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Preliminary Pharmacological Studies of Methanolic Extract of *Cryptolepis buchanani* Leaves

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Abstract

The purpose of this study is to assess the characteristics associated with the depressive, anxiolytic, analgesic and thrombolytic activities of the methanolic extract of *Cryptolepis buchanani* leaves. The writhing test (acetic acid-induced), paw licking test (formalin-induced), tail suspension test (TST), forced swim test (FST), elevated plus maze test (EPM), and hole board test (HBT) were utilized to assess the *in-vivo* analgesic, anxiolytic, and antidepressant effects. An *in-vitro* human blood clot lysis method was also used to test the thrombolytic activity. In animal models, the tail suspension test (TST) and the forced swim test (FST) resulted in a shorter immobility time. The methanolic extract of *C. buchanani* (MECB) was administered at doses of 200 and 400 mg/kg, resulting in a dose-dependent increase in the mice's mobility duration. In both the Elevated Plus Maze (EPM) and the Hole Board Test (HBT), mice were given varying doses of MECB. Shortly after administration, the EPM results showed that the mice spent more time in the open arms and dipped their heads more frequently. The mice's writhing frequency and licking duration were significantly reduced, indicating an analgesic effect throughout the test. For each dose tested, the response varied as the concentration increased. This study concludes that the extract has anxiolytic, antidepressant, analgesic and thrombolytic properties, supporting the plant's traditional use in medicine.

Key words: *Cryptolepis buchanani*, anti-depressant, anxiolytic, analgesic and thrombolytic.

Introduction

From ancient times, people have turned to herbal remedies to alleviate the symptoms of many disorders (Maqsood *et al.*, 2010). Researchers have looked at the many uses and benefits of a wide variety of medicinal plants. In addition to being great sources of novel drugs, medicinal plants can provide beneficial therapeutic options. Many people in developing countries rely on medicinal plants to prevent and treat illness, particularly those who live in rural regions and have low incomes (Haque *et al.*, 2018). A nociception is a cluster of unpleasant feelings, either localized or widespread, that can produce moderate to severe physical discomfort and impulsive anguish. It is usually a result of a problem in the body, like an accident or illness. Several manifestations, including inflammatory, neuropathic, chronic, visceral and acute types (Uritu *et al.*, 2018). Traditional medicinal herbs have been found to have several metabolites that have antinociceptive activity, which makes them good painkillers (Dutta *et al.*, 2020). Worldwide, mental health issues like depression and anxiety are the leading causes of disability. People with neurological illnesses often experience anxiety and

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find it challenging to carry out daily tasks as a result (Adnan *et al.*, 2020). Major depressive disorder (MDD) is related to a host of negative health outcomes, including increased risk of physical illness and even mortality. Yet another prevalent mental disorder globally is anxiety (Shah *et al.*, 2022). According to the World Health Organization (WHO), many nonfatal diseases are caused by depressed illnesses globally (Emon *et al.*, 2021). According to the WHO, 3.8% of the population suffers from depression. With 4% of men and 6% of women making up this demographic, 5.7% are adults and 5% are over the age of 60. A lack of monoamine neurotransmitters such as dopamine (DA), noradrenaline (NE), and 5-hydroxy tryptamine (5-HT) is believed to be present in sad individuals due to physiological functions. On the other hand, research has shown that levels of reactive oxygen species (ROS) in the brains and blood of depressed patients are significantly greater. It has been suggested that oxidative stress could contribute to the onset of depression (Eren *et al.*, 2007). Despite common belief, anxiety is a mental disorder characterized by a wide range of symptoms that have zero correlation to actual medical conditions. According to Hoffman, Dukes, and Wittchen (2008), it has also been associated with a few chronic diseases, a low quality of life and subpar performance. Some estimates put the lifetime occurrence of these issues at 20% of the population. Anxiety is a normal human emotion, but too much of it may be considered a mental illness. The genuine prognosis of the patient may worsen, the efficacy of medication or therapy may be diminished, and the risk of suicide may increase as a result of the anxiety that accompanies depression (Emon *et al.*, 2021). They may also be associated with other health issues. Problems with the thyroid and other endocrine systems, as well as glucose homeostasis issues including diabetes and hypoglycemia, manifest in such cases (Auniq *et al.*, 2021; Raihan *et al.*, 2011).

Thrombosis, in which blood clots develop in the circulatory system, occurs when the homeostatic system of physiological procedures is out of whack. Acute coronary disorders can lead to thrombosis and other arterial diseases, which in turn can cause a host of complications and even death (Kistner *et al.*, 1972; Nicolini *et al.*, 1992). While thrombolytic drugs are useful for dissolving blood clots, they do have the potential to cause serious and even fatal side effects in rare cases (Rahaman *et al.*, 2020).

Traditional medicine in Southeast Asia makes use of the climbing tree *C. buchanani*, which belongs to the Asclepiadaceae family. Traditional Thai medicine makes use of this plant's stem to alleviate arthritic pain, inflammation and other aches and pains experienced by the muscles and joints. A study suggested that ethanol extract of this plant has an anti-inflammatory effect (Laupattarakasem *et al.*, 2006). We were unable to find any previous studies that examined the antidepressant, thrombolytic, analgesic or anxiolytic effects of the methanol extract of *C. buchanani* derived from the Hazarikhil region, Bangladesh. Thus, our study's objective is to investigate and assess MECB's analgesic, anxiolytic, antidepressant and thrombolytic effects.

