (ANOVA) followed by multiple comparison Tukey test). MEB= Methanol extract of *Buchanania lanzan*, DSCG=Disodium Cromoglycate and ns= non-significant.
Peritoneal mast cell

![Graph showing the effect of various treatments on mast cell degranulation](image)

**Fig. 2: Effect of methanol extract of *Buchanania lanzan* on mast cell degranulation in rats.**

All the values are expressed as a mean ± SEM, n=4, *p*<0.05 was considered as significant. *ap*<0.001 as compared with normal group (Student’s *t*-test). *p*<0.05, **p*<0.01, ***p*<0.001 as a comparison to control group (One Way Analysis of Variance (ANOVA) followed by multiple comparison Tukey test). MEB= Methanol extract of *Buchanania lanzan*, DSCG=Disodium Cromoglycate.

---

Estimation of Histamine from blood

![Graph showing the effect of various treatments on blood histamine levels](image)

**Fig. 3: Effect of methanol extract of *Buchanania lanzan* on blood histamine levels in rats.**

All the values are expressed as a mean ± SEM, n=4, *p*<0.05 was considered as significant. *ap*<0.001 as compared with normal group (Student’s *t*-test). *p*<0.05, **p*<0.01, ***p*<0.001 as a comparison to control group (One Way Analysis of Variance (ANOVA) followed by multiple comparison Tukey test). MEB= Methanol extract of *Buchanania lanzan*, DSCG=Disodium Cromoglycate.
Anti-anaphylactic activity
The compound 48/80 induced mortality protection was noteworthy (p<0.001). The standard group of animals showed 70% protection and treated groups at doses 100, 200 and 400mg/kg showed 20, 20 and 40% of protection respectively from mortality. The results are summarized in Table 1.

Mast cell stabilizing activity
There was significant (p<0.001) protection from mast cell degranulation induced by compound 48/80. The standard group of animals showed 63.25% of protection and treated groups of animals at doses 100, 200 and 400mg/kg of MEB showed 48.50, 58.75 and 82.25% of protection from mast cell degranulation respectively. The results are summarized in Table 2 and Fig. 2.

Effect on blood histamine release
The significant increase of histamine release in control group of animals blood as compared to the normal group. The group of animals treated with standard and methanol extract of Buchanania lanzan Spreng. showed were showed significantly (p<0.001) decrease in histamine level in the blood as the contrast to the control group. The results are summarized in Table 2 and Fig. 3.

DISCUSSION AND CONCLUSION:
The Buchanania lanzan Spreng. leaves have anti-allergic properties and it was demonstrated by results of this study. The compound 48/80 induced systemic allergic reactions are inhibited by Buchanania lanzan Spreng. We have observed a significant protection from mast cell degranulation and a significant reduction of the release of blood histamine content in rats and significant reduction in anaphylaxis and pruritis in mice with 5 days of short-term treatment.

In the immediate type of hypersensitivity reactions, the mast cells play a crucial role by releasing a variety of chemicals and cytokines. The compound 48/80 is also used to produce degranulation of mast cells for to understand the process of anaphylaxis with the additional convenience [18]. The compound 48/80 activates mast cell and triggers signal transduction pathway, which causes histamine release from mast cells. The subcutaneous administration compound 48/80 to control and treated group induces antipruritic activity and it was determined by observing the frequency of scratching behavior. The considerable increase in a number of scratching by release of the histamine is due to mast cell degranulation triggered by compound 48/80. The group of animals treated with extract of Buchanania lanzan Spreng. showed significant dose dependent inhibition of scratching as compared to control group.

The compound 48/80 induced anaphylactic shock is a result of the release of vasoactive agents such as histamine from mast cells and basophiles [19]. Anaphylaxis is called as systemic allergic reaction because it is caused by the release of histamine and other inflammatory chemical mediators in blood. The methanol extract of Buchanania lanzan showed significant dose dependent (100-400mg/kg) protection from anaphylaxis produced by compound 48/80 as compared to control group.

The compound 48/80 and other more than two replaceable hydrogen containing compounds activate G-proteins and it is confirmed by a number of recent researches [20]. The Compound 48/80 causes the deviation of a membrane which enhances the lipid bilayer membrane permeability. For the degranulation of mast cells the role of intracellular calcium ions was important because the agents which are involved in increasing the amount of intracellular calcium ions have been responsible to cause mast cell degranulation[21]. The level of cAMP and intracellular calcium pathways plays role in the degranulation of mast cells. The amount of intracellular cAMP increasing agents has been involved in reduction of mast cell degranulation. It is believed that increases of cAMP precede the blocking of release of histamine from mast cells due to the stimulation of IgE receptors [22]. The movements of the calcium ions in mast cells symbolize a main target for efficient anti-allergic drugs, because this is a crucial event related to stimulation of secretion. The methanol extract of Buchanania lanzan Spreng. Inhibits histamine release and stabilize mast cells. It is considered that these actions might be produced by inhibiting G-Protein activation and decreasing intracellular calcium level in mast cells [4]. Several flavonoids have been shown to bronchodilator action and smooth muscle relaxant activity [23].

In the present study, it has been concluded that the methanol extract of Buchanania lanzan Spreng. Has a beneficial effect for the treatment of the allergic disorder. In addition, further studies are required to clear molecular mechanism of this extract of the plant to investigation for the successful development of the drug for clinical use.

ACKNOWLEDGEMENT:
This work was supported by the Department of Pharmacology, H.S.K. College of Pharmacy, Bagalkot-587101, Karnataka, India.
REFERENCES: