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(RESEARCH ARTICLE)

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Phytochemical and pharmacological activity of *Bauhinia acuminata*

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Abstract

Background: Over the history of traditional medicine, medicinal plants have been essential because they offer a wealth of therapeutic agents for a variety of illnesses. The project work has been designed to investigate the plant *Bauhinia acuminata*, a mangrove perennial herb (family Fabaceae), for its phytochemical nature, antioxidant, analgesic, anti-inflammatory, and antidiabetic activity.

Methods: Phytochemical analysis was done by using standard phytochemical reaction methods. We assessed antioxidant potential by employing the free radical scavenging method. The analgesic, anti-inflammatory, and antidiabetic analyses were done by observing writhing movement inhibition, inhibition of paw edema, and blood glucose levels in mice.

Results: Phytochemical analysis of the methanol extract of *B. acuminata* indicated the presence of glycosides, alkaloids, proteins, saponins, and reducing sugars. The IC_{50} value of ascorbic acid was 7.8 µg/ml and that of the extract was 25.02 µg/ml. The oral administration of 250 mg/kg and 500 mg/kg doses of *Bauhinia acuminata* extract inhibited 37.78% and 57.78% of writhing movements compared to the standard drug diclofenac-Na 82.22% writhing movement inhibition. The present study showed 45.45% and 69.07% inhibition of paw edema at the doses of 250 mg/kg and 500 mg/kg which is compared to standard drug Diclofenac-Na (75mg) 76.56% inhibition of paw edema representing significant anti-inflammatory activity. The extract at the doses of 250 mg/kg and 500 mg/kg was also found to lessen blood glucose levels decrease 47.06% and 48.73% in alloxan-induced diabetic mice compared to the standard drug Diclofenac-Na (75mg) 56.12%.

Keywords: *Bauhinia acuminata*; Free radical scavenging assay; Diclofenac-Na; Alloxan; Writhing movement

1. Background

The plants that have therapeutic properties or exert helpful medicinal impact on the living body square measure are usually referred to as “Medicinal plants”. According to the World Health Organization, around 80% of the world's developing population receives their main medical treatment from traditional medicines, which can be derived from a range of medicinal plants. [1] Medicinal plants could also be outlined as a bunch of plants that possess some special properties or virtues that quality then as articles of medicine and therapeutic agents and square measure used for healthful functions. Medicinal plants are used as natural medicinal products. This observation has been occurring since prehistoric times. [2] Modern approaches to deciding the medicinal properties of plants involve cooperative efforts that will embody anthropologists, pharmacists, pharmaceutical, chemists, and physicians. Many different chemical components, such as alkaloids, flavonoids, terpenoids, and phenolic compounds, are found in medicinal plants. These

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substances can affect the human body in a variety of ways through their pharmacological actions, including analgesic, anti-inflammatory, antiviral, antibacterial, and antioxidant qualities. [3]

Bauhinia Acuminata is a species of flowering shrub native to tropical southeast Asia. This plant goes by several common names, such as small white Bauhinia, white orchid-tree, and snowy orchid-tree. This plant is a species of flowering shrub (Family: Fabaceae) renowned for its ornamental and medicinal uses. Relatively common among gardeners, this upright shrub is well-known for its beautiful blossoms that bear a striking resemblance to orchids. [4] This study has been designed to investigate the phytochemical properties, antioxidant potentiality as well as analgesic, anti-inflammatory, and antidiabetic activity of the plant.

2. Methods

2.1. Plant Collection and Identification

The plant *Bauhinia acuminate* was acquired in November 2017 from the Mirpur Botanical Garden, Dhaka, Bangladesh. In the Bangladesh National Herbarium, Dhaka, Bangladesh, a voucher specimen for this plant has been retained (Accession no. 38305). To extract all unwanted plant materials and sand, the leaves were picked and washed with water, air dried under light exposure (27 ° C-30 ° C for 7 days), pulverized in a mill, and stored in an airtight container for further analysis. After the extraction procedure, we decided to evaluate this plant's phytochemical screening, anti-oxidant, flavonoid content, analgesic, anti-inflammatory, and anti-diabetic activity.

