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Antipyretic, Antidiabetic, Thrombolytic and CNS Depressant Potential of Ethanol Extract of *Crotalaria Verrucosa* L. Leaves

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Abstract

Hyperthermia, hyperglycemia, depression are the most common physiological disorders found in third world countries where people are not well aware of their health condition as well as about their health practice. People in rural and tribal areas depend on the traditional system of medicine where allopathic medicines are not the first choice because of ethnicity and belief. The plant *Crotalaria verrucosa* is believed as one of the cures in traditional system of medicine for many ailments. The present study was performed to evaluate the antipyretic, antidiabetic, thrombolytic and depressive potential of the leaf extract of this plant. Yeast induced pyrexia, alloxan induced type II hyperglycemia, HRBC clot denaturation, Hole cross and Open field tests were performed to investigate these activities at moderate to high doses (100, 250 and 500 mg/kg). Results of the study suggests that the extract possess strong antipyretic, antidiabetic and CNS depressant activity in higher doses (250 and 500 mg/kg) but lack to exhibit clear thrombolytic potential.

Keywords: pyrexia, diabetic, clot lysis, locomotor activity, *C. verrucosa*

1. Introduction

Pharmacological investigation of the medicinal plant has become an essential target for the bio-model researchers for their widespread use for different ailments. In Bangladesh thousands of plants are in traditional use and are being investigated for the scientific basis of their activities. Plants used for different properties in different geographical areas trigger the scientists to design different investigation portfolio to identify their right uses. The major objective of these studies are to find or indicate the responsible compounds or fractions and thereby to suggest the best preparation of this traditional

medicine to get optimum effects. The present study was conducted on a plant *Crotalaria verrucosa* which has been reported in many literatures to use in medicinal purpose in different tribal areas of Bangladesh and has shown many firm activities and this act as a driving force for the researchers to investigate its properties in different areas.

Crotalaria verrucosa (Fabaceae) grows well in the districts of Chittagong, Khulna, Mymensingh, Rajshahi and Sylhet of Bangladesh and is used by the native tribal of these districts [1]. *Chakma* and *Marma* tribes use it in case of skin allergies and applies in affected areas [2,3]. This is an erect plant having ovate-deltoid to obtuse leaves [3]. Literature review suggests that the plant is effective for impetigo, scabies, salivation, jaundice, cough, biliousness, dyspepsia, fever, cardiac abnormalities and oral diseases [4-6]. Besides, it is also reported to have hepatoprotective, antifertility activity and also effective against estrogenic implantation [7,8]. It contains anacrotine, crotaverrine and O12-acetyl-crotaverine [6]. The study was designed to investigate its antipyretic, antidiabetic, thrombolytic and depressive potential by established methods.

2. Material and Method

2.1 Collection and preparation of plant material

The plant leaf was collected from the hilly areas of Sylhet on April 2014 and a sample specimen was sent to Bangladesh National Herbarium (BNH) (Accession number: DACB 42010). Leaves were thoroughly washed and dried under ceiling fan. Then these were crushed into powder (approx. 500 gm) and soaked in ethanol (1 L) for 3 days. After that it was filtered and the filtrate was condensed using a rotary evaporator to obtain the viscous ethanol extract (50 gm approx.) of *C. verrucosa* (CVE).

2.2 Drugs and Chemicals

Brewer's yeast, Paracetamol, Alloxan monohydrate, Glibenclamide, Streptokinase and Diazepam used in the experiments were of analytical grade.

Acute toxicity studies

The acute toxicity was performed both for the Wister rats and Swiss Albino mice. Animals were orally dosed with the extract of *C. verrucosa* leaf at the doses of 400, 800, 1200 and 2000 mg/kg body weight. Immediately after dosing, the animals were observed for next 72 hours for behavioral changes and for mortality [9].

2.3 Antipyretic test

Subcutaneous injection of brewer's yeast (20% w/v in dH_2O at 10ml/kg b.w.) was given to five groups (N=5) of Wistar rats each weighing 130-150 to induce pyrexia. Rectal temperature of these animals was recorded by inserting a digital clinical thermometer up to 2cm inside the rectum, before (basal) and 19 hr after (pyrexia) the yeast injection. Then the groups were orally treated with respective test samples (CVE 100, CVE 100, 250, 500 mg/kg b.w.), standard drug (Paracetamol 150mg/kg) and control (received none). Reduction in pyrexia was measured 1, 2 and 3 hr after the treatments [10].