Methods and Materials

Plant collection, identification and extraction: The plant part was collected in June 2018 from Hajarikhil, located in the Fatikchhari region of Chittagong, Bangladesh (Longitude: 091° 40' 839" E; Latitude: 22° 41' 700" N). The collected specimens were identified by Professor Dr. Sheikh Bokhtiar Uddin from the Department of Botany, University of Chittagong, Bangladesh. The leaves were thoroughly cleaned and washed, then dried in a shaded area for approximately 15 days. Once dried, they were ground into a fine powder. A total of 200 g of powdered leaf material was added to 1 liter of methanol in an amber, flat-bottomed glass container. The container was sealed with scotch tape and aluminum foil and then continuously shaken and stirred for 14 days. The resulting mixture was first coarsely filtered using a clean white cotton cloth and subsequently cleaned using filter paper. The filtrate was evaporated in a water bath at 50°C to remove methanol, yielding the desired extract.

Drugs and reagents: Analytical-grade chemicals and reagents were used in the investigation. The solvent methanol and Tween-80 were supplied by Merck of Darmstadt, Germany. The standard drug diazepam and fluoxetine hydrochloride were obtained from Square Pharmaceuticals Ltd. Diclofenac Na and levamisole hydrochloride were sourced from Albion Laboratories Ltd. and Incepta Pharmaceuticals Ltd., respectively. Additionally, the normal saline solution, containing 0.9% NaCl, was procured from the Social Marketing Company Ltd. in Bangladesh.

Experimental animals: In the current investigation, both male and female Swiss albino mice were used. The animals were approximately 6 to 7 weeks old, weighed from 25 to 30 grams, and were sourced from the Department of Pharmacy at Jahangirnagar University, located in Savar, Dhaka. The animals were kept in a natural light-dark cycle at 25±2°C with adequate ventilation, provided distilled water, and fed a conventional laboratory diet. A tranquil, undisturbed environment was used for every one of the experiments. Ten days prior to the test, the animals were acclimated to the laboratory environment. We gave them regular pellets and water that was completely pure. The mice were all kept in cages that mimicked the normal 12-hour light-dark cycle. The experimental protocols were approved by the departmental planning and development and animal ethics committee (Approval Number: Pharm/P&D/255/18-24).

Analgesic activity-Writhing test (Acetic acid-induced): Acetic acid was administered intraperitoneally to test subjects in order to elicit a pain response. As a result, in response to their discomfort, the animals exhibit rhythmic body movements known as "writhing." The animals' writhing continues as long as they are in pain and each instance is recorded as a measure of pain perception. Substances with analgesic properties are expected to reduce the frequency of writhing observed in the animals over a given time period, particularly when compared to a control group. The degree of writhing inhibition in the positive control group serves as a standard for comparing the efficacy of the test samples to the control. A standard non-steroidal anti-inflammatory drug (NSAID), diclofenac Na, is used as the positive control in the current study (Le Bars *et al.*, 2001). Each of the four mouse groups (n = 3), which contained both male and female mice, weighed between 20 and 22 grams. The control group, Group I, was given 10 milliliters per kilogram of body weight of regular saline. As an alternative, Group II, the standard group, received 10 ml/kg of a reference medication (Diclofenac Na). Additionally, oral doses of MECB at 200 and 400 mg/kg/b.w. were administered to Groups III and IV, respectively. Fifteen minutes after the acetic acid injection, the participants' abdominal muscle contractions were observed to assess the effect of MECB (Ahmad *et al.*, 2018; Gupta *et al.*, 2015).

The following formula was used to calculate the analgesic effect:

$$\% \text{ of Efficacy in reducing pain} = \frac{\text{Control Animal Writhing} - \text{Test Animal Writhing}}{\text{Control Animal Writhing}} \times 100$$

Paw licking test (Formalin-induced): The paw-licking test involved injecting 20 µl of a solution containing 2.5% formalin in saline into the ventral surface of the right hind paw. The mice were observed during the neurogenic phase (0–5 min) and the inflammatory phase (15–30 min), and the licking time (sec) of the injected paw was recorded as a measure of nociception (Uddin *et al.*, 2017). Thirty minutes prior to the start of the experiment, the mice were administered 200 and 400 mg/kg of MECB. The standard group received the conventional medication (diclofenac Na) at a dose of 10 mg/kg body weight, while the control group received saline solution (Uddin *et al.*, 2017).

Anxiolytic activity-elevated plus maze test (EPM): The EPM apparatus is 35 × 5 cm^2 with two open sides and 35 × 5 × 15 cm^3 with two closed sides. The two arms were connected by a 5 × 5 cm^2 central square. The device was placed in a room with low lighting. Following an overnight fast, the mice, each weighing 20–25 grams, were divided into four groups of three. The negative control group received

10 ml/kg of distilled water, while the positive control group received 1 mg/kg of diazepam intraperitoneally. Two experimental groups were evaluated: prior to the 30-minute trial, each group was administered 200 and 400 mg/kg of MECB. The frequency and duration of entries into the open and closed arms were measured over a 5-minute period (Uddin *et al*., 2020).