2.2. Experimental Animals

Eight-week-old Swiss albino mice (27-30g) were purchased from Jahangirnagar University, Dhaka, Bangladesh, and were housed in animal cages under standard environmental conditions (22-25°C, humidity 60-70%, 12 hr. light: 12 hr. dark cycle). The mice were fed with a standard pellet diet taken from, Jahangirnagar University Dhaka. The animals used in this study were cared for in accordance with the guidelines on animal experimentation of our institute.

2.3. Drugs and chemicals

Acetic acid, HCl, H_2SO_4, NaOH, $FeCl_3$, potassium dichromate, metal magnesium, chloroform, DPPH were collected from the laboratory of Bangladesh University. The reagents were prepared by following standard methods. The standard drug Diclofenac-Na was purchased from Opsonin Pharma Limited of Bangladesh. Carrageenan was purchased from Otto Chemical, India. The standard drug, Metformin hydrochloride was purchased from Beximco Pharmaceuticals Ltd of Bangladesh. Alloxan monohydrate was purchased from Loba Chemie, India.

2.4. Phytochemical screening

2.4.1. Detection of Alkaloid

Mayer's Test

With 2ml aqueous extract solution in a test tube, 0.5 ml HCl (1%) was applied. Then they added 1ml of Mayer's reagent. A precipitate of creamy or white color was found which indicates alkaloid presence.

Dragendroff's Test

In a test tube, 2 ml of solution of the extract and 5 ml of dilute HCl (1%) were taken. Then 1ml of Dragendroff reagent was inserted. The presence of alkaloids suggests the orange-brown precipitate.

Hager's Test

0.5 ml HCl (1%) was added with 2ml aqueous extract solution in a test tube. Then 1ml of Hager's reagent was added. A yellow color precipitate was found which indicates the presence of alkaloids.

2.4.2. Detection of carbohydrates

Molisch's Test

5 ml solution of the extract was taken and then Molish's reagent and H_2SO_4 were added. A red-violet ring was produced at the junction of two liquids indicating the presence of carbohydrates.

2.4.3. Detection of Glycoside

1ml aqueous solution of the plant extract was treated with a few drops of NaOH solution. A yellow color indicates the presence of glycosides.

2.4.4. Detection of Steroid

Sulphuric acid test

1ml solution of extract was taken and then added 1ml H_2SO_4 acid. A red color was produced indicating the presence of steroids.

2.4.5. Detection of Tannin

Ferric Chloride Test

5ml solution of the extract was taken in a test tube. Then 1 ml of 5% $FeCl_3$ solution was added. A greenish-black precipitate was produced indicating the presence of tannins.

Potassium Dichromate test

5ml solution of the extract was taken in a test tube. Then 1 ml of 10% potassium dichromate solution was added. A yellow precipitate was produced indicating the presence of tannins.

Detection of Flavonoid

3 drops of 37% HCl were added with 2ml aqueous solution of extract and 0.5 gm metal magnesium was also added. A reddish color precipitate was not found which indicates the absence of flavonoids.

Detection of Terpenoids

1ml aqueous solution of extract was added with 2ml chloroform and 3ml Sulphuric acid was carefully added to form a layer. A reddish-brown coloration at the interface was produced indicating the presence of terpenoids.

2.4.6. Detection of Reducing sugar

Fehling's test

2ml of aqueous extract of the plant material was added to 1ml of a mixture of equal volumes of Fehling's solution A and B. A red or brick red color precipitate formation indicates the presence of a reducing sugar.

2.5. Test for Quantitative Antioxidant activity

Stock solution of the plant extract was prepared in ethanol from which a serial dilution was carried out to obtain the concentration of 1, 5, 10, 50, 100, 500 µg/ml. Diluted solutions (2ml) were added to 3 ml of a 0.004% ethanol solution of DPPH, mixed, and allowed to stand for 30 minutes for the reaction to occur. The absorbance was determined at 517nm and from these values corresponding percentage of inhibitions was calculated. Then % inhibitions were plotted against log concentration and from the graph IC_{50} was calculated. The experiment was performed 3 times and average absorption was noted for each concentration.

