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In Vivo Pharmacological Evaluation of the Therapeutic Potential of *Ageratum conyzoides* Methanolic Leaf Extract: Analgesic, Anti-Inflammatory, And Antidiabetic Activities

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ABSTRACT

Ageratum conyzoides is an extensively distributed medicinal plant which has been used in various folk medical practices worldwide for pain relief and treatment of inflammatory conditions, fever, and metabolic syndromes. The aim of this study was to scientifically validate the in vivo pharmacological effects of methanolic extract of the leaves of *Ageratum conyzoides*, mainly its analgesic, anti-inflammatory, and antidiabetic properties. The analgesic activity was tested on mice using acetic acid induced writhing method. The methanolic extract showed 44.45% inhibition of writhing behavior in mice at a dose of 500 mg/kg body weight, while diclofenac sodium caused 82.22% inhibition of writhing. In the anti-inflammatory evaluation using carrageenan induced paw edema method, the extract showed 54.02% inhibition, whereas diclofenac sodium caused 80.39% inhibition. Antidiabetic effect was determined from the measurement of plasma glucose concentrations in diabetic mice at different periods of time. Blood glucose concentration was decreased by 46.03% due to the action of methanolic extract, and metformin decreased the plasma glucose concentration by 81.23%. From the above experiments, it was concluded that the methanolic extract of leaves of *Ageratum conyzoides* has potent analgesic and anti-inflammatory properties as well as moderate hypoglycemic activity. It might be due to the bioactivity of some phytochemicals. Further studies should be carried out on this plant for better characterization of its phytochemical components and mechanism of action.

Keywords: *Ageratum conyzoides*; Analgesic Activity; Anti-Inflammatory Activity; Antidiabetic Activity; Methanolic Extract; Carrageenan-Induced Paw Edema; Writhing Test; Phytochemicals.

1.0. Introduction

Natural medicinal herbs have been extensively utilized as sources of therapeutic agents for managing various human ailments. Many modern drugs have been developed based on natural compounds isolated from these plants. The recent increase in the number of research conducted on medicinal herbs is primarily due to the safety, cost-effectiveness, and multiple pharmacological activities of the plants [1]. One of the medicinal herbs that have received significant attention for its traditional usage in the management of pain, wound, inflammation, and metabolism-related diseases is *Ageratum conyzoides* of family Asteraceae [2].

Ageratum conyzoides is a popular medicinal herb that occurs widely across tropical and subtropical regions. The different parts of the herb have been traditionally utilized for treating fever, diarrhea, skin disease, and inflammation [2]. It is known to contain bioactive compounds such as flavonoids, alkaloids, tannins, saponins, and phenolic substances, which show a range of biological actions like analgesia, anti-inflammatory, anti-histamine, antioxidant, antimicrobial, and anti-diabetic activity [3-5].

Pain and inflammation are common ailments affecting many individuals across the globe. Pain often results from inflammatory reactions involving chemicals such as prostaglandins, histamine, and serotonin while inflammation, in turn, is a defensive reaction that leads to tissue damages if left unmanaged. Diabetes mellitus is another medical condition characterized by increased blood sugar level, leading to serious complications, including death. There are

various anti-diabetic medications and analgesics; however, their prolonged usage can cause adverse effects, leading to the need for developing safer options [6-7].

Although traditional medicinal herbs, including *Ageratum conyzoides*, are increasingly used for managing pain, inflammation, and diabetes, little is known about their scientific validation in relation to their medicinal values and applications. For example, despite its well-established traditional uses, very little data exist on the in vivo pharmacological potentials of *Ageratum conyzoides*. In this context, the present investigation has been conducted to explore the effect of methanolic extract of leaves of *Ageratum conyzoides* on analgesia, inflammation, and diabetes. This research will provide scientific support for the ethnobotanical importance of this plant and help develop new drug candidates.

1.1. Study Objectives

The primary aim of the current investigation was to determine the possible pharmacological potentials of the methanolic leaf extract of *Ageratum conyzoides* through animal experiments. The objectives were to:

1) Determine the analgesic potential of the methanolic leaf extract through the acetic acid-induced writhing assay on mice.

2) Determine the anti-inflammatory potential of the methanolic leaf extract through the carrageenan-induced paw edema assay.