Hole board test (HBT): The anxiolytic test apparatus consisted of a 40 × 40 × 25 cm^3 hardwood chamber with sixteen 3 cm diameter holes. The mice could see through the holes as the equipment was elevated 25 cm from the ground. Swiss albino mice weighing 20–25 grams were subjected to overnight fasting. The negative control group received 10 ml/kg of distilled water, while the positive control group received 1 mg/kg of diazepam intraperitoneally. Two groups of mice were evaluated: prior to the 30-minute trial, each group was administered 200 and 400 mg/kg of MECB. The board was divided into individual spots for each mouse, allowing them to freely roam and dip their heads into the holes at will. The mice's head dips were recorded over a 5-minute period (Rahman *et al.*, 2024).

In vivo antidepressant activity-tail suspension test (TST): Four groups of mice, each consisting of three individuals, were subjected to a period of overnight fasting. The Swiss albino mice weighed between 20 and 25 grams. Each group received different treatments: MECB at 200 mg/kg and 400 mg/kg, fluoxetine-20 at 1 mg/kg intraperitoneally (i.p.), and distilled water at 10 ml/kg for the control group. During the test, the mice were suspended 50 cm above the ground using sticky tape placed around 1 centimeter from the tip of their tails. The suspension was carried out for five minutes, thirty minutes after the administration of the drugs. The number of seconds that each mouse remained motionless while suspended was recorded (Rahman *et al.*, 2024).

Forced swim test (FST): In this case, mice weighing between 20 g and 25 g were subjected to an overnight fasting protocol. Following this period, the mice were divided into four separate groups, each consisting of three individuals. The negative control group received 10 ml/kg of distilled water, whereas the positive control group received an intraperitoneal injection of 1 mg/kg of diazepam. Two experimental groups were administered 200 mg/kg and 400 mg/kg of plant extracts, respectively. After 30 minutes of pre-treatment at 23 ± 1°C, the mice were placed individually in a glass cylinder chamber that was 25 cm high and 10 cm in diameter, filled with water to a depth of 19 cm. During the last four minutes of the test, the animals' immobility after ceasing to swim was assessed in seconds (Rahman *et al.*, 2024).

Thrombolytic activity: To investigate thrombolytic activity, an *in-vitro* human blood clot lysis technique was used (Rahaman *et al.*, 2020). MECB from the plant was added to a volumetric flask with 0.9% NaCl to create a 10 mg/ml stock solution. Venous blood (5 ml) was drawn from six healthy human subjects. From this, 500 µl of blood was added to each microcentrifuge tube and allowed to coagulate. Following 45 minutes of incubation at 37°C, clots were formed, and the serum was carefully aspirated from each tube. The weight of the tube was recorded, and the clot weight was determined by subtracting the empty tube weight. Distilled water served as the negative control, MECB was the test group, and 15,000 IU of streptokinase was used as the positive control. Each tube was kept in an incubator set at 37°C for 90 minutes. After incubation, the tubes were weighed again after carefully removing the remaining fluid. The percentage of clot lysis was calculated using the formula:

$$\% \text{ of Clot lysis} = \frac{\text{Weight of release clot}}{\text{Weight of clot}} \times 100$$

Statistical analysis: The data were presented as mean ± standard error of the mean (SEM). Statistical data were graphically represented using GraphPad Prism.

Results and Discussion

Analgesic activity-acetic acid-induced writhing test: Mice that received an oral administration of methanolic extract of *Cryptolepis buchanani*

(MECB) exhibited a notable reduction in the incidence of writhing. Specifically, MECB administered at dosages of 200 mg/kg and 400 mg/kg of body weight resulted in writhing inhibition of 57.89% and 42.10%, respectively. Moreover, at a dosage of 10 mg/kg of body weight, the analgesic efficacy of MECB was comparable to that of the conventional analgesic diclofenac sodium, which produced a 66.66% reduction in writhing, as illustrated in table 2 and figure 2.

Table 1. The results of analgesic activity on acetic acid-induced writhing test

Group	Dose	Frequency of writhing	Inhibition (%)
1% tween 80 in water	10 ml/kg	57	-
Diclofenac Na	10 mg/kg	19	66.66
MECB-200	200 mg/kg	24± 1.673	57.89
MECB-400	400 mg/kg	33 ± 1.231	42.10

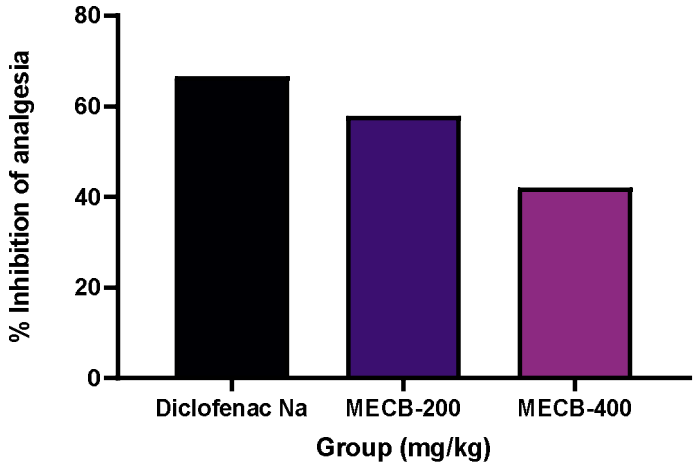

Figure 1. Percent inhibition of analgesia on acetic acid-induced writhing test

Formalin-induced paw-licking test: The frequency of formalin-induced licking in mice decreased significantly and dose-dependently following oral administration of *C. buchanani* in the formalin-licking test. In the early phase, the extract inhibited licking time by 34.30% at a dosage of 200 mg/kg body weight, while in the late phase, the inhibition was 40.71%. At a dosage of 400 mg/kg body weight, the extract reduced licking time by 41.74% in the initial phase and 38.52% by the end of the test. As shown in table 2 and figure 2, at a dosage of 400 mg/kg body weight, the plant extract exhibited analgesic effects nearly comparable to those of the conventional drug diclofenac Na, which showed analgesic effects of 68.82% and 62.53% in the early and late phases, respectively.