2.6. Procedures

- At first 6 volumetric flasks are taken to make 6 different types of concentration (1, 5, 10, 50, 100 and 500 µg/ml)
- Test tubes and volumetric flasks are wrapped with foil paper.
- In 6 volumetric flaks serial dilution of extract is done and marked them respectively.
- 1 ml of sample from each concentration and 3 ml of 0.004% DPPH solution are taken with the help of a pipette in 6 test tubes respectively.
- Then the solution is kept in a dark place for 30 minutes raping each test tube with foil paper.
- In another test tube, 3ml 0.004% DPPH and 1ml methanol are taken to prepare a blank solution.

Then absorbance is taken by UV Spectroscopy.

The percent of inhibition is calculated by using the following formula____

$$\% \text{ inhibition} = \frac{(Blank\ absorbance - \ sample\ absorbance)\ X100}{Blank\ absorbance}$$

2.7. Test for Analgesic activity

2.7.1. Acetic acid-induced writhing test for Analgesic activity

The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice. Test samples and vehicle were administered orally 30 mins before intraperitoneal administration of 1% acetic acid but Diclofenac-Na was administered intraperitoneally 15 mins, the mice were observed for specific contraction of a body referred to as "writhing" for the next 10 mins.

2.8. Test for Anti-inflammatory activity

2.8.1. Induction of Inflammation

Inflammation in mice was induced by injecting 0.1ml of 1% carrageenan in physiological saline into the sub-plantar tissue of the left hind paw of each mouse. Paw edema was induced by injecting 0.1ml of 1% Carrageenan in physiological saline into the sub-plantar tissues of the left hind paw of each mouse.

The methanol extract of *Bauhinia acuminata*. Leaves 500 mg/kg were administered orally 30 min prior to Carrageenan administration. The paw volume was measured at 60, 120, 180, and 240 minutes by using a micrometer screw gouge. The percentage inhibition of paw volume in drug treated group was compared with the control group. Diclofenac sodium (10 mg/kg p.o.) was used as the reference standard.

2.9. Test for Anti-Diabetic activity

2.9.1. Induction of Diabetes

After fasting for 16 hr., diabetes was induced in mice by an intra-peritoneal injection (i. p.) of alloxan monohydrate (90 mg/kg), dissolved in saline (100 µl/mice, ip.). After 48 hours, plasma glucose levels were measured by OK meter Match glucometer (Hsinchu, Taiwan) using a blood sample from the tail-vein of mice. Mice with blood sugar levels higher than 08.5-11.5 mmol/l are considered as diabetic. Age-matched healthy mice were used to examine the effects of the extract on normal mice.

2.9.2. Anti-Diabetic Test in Alloxan-Induced Diabetic Mice

All mice received after half an hour of the feeding of extract and/ drug and four-time blood samples were collected at 60-, 120-, 180- and 240-minute intervals, and blood glucose levels were estimated in all the experiments by using the OK meter (Match glucose test meter).

3. Results

Table 1 Results of phytochemical tests

Test	Result
Alkaloid	+
Carbohydrate	+
Glycoside	+
Steroid	+
Tannin	+
Flavonoid	-
Terpenoid	+
Reducing Sugar	+
Phenolic Compound	+

(+) = Presence; (-) = Absence

The tests carried out on *Bauhinia acuminata* methanol extract revealed the presence of several important phytochemicals which might be responsible for its medicinal properties.

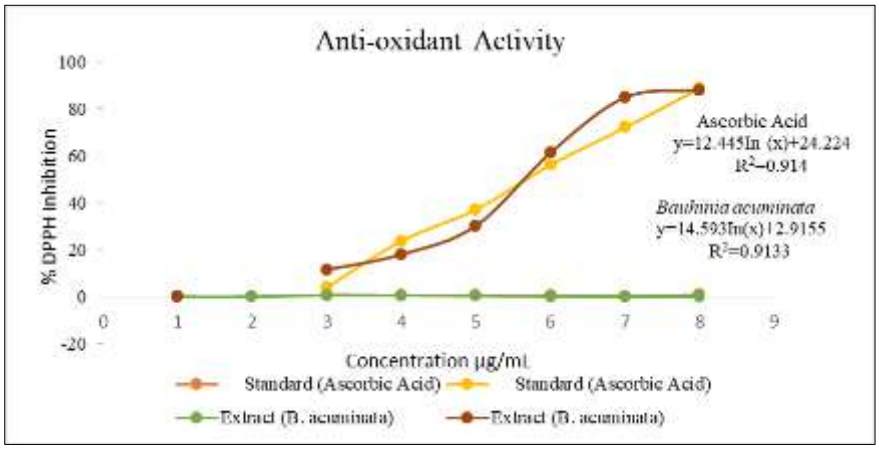

Figure 1 Anti-oxidant activity of *Bauhinia acuminata* and ascorbic acid

From the graph IC_{50} values are:

Ascorbic Acid	7.8µg/ml
Bauhinia acuminata	25.02µg/ml

Values were expressed in (mean ± SEM) value. Each group comprised 4 animals (n=4); compared to the Control Group. Control Group (normal water), Standard Group received Diclofenac-Na(10mg/kg) body weight, Extract Group was treated with 250 mg/kg and 500mg/kg body weight (p.o) of the crude extract of *Bauhinia acuminata.*

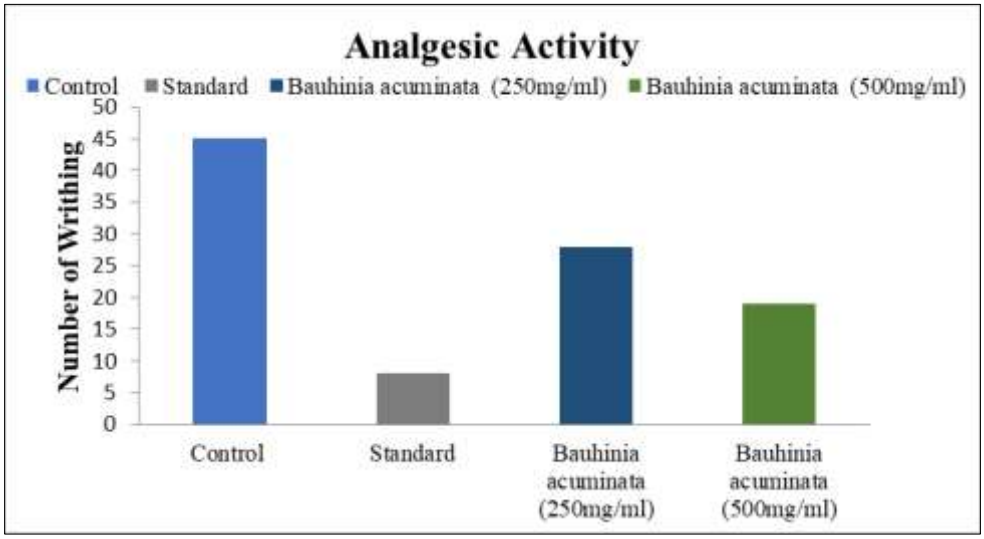

Figure 2 Effects of the Methanol extract of *Bauhinia acuminate*

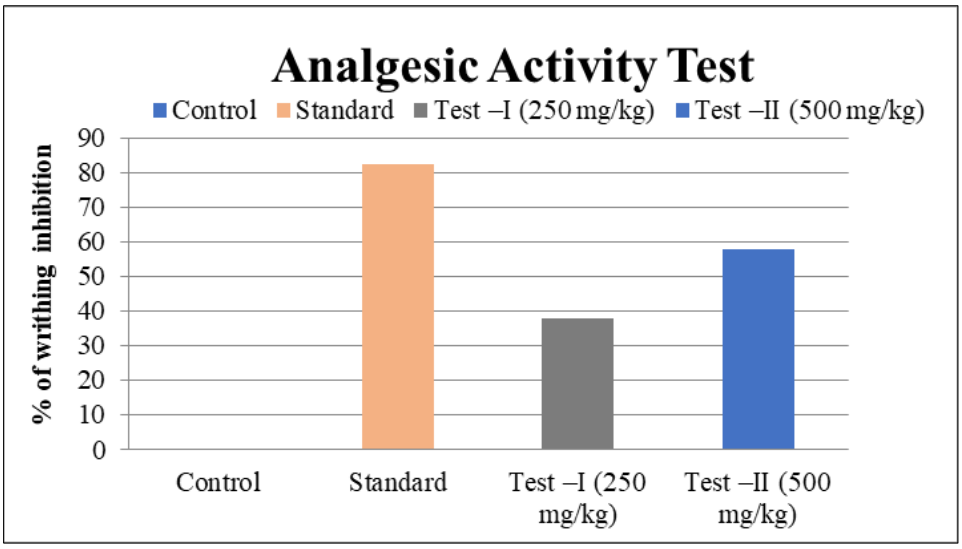

Figure 3 % inhibition of methanol extract of *Bauhinia acuminata*

Table- 3 and Figure-2 and 3 show the effects of the extract of acetic acid-induced writhing in mice. The oral administration of doses 250 mg/kg and 500 mg/kg of