3) Determine the antidiabetic potential of the methanolic leaf extract through determination of blood glucose concentration in diabetic mice.

4) Compare the obtained results with those of standard drugs, namely diclofenac sodium as the standard analgesic and anti-inflammatory drug and metformin as the antidiabetic drug.

2.0. Literature Review

The plant continues to attract scientific interest due to its multifunctional pharmacological activities such as antimicrobial, anti-inflammatory, antithrombotic, and cytotoxic effects. Recent works have shown new aspects of *Ageratum conyzoides* medicinal importance based on experiments involving both in vitro and in vivo techniques.

Recent studies of phytotherapeutics have shown that *Ageratum conyzoides* leaf extracts possess potent antibacterial and antibiofilm properties against Staphylococcus aureus and Escherichia coli bacteria. The GC-MS metabolomics approach revealed a number of active substances among which precocene-II and other terpenoids can be highlighted. Thus, the pharmacological significance in treating infectious diseases is proved [8].

Furthermore, a study published in 2025 showed that leaf extracts of *Ageratum conyzoides* demonstrated significant analgesic and anti-inflammatory effects based on the tests of rodent models using carrageenan-induced paw edema and tail flick tests. Thus, the presence of dose-dependent effects is confirmed in experiments [9].

Another in vivo study published in 2026 showed that aqueous extracts of *Ageratum conyzoides* significantly reduced inflammatory reactions in Wistar rats based on the carrageenan-induced paw edema and formalin tests.

Also, an in-silico study conducted in 2026 identified a variety of bioactive compounds with strong GPCR binding properties related to the metabolic and anti-aging pathways [10].

Overall, recent research has provided evidence for the existence of numerous pharmacological activities of *Ageratum conyzoides* such as anti-inflammatory, analgesic, antimicrobial, antithrombotic, and metabolic regulation effects. However, at the same time, there are no studies combining analgesic, anti-inflammatory, and antidiabetic effects together in one experiment.

3.0. Materials and Methods

3.1. Chemicals and Drugs

Acetic acid, diclofenac sodium, methanol, carrageenan, metformin, and alloxan monohydrate were purchased from Merck Co., Germany. All reagents were analytical grade.

3.2. Experimental animals

In the current research work, five-week-old Swiss albino mice weighing around 18-25 grams were used. The mice were procured from Jahangirnagar University, Dhaka, Bangladesh. Acclimatization was done for two weeks and then fed with conventional pellet food and water.

3.3. Collection of plants and preparation of extracts

Ageratum conyzoides plants were collected from Narayangonj, Dhaka, Bangladesh. Cold extraction or methanol extraction was carried out to clean up the resulting plant parts- the leaves- from any impurities like other plants or plant particles. They were cut into fine particles and left to dry under sunlight for a week. A proper grinders' aid was taken to crush the particles into fine powder. Then it was kept in an airtight container, away from moisture and heat, till required. A clean glass jar having flat bottom surface was used to take up around 430 gm of the dried powder substance which was steeped into 1200 mL methanol. After sealing the lid, the mixture inside was shaken periodically for ten days. This mixture was sieved roughly using a piece of white cotton cloth. Then the solution was poured onto the Whatman filter paper. For the crude extract, the paper filter was left aside under sun for the solvent to evaporate [11].

3.4. Analgesic activity

The writhing response was studied in mice model caused by acetic acid, in order to assess the analgesic potential of the extract. Five numbers of mice were used for each of six randomly selected groups. Group one, acting as the control group was provided with 10 mL per kg of body weight of 1% Tween-80 in distilled water. The standard drug, Diclofenac Sodium was given at a dose of 25 mg/kg body weight to group two. Group three to six were administered with extracts 250 and 500 mg/kg body weight, respectively. The experimental extracts, drug, and control solvent were administered through oral route 30 minutes prior to an intraperitoneal injection of 0.7% v/v acetic acid. Five minutes after, the number of writhes was recorded after 15 minutes. The percent writhing inhibition calculated in the experimental and standard treatments was compared to the writhing means in the control treatment through this formula:

$$\text{Writhing inhibition } (\%) = 1 - \frac{W0}{W1} \times 100 \text{ ----------(1)}$$

Where W_0 and W_1 are the writhing means of the control and sample/standard treatments, respectively [12-13].