To determine the analgesic potential of MECB, an *in-vivo* acetic acid-induced writhing test was performed using a mouse model. Following treatment with acetic acid, the mice exhibited writhing behavior indicative of abdominal discomfort. This experiment provides insights into the body's pain perception by evaluating the levels of endogenous mediators that promote inflammation in peripheral tissues and fluids. These mediators include prostaglandins, serotonin, cyclooxygenase (COX), and cytokines. They exacerbate inflammation by enhancing prostaglandin activity within the peritoneal compartments and increasing capillary permeability

(Shovo *et al.*, 2021). Similar mechanisms underlie the analgesic effects (Karbab *et al.*, 2020). The nociceptive (biphasic) response, which comprises two separate stages controlled by several mechanisms, can be studied using the formalin-induced paw-licking test (Antonio Guerrero-Solano *et al.*, 2022). The intraperitoneal injection in mice triggers the early preparatory phase (0-5 min), as it gradually chemically stimulates sensory C-fibers. Centrally acting anti-inflammatory drugs have the potential to shorten the last 15-30 minutes of an opioid-based drug's half-life, which is expected to provoke an inflammatory response (Hirota *et al.*, 2023). In this experiment, the administration of MECB significantly reduced formalin-induced pain sensitivity in both stages, with the effect being dose-dependent. This evidence supports the conclusion that the extract has analgesic effects on both the central nervous system and the periphery.

Table 2. Analgesic activity of MECB on formalin-induced licking test

Group	Dose	Early phase	Inhibition (%)	Late phase	Inhibition (%)
1% tween 80	10 ml/kg	53.76±2.20	-	39.32± 1.78	-
Diclofenac Na	1 mg/kg	16.76±1.24	68.82	14.73± 1.42	62.53
MECB	200 mg/kg	35.32±1.45	34.30	23.31± 1.10	40.71
MECB	400 mg/kg	31.32±1.56	41.74	24.21± 0.88	38.42

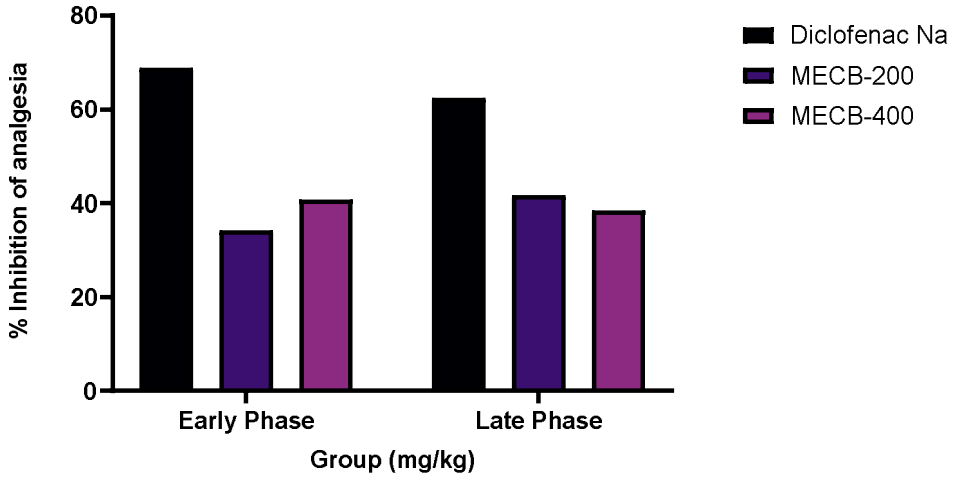

Figure 2. Percent inhibition of analgesia on formalin-induced writhing test

Anxiolytic activity-elevated plus maze test (EPM): Table 3 shows the impact of MECB on the total entries and time spent (sec) in the open arms of the elevated plus maze (EPM) test. Mice administered diazepam demonstrated a markedly longer duration spent in the open arms compared to the control group. Additionally, mice given doses of 200 or 400 mg/kg of MECB showed a tendency to remain in the open arms for extended periods within the treatment groups. In comparison to the negative control group, the mice treated with MECB spent considerably more time in both the open and closed arms.

Hole board test (HBT): The anxiolytic effects of MECB were assessed using the hole board test (HBT), which showed a dose-dependent relationship (Table 4). A higher number of head dips (24.52±0.94) was observed in the mice treated with 400 mg/kg of MECB compared to those treated with 200 mg/kg

(17.33±1.201). The control group exhibited the lowest amount of head dipping, while the traditional medication diazepam (1 mg/kg) resulted in the highest amount of dipping at 63.41 ± 2.13.