extract of *Bauhinia acuminata* inhibited 37.78% and 57.78% of writhing movements compared to standard drug diclofenac-Na 82.22% writhing movement inhibition.

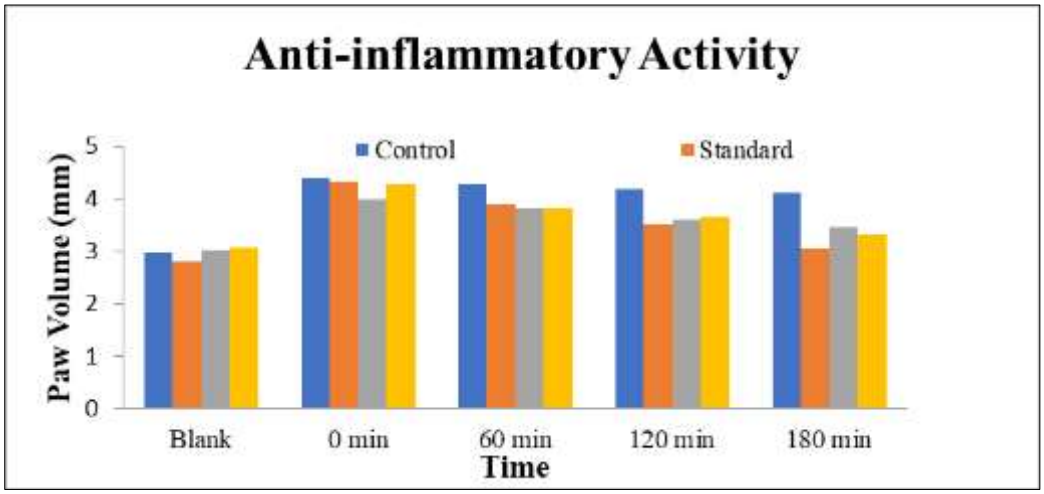

Figure 4 Effects of the methanol extract of *Bauhinia acuminata* on Carrageenan-induced paw edema in mice

Values were expressed in (Mean±SEM) value. Each group comprised 4 animals. The Control Group received 0.5% Methylcellulose and the Standard Group received 5 mg/kg Diclofenac sodium. Extract Group was treated with 250mg/kg and 500mg/kg of the crude extract of *Bauhinia acuminata*

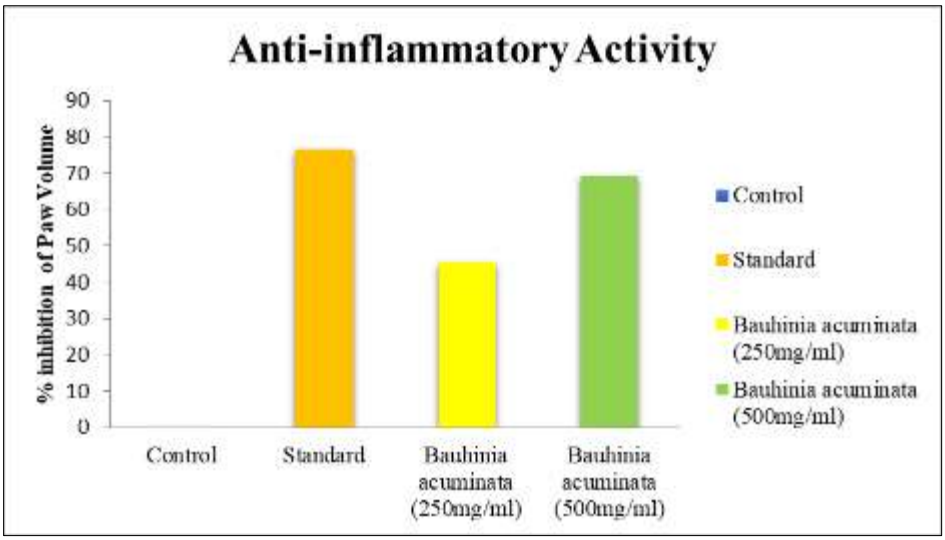

Figure 5 Percent of inhibition effects of the methanol extract of *Bauhinia acuminata* on Carrageenan induced paw edema in mice