2.4 Antidiabetic test

The method was performed on artificially developed type II diabetic model by single intraperitoneal injection of alloxan monohydrate (120mg/kg b.w.) on Wistar rats. After measuring the blood glucose level at 72 hr, diabetic condition was confirmed for the rats having more than 140mg/dL glucose level. Normal water, test samples (CVE 100, CVE 100, 250, 500 mg/kg b.w.), standard drug (Glibenclamide 2.5 mg/kg b.w.) were orally administered for 21 days. Decrease in blood glucose level was measured at 0, 7, 14 and 21 days using commercially available glucose test kits [11].

2.5 Thrombolytic test

100 µl of previously prepared extract solution (100 mg in 10 ml dH_2O, filtered through 0.22 micron syringe filter) was taken for each alpine tube containing thrombus, blood drawn from healthy volunteers (500 µl each, incubated at 37°C for 45 minutes). The weight of the tube containing clot was measured against the weight of empty tube. Equal volume of streptokinase and dH_2O were served as positive and negative

control respectively. Incubation of all tubes at 37°C for 90 minutes was undertaken for clot lysis. After removing the supernatant, tubes were again weighed to observe clot disruption [12].

2.6 CNS depressant tests

2.6.1 Hole cross test

The methodology was based on the procedure described by Khatoon *et al*. with modification [13]. Swiss Albino mice weighing 22-28 gm of both sex were selected for this experiment. 20 minute after oral gavage of the test (CVE 100, 250, 500 mg/kg b.w.) and standard (Diazepam 1 mg/kg b.w.) drugs, animals were observed for 20 minutes for their activity of hole crossing in specially designed apparatus. It was a rectangular box with wall height of 14 cm each side and having a 3 cm circular hole at 5 cm height on the partition inside the box which divided the box into two equal chambers with harmonized illumination. Reduction in hole cross activity was considered as CNS depressant property of the administered sample.

2.6.2 Open field test

Open field test was performed according to the method described by Shams-Ud-Doha *et al*. with modifications [14]. Albino mice were orally treated with the test (CVE 100, 250, 500 mg/kg b.w.) and standard (Diazepam 1 mg/kg b.w.) drugs and then placed in a open field floor designed as several square which were the measurement unit for the distance travelled by the animal. Mice were observed for 20 minutes after a 20 minute of lag phase.

2.7 Statistical Presentation

All data were statistically examined for significance of the result in 95%, 99%, and 99.99% confidence level using SPSS. Values are presented as mean ± S.E.M., (n = 5) for all tests; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. One Way ANOVA followed by Dunnet t-test was performed as compared to control.

3. Results

3.1 Antipyretic activity

Table 1 shows that CVE 500 exerts a significant reduction of temperature ($P > 0.001$) which is comparable to the standard paracetamol. CVE 250 also showed moderate activity against hyperthermia. There was gradual decrease in temperature observed form the 1st to 3rd hr after oral administration of the samples which can be attributed for the duration of action.

Table 1: Effect of *C. verrucosa* leaf against yeast induced pyrexia in reducing the temperature (˚C)

Groups	Initial Body temperature (˚C)	19 hr after yeast injection (hyperthermia)	After oral administration of drugs and samples		
			1 hr	2 hr	3 hr
Control	37.48±0.170	40.47±0.149	40.61±0.108	40.65±0.156	40.52±0.238
Paracetamol (150mg/kg)	37.80±0.206	40.42±0.225	38.48±0.173***	37.45±0.123***	37.51±0.136***
CVE 100	37.46±0.164	40.27±0.125	40.22±0.113	39.59±0.120***	39.63±0.183**
CVE 250	37.40±0.374	40.67±0.105	39.62±0.189***	38.54±0.175***	38.55±0.124***
CVE 500	37.71±0.285	40.33±0.173	38.70±0.099***	37.64±0.120***	37.48±0.164***

Table 2: Activity of *C. verrucosa* against alloxan induced diabetic model in decreasing the blood glucose level (mg/dL)