Table 3. Effect of MECB on *Swiss albino* mice (n=3) in EPM.

Treatment	Dose	% Entry into the open arm	Time spent in open arm (sec)
1% Tween solution	10 (ml/kg)	56.25 ± 1.32	35.25 ± 2.654
Diazepam	1 mg/kg	90.31 ± 0.67	125.27 ± 1.456
MECB	200 mg/kg	23.67 ± 0.954	43.00 ± 1.527
MECB	400 mg/kg	80.57 ± 0.79	61.12 ± 1.874

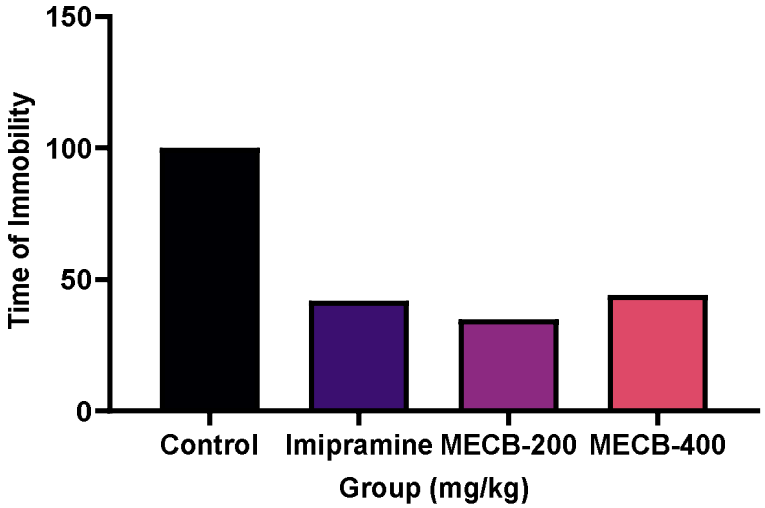

Figure 3. Effect of MECB on *Swiss albino* mice (n=3) in TST.

This research employed the elevated plus maze (EPM) and hole board test (HBT) to evaluate anxiolytic effects. Mice were administered two oral doses of MECB (200 and 400 mg/kg) during the EPM and HBT assessments. The results demonstrated a notable increase in both the time spent in the open arms and the frequency of entries, suggesting a possible alleviation of anxiety behaviors. The observed effects were compared to those of diazepam, the standard drug.

a potential correlation has been identified between the head-dipping behavior of animals and their emotional states (Takeda *et al.*, 1998). Anxiety levels in humans may rise in response to disturbances within the GABA-ergic circuits or an imbalance in neurotransmitter levels. The alleviation of anxiety symptoms can be attributed to a strong activation of GABA receptors, which is facilitated by the opening of chloride channels (Auniq *et al.*, 2021).

Table 4. Effect of MECB on *Swiss albino* mice (n=3) in HBT.

Group	Dose	No. of head dipping (Mean ± SEM)
1% Tween solution	10 ml/kg	24.52 ± 0.94
Diazepam	1 mg/kg	63.41 ± 2.13
MECB	200 mg/kg	17.33 ± 1.201
MECB	400 mg/kg	35.66 ± 0.576

Antidepressant activity test-tail suspension test (TST): The decreased immobility time (s) in the TST

is shown in figure 3. Antidepressant-like effects were observed in mice when MECB was administered. Mice in the 200 mg/kg and 400 mg/kg groups were immobile for 67.66 ± 1.763 and 85.66 ± 3.88 seconds, respectively, while the mice in the control group were immobile for 194.66 ± 2.46 seconds. A smaller dose of MECB showed a greater reduction in immobility than conventional therapy, with an immobility time of 81.56±2.309 seconds.

Force swimming test: Mice administered doses of 200 mg/kg and 400 mg/kg exhibited immobility durations of 60.66 ± 1.20 seconds and 84.24 ± 2.645 seconds, respectively. In contrast, the control group of mice demonstrated an immobility duration of 142.24 ± 1.18 seconds. Notably, the lower dose of MECB resulted in a more significant decrease in immobility compared to the conventional treatment (Figure 4).

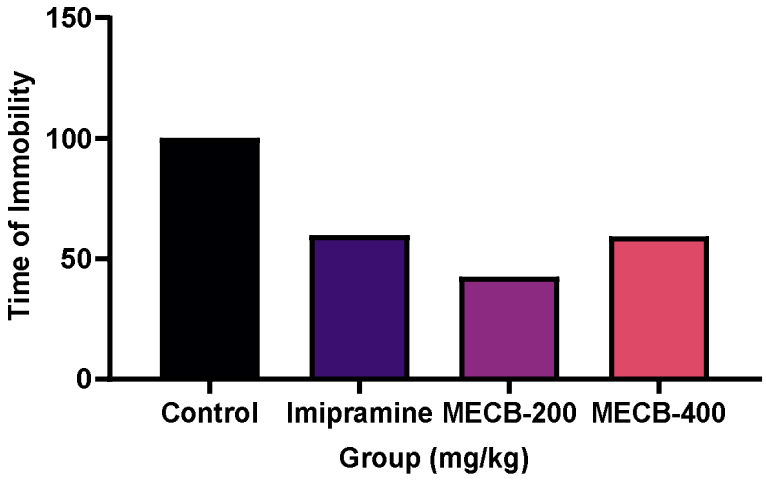

Figure 4. Effect of MECB on *Swiss albino* mice (n=3) in FST.