3.5. Anti-inflammatory activity

To produce the paw edema (inflammation) of the plantar tissue of the left hind paw, an injection of 0.1 mL of 1% of carrageenan in physiologic saline was administered for each of the mice. Each animal was administered with oral dosage of 500 mg/kg of extract rind methanol extract thirty minutes prior to the injection of carrageenan. The measurement of the size of the paw edema using the sliding calipers was carried out after 0, 1, 2, 3 and 4 hours [14]. The percentage reduction in the size of paw edema was calculated when the drug was compared with the control group. Diclofenac Sodium, the reference drug, was used at a dosage of 5 mg/kg. The average size of paw was obtained at hour zero. The percent inhibition of the paw edema and the thickness of paws in the treatment groups were obtained [15-17]. The anti-inflammatory activity in percentage can be obtained from the following equation:

$$\text{Anti} - \text{inflammatory activity } (\%) = 1 - \frac{T}{C} \times 100 \text{ ----------(2)}$$

Where, T = Treatment Group, and C = Control Group [18].

3.6. Hypoglycemic Activity

Mice were intraperitoneally injected with 90 mg/kg of alloxan monohydrade in the form of solution in the saline following a fasting period of 16 hours. After 72 hours, blood glucose levels were measured through glucometer, Tyson, Taiwan from the sample taken from tail vein of the mouse. Mice whose blood glucose level exceeds 11.5 mmol/L were considered diabetic. All the mice were classified into four groups. Diabetic mice treated with methanol extract; standard, diabetic mice treated with 100 mg/kg Metformin; and normal control groups were formed. The following day after overnight fasting of mice, blood samples from each group were collected to measure blood glucose levels before feeding (0 min). Glucose 2 g/kg was given to each mouse. Then, extract was orally administered immediately. In each experiment, glucometer was used to measure blood glucose level and additional four samples of blood were collected at different times (60, 120, 180, and 240 min) [19-20].

4.0. Result and Discussion

4.1. Analgesic Activity

Analgesic effects of methanolic leaf extract of Ageratum conyzoides were investigated in mice using the acetic acid-induced writhing model. According to Table 1, the number of writhes in the control group was 45. When standard drug diclofenac sodium was used, the number of writhes decreased to 8, showing 82.22% inhibition. Treatment of mice with Ageratum conyzoides extract at a dose of 500 mg/kg reduced the number of writhes to 25, which corresponded to 44.45% inhibition, which is calculated by equation 1. The findings are demonstrated in Table 1 and shown in Figure 1.

Acetic acid-induced writhing test is one of the commonly used models for assessing peripheral analgesia. Writhing is caused by an intraperitoneal injection of acetic acid leading to the development of pain. Acetic acid triggers an

inflammatory response that involves an increase in prostaglandin levels resulting in writhes. Therefore, an inhibition of writhing suggests analgesic activity of the tested substance [13].

Methanolic leaf extract of *Ageratum conyzoides* showed moderate analgesic effects in comparison with the standard analgesic drug. Though the extract was less efficient than diclofenac sodium, it still inhibited the number of writhes in 44.45%. Thus, the results obtained confirm the presence of bioactive components in the extract that can inhibit pain. These effects may be due to the ability of the compound to act on pain-mediating substances, such as phytochemicals including flavonoids, phenolics, tannins, and alkaloids.

Thus, based on the results obtained, *Ageratum conyzoides* can be used for relieving pain and inflammation. The analgesic effects obtained provide grounds for considering *Ageratum conyzoides* as a source of natural analgesics. Further research is required to identify active components of the extract and study their modes of action.

Table 1. Effect of methanolic leaf extract of *Ageratum conyzoides* on acetic acid-induced writhing in mice. The number of writhes and percentage inhibition were recorded following treatment with the plant extract (500 mg/kg) and diclofenac sodium (25 mg/kg) compared with the control group.