After oral administration of 1% Carrageenan, paw edema in mice was significantly higher in control and experimental groups of mice as shown in Table-4 and 5 and Figure-4 and 5. Mice treated with the extract of *Bauhinia acuminata* (250 mg/kg and 500mg/kg) posed a significant decrease in paw volume from 0 min to 180 min compared with mice of the control group. Here highly pronounced effects of 45.45% and 69.07% inhibition of paw edema at the doses of 250 mg/kg and 500mg/kg extract were observed respectively and this effect is closely similar to that of standard group 76.56% inhibition of paw edema.

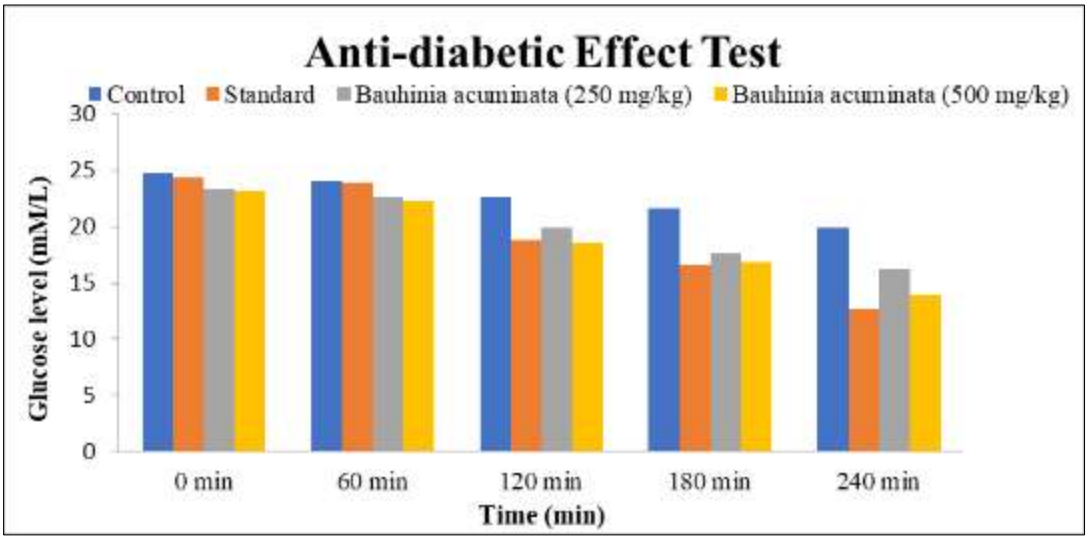

Figure 6 Effects of the methanol extract *Bauhinia acuminata* on diabetic mice

Values were expressed in (Mean ± SEM) value. Each group comprised 4 animals. Control Group (Normal water) and Standard Group received 100mg/kg Metformin. The extract group was treated with 250mg/kg and 500mg/kg body weight of the crude extract of *Bauhinia acuminata.*