Worldwide, underlying mental health conditions such as anxiety and depression are among the most pressing medical concerns in the modern era. According to the results of these studies, MECB exhibits effects similar to those of antidepressants and anxiety medications. To determine if mice were depressed, researchers utilized the FST and the TST (Islam *et al.*, 2021). Characteristic behaviors, such as inactivity, are considered indicative of clinical depression in these investigations. Similar to its antidepressant potential, the medication can reduce the amount of time that mice remain immobile (Diniz *et al.*, 2019). Several substances with antidepressant potential were discovered once swimming was established as an endpoint. One way to alter the swimming behavior is by using drugs that block certain receptors. The proper production of 5-HT is ensured by selective serotonin receptor inhibitors (SSRIs). This is because 5-HT (5-hydroxytryptamine) modulates the swimming behavior in the motility test (He *et al.*, 2015; Rahman *et al.*, 2024). Additionally, it is believed that the antioxidants in MECB facilitate cell migration through the FST and TST by enhancing 5-HT flow. During the FST, the GABA transmitter and NOS were unable to exert their psychological effects due to a lack of 5-HT synthesis. This suggests that their antidepressant activity is associated with enhanced 5-HT transmission (Emon *et al.*, 2021). The specific process remains unclear; however, this research provides valuable insight. To clarify the matter, further comprehensive mechanistic research is necessary.

Thrombolytic activity: The results demonstrated that the concentration of the extract, in relation to incubation duration, had a dose-dependent effect on clot lysis. Blood clot percentages decreased by

78.42% and 13.78%, respectively, when conventional streptokinase and MECB concentrations were lowered in this investigation (Figure 5). Results like these suggest that MECB was not very effective at preventing blood clots.

Herbs and natural products have been found to possess thrombolytic activity in numerous studies (Basta *et al.*, 2004). Thrombolytic agents are classified into three categories: tissue plasminogen activator (t-PA), streptokinase (SK), and urokinase (UK), with t-PA being the most commonly used. Hemorrhage or bleeding is the most common side effect of these medications, and it can be fatal (Ansari *et al.*, 2012). Several thrombolytic agents have recently been introduced into clinical practice to aid in the removal of pre-existing blood clots from arteries. Nevertheless, these treatments have limitations and, in rare cases, can be fatal (Rahaman *et al.*, 2020).

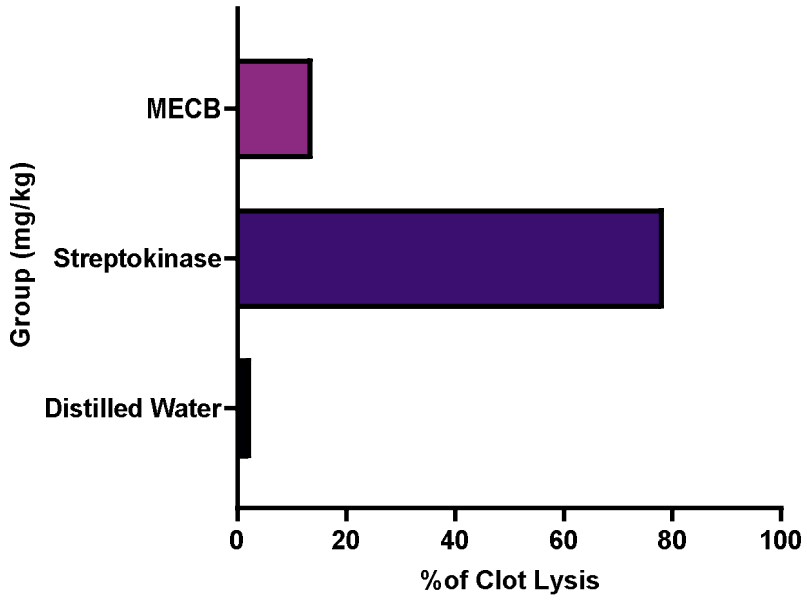

Figure 5. Percent of clot lysis on thrombolytic activity

Conclusion

Throughout millennia, a diverse array of health issues has been addressed through the utilization of medicinal plants. Numerous species of these plants, possessing significant analgesic, thrombolytic and various other therapeutic properties, can be found in the natural environment. This study shows that *C. buchanani* methanolic leaves extract has significant neuropharmacological effects, including antidepressant and anxiolytic effects. These findings point to the extract's marked influence on the CNS. Furthermore, the study presents compelling evidence of its antinociceptive qualities, as demonstrated by its efficacy in the paw-licking and writhing tests. The thrombolytic activity of MECB was mild, in contrast. Despite the encouraging findings, additional studies are needed to determine the exact active ingredients and thoroughly assess the extract's biological effects in animal models.