Group	Number of Writhing	% Writhing Inhibition
Control	45	0
Standard	08	82.22
Ageratum conyzoides	25	44.45

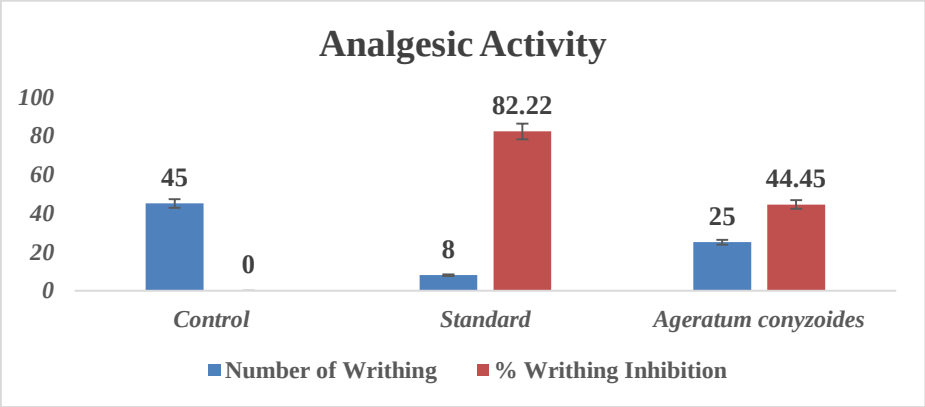

Figure 1. Percentage inhibition of acetic acid-induced writhing in mice after treatment with methanolic leaf extract of *Ageratum conyzoides* (500 mg/kg) and diclofenac sodium (25 mg/kg). Writhing inhibition was calculated relative to the control group to assess analgesic activity.

4.2. Anti-inflammatory Activity

The anti-inflammatory effect of the methanolic leaf extract of *Ageratum conyzoides* was determined using the carrageenan-induced paw edema technique on mice. As can be seen from the data obtained in Table 2, carrageenan injection successfully led to paw inflammation in all groups of animals. In the control group, paw inflammation was stable during the entire experiment period, where the paw volume reduced only minimally from 4.40 mm at minute 0 to 4.10 mm at minute 180.

In turn, the treatment with diclofenac sodium (10 mg/kg), which is a commonly used anti-inflammatory agent, reduced paw edema in animals significantly. The reduction was from 4.35 mm to 2.82 mm, or 80.39% inhibition of

paw inflammation. Likewise, the mice exposed to treatment with Ageratum conyzoides extract (500 mg/kg) experienced an increase in paw volume from 3.93 mm at minute 0 to 3.06 mm at minute 180. This provided a 54.02% inhibition of paw edema, which is quite high compared to the control group. The results are shown in Fig. 2.

Thus, the carrageenan-induced paw edema test is considered the established model of determination of the anti-inflammatory activity, which is calculated by equation 2. As mentioned above, the process of inflammation is associated with the production of inflammatory mediators such as histamine, serotonin, bradykinin, and prostaglandins, which are released from tissues. Hence, the reduction of paw edema seen after the exposure to the plant extract suggests that it interfered with the inflammatory processes.

As could be noticed, the effect seen from the anti-inflammatory action of *Ageratum conyzoides* was not as high as that of diclofenac sodium (80.39%). Nevertheless, the 54.02% of paw edema inhibition suggests a fairly good activity of the studied compound (Fig. 2). This may be linked with the presence of such phytochemicals as flavonoids, phenolics, tannins, and alkaloids, which have anti-inflammatory effects. The substances have been shown to have an ability to inhibit inflammatory reactions through inhibition of prostaglandins formation and decrease in oxidative stress within the inflamed tissue.

The results obtained have shown that the methanolic leaf extract from *Ageratum conyzoides* has substantial anti-inflammatory activity and suggest that the use of the plant is effective against inflammation.

Table 2. Anti-inflammatory activity of methanolic leaf extract of *Ageratum conyzoides* in carrageenan-induced paw edema in mice. Paw volume was measured at different time intervals after carrageenan administration, and the percentage reduction in paw edema was determined relative to the control group.