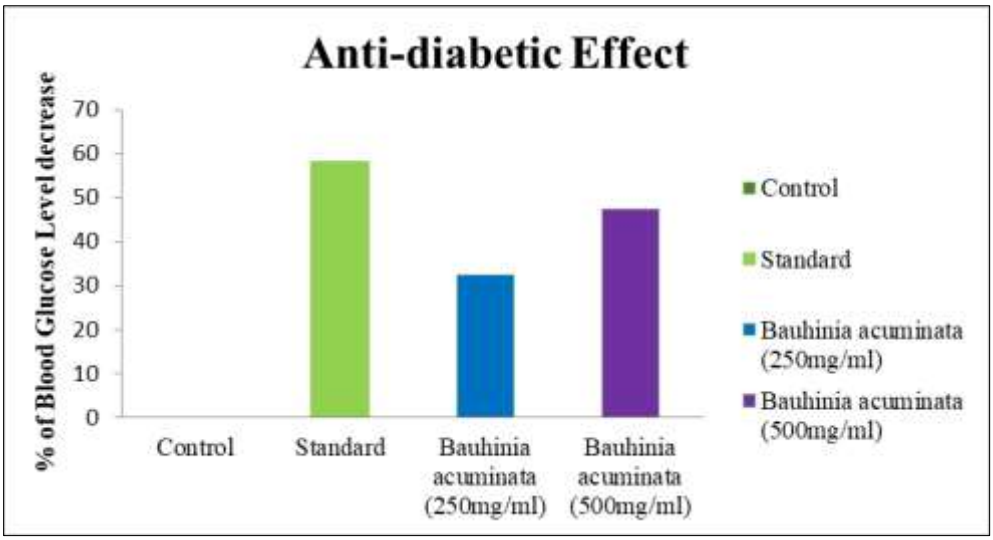

Figure 7 % of Blood Glucose Level decrease

After administration of Alloxan, the blood glucose levels were significantly higher in control and experimental groups of mice as shown in Tables 6-7 and Figure 6-7. The increase in blood glucose concentration was observed after 48 hours. Mice treated with extract in Group (250 mg/kg and 500 mg/kg), showed a significant decrease (32.36% and 47.35%) in blood glucose concentration from 60 min to 240 min. Prominent effects were observed with the extract group (250 mg/kg and 500 mg/kg) and this effect is like that of the standard group (58.30%).

4. Discussion

The phytochemical analysis of the methanol extract of *Bauhinia acuminata* conducted in this study revealed the presence of glycosides, alkaloids, proteins, saponins, and reducing sugars. Phytochemicals, including alkaloids and glycosides, are known for their diverse biological activities and have been extensively studied for their medicinal properties. The presence of these compounds in *B. acuminata* suggests its potential as a source of bioactive molecules.

The assessment of antioxidant activity is crucial due to the role of antioxidants in neutralizing harmful free radicals in the body. [5] The observed IC_{50} value of 25.02 µg/ml for the extract indicates moderate antioxidant potential, comparable to the standard ascorbic acid. Antioxidant properties are often linked to the presence of phenolic compounds, flavonoids, and other secondary metabolites, which might be present in the extract, contributing to its antioxidant activity. [6]

Furthermore, the analgesic activity of *B. acuminata* extract was evaluated using writhing movements in experimental animals. The significant inhibition of writhing movements at doses of 250 mg/kg (37.78%) and 500 mg/kg (57.78%) suggests its potential analgesic effect. This finding is particularly noteworthy considering the well-established analgesic property of the standard drug diclofenac-Na, which exhibited 82.22% writhing movement inhibition.

The anti-inflammatory activity of the extract was evidenced by its ability to inhibit paw edema. At doses of 250 mg/kg and 500 mg/kg, the extract demonstrated inhibition rates of 45.45% and 69.07%, respectively, comparable to diclofenac-Na's inhibition of 76.56%. This significant reduction in paw edema indicates the extract's potential to mitigate inflammatory responses, highlighting its anti-inflammatory efficacy.

Moreover, the study explored the antidiabetic potential of *B. acuminata* extract in alloxan-induced diabetic mice. The extract, administered at doses of 250 mg/kg and 500 mg/kg, exhibited a considerable decrease in blood glucose levels (47.06% and 48.73%, respectively). Although slightly lower than diclofenac-Na's effect (56.12%), this reduction underscores the extract's ability to modulate blood glucose levels, suggesting its antidiabetic activity.

The findings of this study demonstrate the diverse pharmacological activities of *Bauhinia acuminata*. The presence of phytochemicals like alkaloids and glycosides in the extract may contribute to its observed analgesic, anti-inflammatory, and antidiabetic properties. However, further studies are warranted to isolate and identify specific bioactive

compounds responsible for these activities. This research provides valuable insights into the therapeutic potential of *B. acuminata*, emphasizing its significance in the development of natural remedies and pharmaceutical agents.

5. Conclusion

According to this study, it is stated that natural products exhibited many pharmacological activities like analgesic, anti-inflammatory, anti-diabetic activities. By proceeding with further studies, we can observe many beneficial activities that could be used in state of numerous medications with various adverse effects.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have no conflict of interest to disclose.

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