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References

Adnan, M., Chy, M.N.U., Kamal, A.M., Azad, M.O.K., Chowdhury, K.A.A., Kabir, M.S.H., Gupta, S.D., Chowdhury, M.A.R., Lim, Y.S. and Cho, D.H. 2020. Comparative study of *Piper Sylvaticum* Roxb. leaves and stems for anxiolytic and antioxidant properties through *In vivo*, *In vitro*, and in silicon approaches. *Biomedicines.* **8**, 68. https://doi.org/10.3390/biomedicines8040068

Ahmad, B., Naz, S., Rauf, A., Bashir, S., Khan, A., Farooq, U., Khan, F.A., Patel, S., Khan, Z., Hussain, J. and Al-Harrasi, A. 2018. *In vivo* Study on analgesic, gastrointestinal tract (GIT) Motility and anti-termite potential of methanolic extract of *Sarcococca Saligna* (D. Don) muell. fruits. *S. Afr. J.* **114**, 40-43.

Ansari, V.A., Siddiqui, H.H. and Singh, S.P. 2012. Antithrombotic and thrombolytic activity of terminalia belerica fruit extracts. *Res. J. Pharm. Biol. Chem. Sci.* **3**, 471-478

Antonio Guerrero-Solano, J., Bautista, M., Vidal Espinosa-Juarez, J., Alfonso Moreno-Rocha, L., Betanzos-Cabrera, G., Claudia Salanta, L., Arciniega, M.D.L.O., Olvera-Hernandez, E.G. and Antonio Jaramillo-Morales, O. 2023. Differential antinociceptive efficacy of peel extracts and lyophilized juices of three varieties of mexican pomegranate (punica granatum l.) in the formalin test. *Plants-basel*. ***12***, 131. https://doi.org/10.3390/plants12010131

Auniq, R.B.J., Chy, M.N.U., Adnan, M., Chowdhury, M.R., Dutta, T., Ibban, S.S., Jahan, I., Tona, M.R., Shima, M., Sawon, M.I.H. and Chakrabarty, N. 2021. Evaluation of anxiolytic, sedative, and antioxidant activities of vitex peduncularis wall. leaves and investigation of possible lead compounds through molecular docking study. *Adv. Tradit. Med.* **21**, 507–518.

Basta, G., Lupi, C., Lazzerini, G., Chiarelli, P., L'Abbate, A. and Rovai, D. 2004. Therapeutic effect of diagnostic ultrasound on enzymatic thrombolysis. an in vitro study on blood of normal subjects and patients with coronary artery disease. *J. Thromb. Haemost*. **91**, 1078-1083.

Diniz, T.C., de Oliveira Junior, R.G., Medeiros, M.A.M.B., e Silva, M.G., de Andrade Teles, R.B., dos Passos Menezes, P., de Sousa, B.M.H., Frank, L.A., de Souza Araujo, A.A., Serafini, M.R. and Guterres, S.S. 2019. Anticonvulsant, sedative, anxiolytic and antidepressant activities of the essential oil of annona vepretorum in mice: involvement of gabaergic and serotonergic systems. *Biomed Pharmacother.* **111**,1074-1087.

Dutta, T., Paul, A., Majumder, M., Sultan, R.A. and Emran, T.B. 2020. Pharmacological evidence for the use of *Cissus Assamica* as a medicinal plant in the management of pain and pyrexia. *Biochem. Biophys Rep.* **21**,100715.

Emon, N.U., Alam, S., Rudra, S., Riya, S.R., Paul, A., Hossen, S.M., Kulsum, U. and Ganguly, A. 2021. Antidepressant, anxiolytic, antipyretic, and thrombolytic profiling of methanol extract of the aerial part of *Piper Nigrum : In vivo, in vitro*, and in *Silico* Approaches. *Food Sci. Nutr.* **9**, 833-846.

Eren, İ., Nazıroğlu, M. and Demirdaş, A. 2007. Protective effects of lamotrigine, aripiprazole and escitalopram on depression-induced oxidative stress in rat brain. *Neurochem. Res.* **32**,1188–1195.

Gupta, A.K., Parasar, D., Sagar, A., Choudhary, V., Chopra, B.S., Garg, R., Ashish and Khatri, N. 2015. Analgesic and anti-inflammatory properties of gelsolin in acetic acid induced writhing, tail immersion and carrageenan induced paw edema in mice. *PloS One.* **10**, e0135558.

Haque, M.I., Chowdhury, A.A., Shahjahan, M. and Harun, M.G.D. 2018. Traditional healing practices in rural Bangladesh: a qualitative investigation. *BMC Complement. Altern. Med.* **18**, 62.

He, D., Wang, X., Zhang, P., Luo, X., Li, X., Wang, L., Li, S. and Xu, Y. 2015. Evaluation of the anxiolytic and antidepressant activities of the aqueous extract from *Camellia euphlebia* Merr. ex Sealy in mice. *J. Evid Based Complementary Altern Med.* **2015**, 1-8.

Hirota, I., Koyama, Y. and Shimada, S. 2023. Histochemical analysis of the biphasic properties of formalin pain-induced behavior. *Biochem Biophys Rep.* **34**, 101467.

Islam, N., Khan, M.F., Khatun, M.R., Nur, S., Hanif, N.B., Kulsum, U., Arshad, L., Lyzu, C., Cacciola, N.A., Capasso, R. and Haque, M.A. 2021. Neuropharmacological insights of African oil palm leaf through experimental assessment in rodent behavioral model and computer-aided mechanism. *Food Biosci*. **40**, 100881.