Group	Blank	0 min	60 min	120 min	180 min	% Decrease in paw volume
Control	2.98	4.40	4.28	4.18	4.10	
Diclofenac-Na 10mg/kg	2.65	4.35	3.88	3.50	2.82	80.39
Ageratum conyzoides	2.72	3.93	3.63	3.40	3.06	54.02

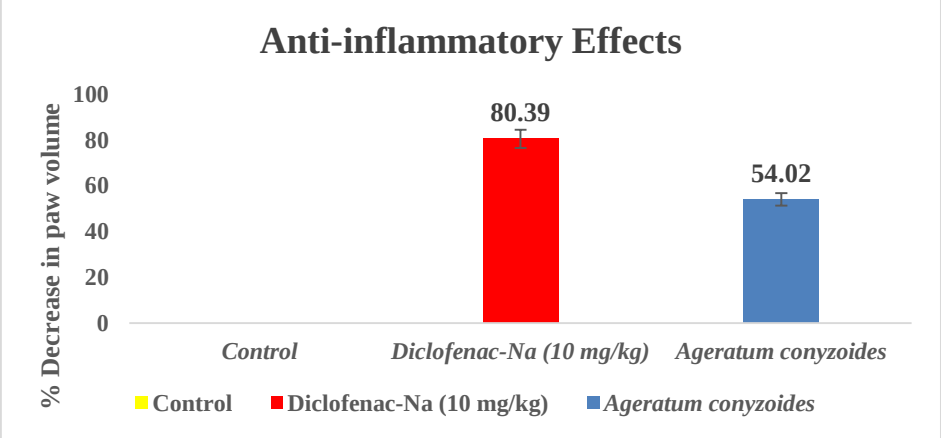

Figure 2. Effect of methanolic leaf extract of *Ageratum conyzoides* (500 mg/kg) and diclofenac sodium (10 mg/kg) on carrageenan-induced paw edema in mice. The graph presents the percentage reduction in paw volume compared with the untreated control group.

4.3. Anti-diabetic Activity

Evaluation of the antidiabetic activities of the methanolic extract of *Ageratum conyzoides* leaves on plasma glucose levels in diabetic mice for 240 minutes is given below. Table 3 shows the results obtained after conducting the experiment. It was observed that all experimental groups experienced an increase in blood glucose after glucose ingestion followed by a gradual decrease in the plasma glucose level.

The control group showed only a very minor change in blood glucose levels, suggesting the lack of glucose lowering activity. However, Metformin 100 mg/kg as the positive control showed marked hypoglycemic activity in this study with the decrease in plasma glucose from 26.96 mmol/L at 0 minutes to 6.67 mmol/L at 240 minutes, i.e., 81.23% decrease in plasma glucose level.

In comparison, the methanolic extract of *Ageratum conyzoides* 500 mg/kg caused a 46.03% decrease in plasma glucose level, which is displayed in Fig. 3. From the results obtained, *Ageratum conyzoides* can be said to have moderate antidiabetic activity. Though less effective compared to metformin, it still showed marked reductions in plasma glucose levels. Antidiabetic activity of this plant extract could be attributed to various bioactive constituents including flavonoids, phenolics, tannins, and alkaloids [21-22].

Table 3. Effect of methanolic leaf extract of *Ageratum conyzoides* on blood glucose levels in alloxan-induced diabetic mice. Blood glucose concentrations were measured over a 240-minute period and compared with those of metformin-treated and control groups.

Group	0 min	60 min	120 min	180 min	240 min	% lessening of Plasma glucose level
Control	24.75	24.03	22.6	21.68	20.94	
Metformin 100mg/kg	26.96	20.88	14.8	10.65	6.67	81.23
Ageratum conyzoides	27.96	26.43	23.73	21.06	20.90	46.03