Groups	0 day	7 day	14 day	21 day
Diabetic control	283.13±4.52	321.50±10.44	356.02±13.97	381.95±6.02
Glibenclamide (2.5 mg/kg)	281.49±5.69	248.50±5.85***	218.18±5.89***	177.12±10.99***
CVE 100	289.55±10.33	316.72±10.81	307.30±8.32**	294.07±7.54***
CVE 250	278.46±5.27	260.70±6.17***	237.33±3.68***	211.07±8.85***
CVE 500	284.54±6.80	252.67±5.89***	228.98±9.42***	190.33±11.09***

Table 3: Thrombolytic activity of CVE against HRBC clot denaturation

Groups	% clot lysis (mean ± S.E)	P value
Control	11.39 ± 8.22	---
Streptokinase (100 µl)	80.65 ± 11.23	< 0.001
CVE (100 µl)	26.81 ± 6.41	< 0.01

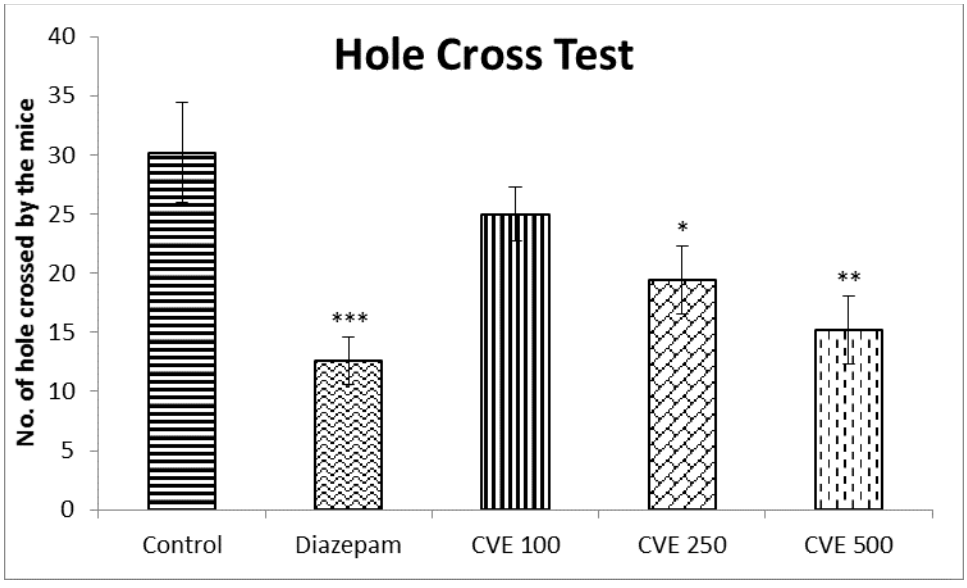

Figure 1: Comparison in number of hole crosses by the mice as a function of locomotor activity by the standard and extracts of *C. verrucosa*. Values are presented as mean ± S.E.M., (n = 5); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Dunnet t-test as compared to control.