Karbab, A., Mokhnache, K., Ouhida, S., Charef, N., Djabi, F., Arrar, L. and Mubarak, M.S. 2020. Anti-inflammatory, analgesic activity and toxicity of Pituranthos scoparius stem extract: An ethnopharmacological study in rat and mouse models. *J. Ethnopharmacol*. **258**, 112936.

Kistner, R.L., Ball, J.J., Nordyke, R.A. and Freeman, G.C. 1972. Incidence of pulmonary embolism in the course of thrombophlebitis of the lower extremities. *Am. J. Surg*. **124,** 169-176.

Laupattarakasem, P., Wangsrimongkol, T., Surarit, R. and Hahnvajanawong, C. 2006. *In vitro* and *in vivo* anti-inflammatory potential of *Cryptolepis buchanani*. *J. Ethnopharmacol.* **108**, 349-54.

Le Bars, D., Gozariu, M. and Cadden, S.W. 2001. Animal models of nociception. *Pharmacol. Rev.* **53,** 597-652.

Maqsood, S., Singh, P., Samoon, M.H. and Khansaheb Balange, A. 2010. Effect of dietary chitosan on non-specific immune response and growth of Cyprinus carpio challenged with *Aeromonas hydrophila*. *Int. Aquat. Res.* ***2***,77-85.

National Research Council, Division on Earth, Life Studies, Institute for Laboratory Animal Research, Committee for the Update of the Guide for the Care and Use of Laboratory Animals. 2011. Guide for the care and use of laboratory animals. *Eighth Edition*. Washington, D.C.: National Academies Press. https://doi.org/ 10.17226/12910

Nicolini, F.A., Nichols, W.W., Mehta, J.L., Saldeen, T.G., Schofield, R., Ross, M., Player, D.W., Pohl, G.B. and Mattsson, C. 1992. Sustained reflow in dogs with coronary thrombosis with K2P, a novel mutant of tissue-plasminogen activator. *J Am Coll Cardiol* **20**, 228–235. https://doi.org/10.1016/0735-1097(92) 90164-I

Rahaman, M.Z., Akhter, S., Islam, M.R., Begum, S., Mondal, K.K., Mottakin, M., Hossain, M.S., Bayen, S. and Das, M. 2020. Assessment of thrombolytic, antioxidant and analgesic properties of a medicinal plant of Asteraceae family growing in Bangladesh. *Disc. Phytomed.* **7**, 47-52.

Rahman, R., Siddique, T., Nipa, F.A., Sultana, S., Devi, P., Islam, F., Nainu, F., Obaidullah, A.J., Emran, T.B. and Khatun, M.R. 2024. Bark extract of *Chaetocarpus castanocarpus* (Roxb.) exhibits potent sedative, anxiolytic, and antidepressant effects through an in vivo approach in *Swiss albino* mice. *Eur Rev Med Pharmacol Sci.* **28**, 1202-1212.

Raihan, M.O., Habib, M.R., Brishti, A., Rahman, M.M., Saleheen, M.M. and Manna, M. 2011. Sedative and anxiolytic effects of the methanolic extract of *Leea indica* (Burm. f.) Merr. *leaf. Leaf. Drug Discov.* **5**, 185-189.

Shah, M.S., Tayab, M.A., Rahman, A., Hasan, M.N., Talukder, M.S.H., Uddin, A.K., Jabed, M., Chy, M.N.U., Paul, A., Rahman, M.M. and Emran, T.B. 2022. Anxiolytic, antidepressant and antioxidant activity of the methanol extract of *Canarium resiniferum* leaves. *J. Tradit Complement Med.* **12**, 567-574. https://doi.org/10.1016/j.jtcme.2022.07.001

Shovo, M.A.R.B., Tona, M.R., Mouah, J., Islam, F., Chowdhury, M.H.U., Das, T., Paul, A., Ağagündüz, D., Rahman, M.M., Emran, T.B. and Capasso, R. 2021. Computational and pharmacological studies on the antioxidant, thrombolytic, anti-inflammatory and analgesic activity of *Molineria Capitulata*. *Curr. Issues Mol. Biol.* **43**, 434-456.

Takeda, H., Tsuji, M. and Matsumiya, T. 1998. Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. *Eur. J. Pharmacol.* ***350,*** 21-29.

Uddin, M.M.N., Ahmed, S., Kabir, M.S.H., Rahman, M.S., Sultan, R.A. and Emran, T.B. 2017. *In vivo* analgesic, anti-inflammatory potential in *Swiss albino* mice and *in vitro* thrombolytic activity of hydroalcoholic fruits extract from *Daemonorops Robusta* Warb. *J. Appl. Pharm. Sci.* ***7***, 104-113.

Uddin, M.S., Millat, M.S., Islam, M.S., Hussain, M.S., Uddin, M.G., Siddiqui, S.A. and Ferdous, M. 2020. Exploration of *in vitro* thrombolytic, anthelminthic, cytotoxic and *in vivo* anxiolytic potentials with phytochemical screening of flowers of *Brassica nigra*. *Future J. Pharm. Sci.* **6**, 73.

Uritu, C.M., Mihai, C.T., Stanciu, G.D., Dodi, G., Alexa-Stratulat, T., Luca, A., Leon-Constantin, M.M., Stefanescu, R., Bild, V., Melnic, S. and Tamba, B.I. 2018. Medicinal plants of the family Lamiaceae in pain therapy: a review. *Pain Res Manag.* **2018**, 7801543.