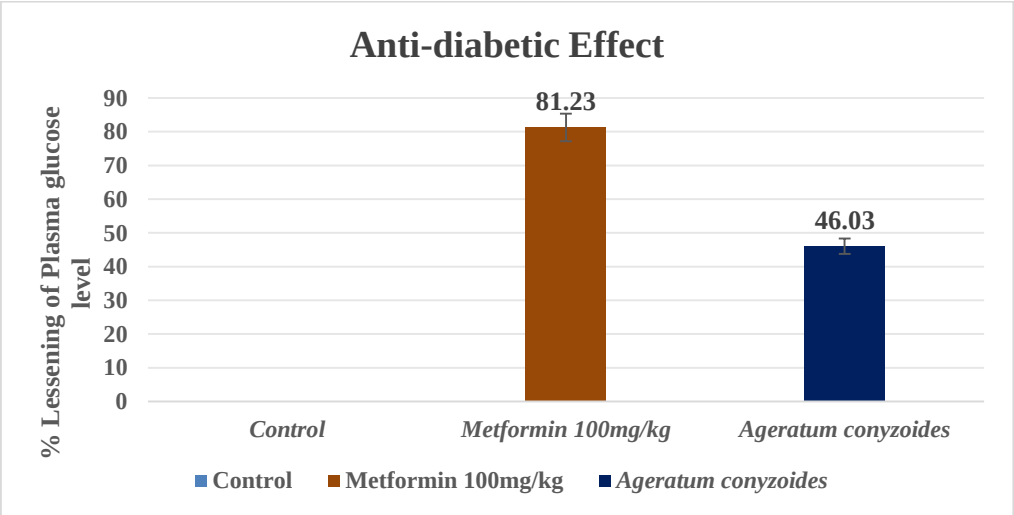

Figure 3. Antidiabetic activity of methanolic leaf extract of *Ageratum conyzoides* (500 mg/kg) and metformin (100 mg/kg) in alloxan-induced diabetic mice. The graph shows the percentage reduction in plasma glucose levels during the experimental period.

5.0. Conclusion and Further Studies

It is evident from this study that methanolic leaf extract of *Ageratum conyzoides* has remarkable pharmacological activity, such as analgesic, anti-inflammatory and antidiabetic properties. The extract showed significant analgesic activity with a percentage inhibition of writhing at 44.45% against the percentage inhibition of writhing of diclofenac sodium, which was 82.22%. In the experiment performed using the paw edema model induced by carrageenan, the percentage inhibition of inflammation achieved through the extract was 54.02%, whereas the percentage inhibition of inflammation by diclofenac sodium was 80.39%. Finally, the effect of the extract on plasma glucose levels was studied in an animal model, and it was noted that the percentage decrease in plasma glucose levels with the extract was 46.03%, while the percentage decrease in plasma glucose levels with metformin was 81.23%. Although the percentage of inhibition of these effects achieved through the extract was less than those observed with the standard drugs used, but still, it shows the biological significance of *Ageratum conyzoides* as a source of active phytoconstituents.

Future research directions will be critical in fully understanding the pharmacological actions of *Ageratum conyzoides*. These include:

1. Isolation and identification of bioactive components: This involves isolating and identifying the bioactive phytochemicals that are responsible for the medicinal effects of *Ageratum conyzoides*.
2. Studying the mechanism of action: Studies need to be conducted to understand how this plant produces pharmacological effects on the body.
3. Evaluation of the dose-response relationship: Research needs to be conducted on various doses of the plant to determine the optimal dose and the dose-response relationship.
4. Studies on the toxicology of the plant: To establish the safe usage levels of this plant, toxicity studies at various intervals should be conducted.
5. Pharmacological studies: This includes studying further the pharmacological effects of the plant on other diseases.
6. Clinical formulation of the plant: The formulation of appropriate medications based on the use of the plant and conducting clinical trials to determine the therapeutic potential of this herb.

Declaration

Source of Funding

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare that they have no competing interests related to this work.

Consent for Publication

The authors declare that they consented to the publication of this study.

Authors' Contributions

Fahamida Binta Anower and Md. Mahbubol Alam took part in literature review, conceptualization, methodology, investigation, analysis, and manuscript writing, editing and reviewed the manuscript. Lamia Akther Enni, Alamgir Hossain, Sadia Afrin Sorna, Shourav Nandi and Mahbuba Alam analyzed data. Md. Mahbubol Alam was involved in the design of the manuscript. All authors have read and approved the final manuscript.

Availability of Data and Materials

Supplementary information is available from the authors upon reasonable request.

Institutional Review Board Statement

Not applicable.

Ethical Approval

All animal experiments were conducted in accordance with the institutional guidelines for the care and use of laboratory animals. Every effort was made to ensure the humane handling of animals and to minimize pain, discomfort, and stress throughout the experimental procedures.

Informed Consent

Not applicable for this study.

Acknowledgement

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Declaration of Artificial Intelligence

Not applicable for this study.

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