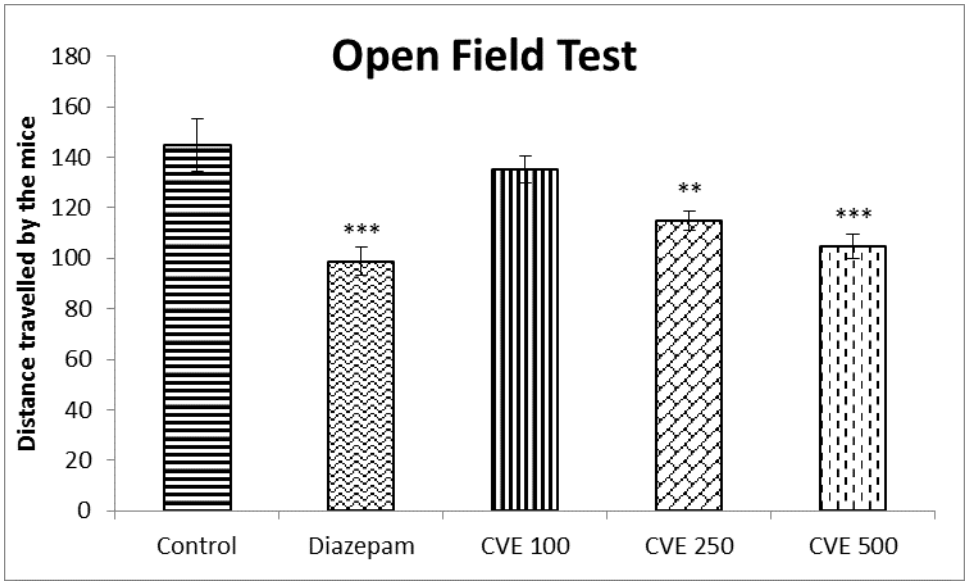

Figure 2: Comparison in number of square travelled by the mice as a function of locomotor activity by the standard and extracts of *C. verrucosa*. Values are presented as mean ± S.E.M., (n = 5); ** $P < 0.01$, *** $P < 0.001$. Dunnet t-test as compared to control.

3.2 Antidiabetic activity

In higher doses the plant leaf of *C. verrucosa* possess a significant anidiabetic property (Table 2). CVE 500 showed almost similar trend in reducing the blood glucose level as shown by the standard glibenclamide. CVE 250 also produce moderate to high activity however, CVE 100 was not able to produce optimum activity.

3.3 Thrombolytic activity

From Table 3 it can be suggested that the ethanol leaf extract of *C. verrucosa* failed to produce a strong clot lysis activity compared with the standard drug Streptokinase. However the results were statistically significant.

3.4 CNS depressant activity

Reduction in the motor activity of central nervous system by the CVE extracts at different doses was evident from the two tests performed. Figure 1 shows the reduction in number of hole crossed by the mice in a hole cross apparatus after oral treatment of the sample. Moreover, this activity was supported by the decrease in the distance travelled by the mice in an open field (Figure 2). Maximum decrease in motor function was exhibited by CVE 600 in both the experiments.

4. Discussion

Fever is a complex biological response attributed to various infections or aseptic stimuli. It is characterized by elevation of bodily temperature due to excessive production of prostaglandin E2 (PGE2) in hypothalamic region of brain. This causes a remarkable alteration in the firing rate of neurons that control the thermoregulation process in the hypothalamus [15]. Antipyretic drugs functions by inhibiting the enzymatic activity of cyclooxygenase as well as reducing the levels of PGE2 within this region [16]. Subcutaneous administration of yeast produced excessive PGE2 which set the thermoregulatory center temperature to a higher level [17]. Therefore, it can be suggested that the *C. verrucosa* ethanol extract at higher dose successfully inhibited the production of prostaglandin thereby reduced the hypothalamic temperature.

Administration of alloxan destroys β-cells of the islets of Langerhans in pancreas which causes considerable decrease in serum insulin level [18]. The activity of Glibenclamide was due to its secretogogue action stimulating rest number of β-cells present [19]. The extract showed a strong antidiabetic activity either by increasing the

glucose utilization in the periphery or by decreasing the endogenous glucose production in the liver.

Thrombolytic agents function by activating plasminogen which produce plasmin [20]. Plasmin lyses clot by breaking down the fibrin mesh. Streptokinase generates a 1:1 stoichiometric complex with plasminogen that convert additional plasminogen to plasmin (Banerjee et al., 2004) [21]. The result does not support any prominent potential of the extract to act through this antithrombotic mechanism.

Changes in behavioral parameters or exploratory activities in Hole cross and Open field tests validates the effect of administered drug or test sample [22]. Gamma-amino-butyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system [23]. GABA mediated inhibition of locomotors activities is due to either decrease in the firing rate of critical neurons in the brain or direct activation of GABA receptor by any agent [24]. Motor functions like hole crossing and distance travel has been reported as an indication for increase in alertness [13]. Therefore, decrease in motor function is considered as depressive effect. In both type of tests for impaired motor functions of the animal, the plant extract demonstrated significant potential which implies to its GABA mediated involvement in CNS.

5. Conclusion

Form the study it can be proposed that the ethanol leaf extract of *C. verrucosa* possess strong antipyretic, antidiabetic as well as CNS depressive activity. However, the extract lack to produce a firm thrombolytic activity. Further study is required to indicate the responsible compounds for their specific activities and mechanistic involvement. The present study also concludes the extract as a safe and beneficial for human use in medicinal purposes.

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