

PHARMACOLOGICAL EVALUATION OF CUCUMIS SATIVUS LINN FRUITS EXTRACT FOR ANTI ANXIETY AND ANTIDEPRESSANT ACTIVITY IN EXPERIMENTAL RATS

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ABSTRACT

INTRODUCTION

Depression and anxiety are common mental health disorders, with a lifetime prevalence of 10% in the general population. *Cucumis sativus* mostly contain alkaloids, glycosides, terpenoids, flavonoids, tannin used for its diabetic, purgative, skin disease, ulcer protective, laxative, anthelmintic effect. The present study was designed to extract the chemical constituents and evaluation of anti-anxiety and antidepressant activity of *Cucumis sativus* fruits using experimental rats.

MATERIAL AND METHODS

The fruits of the *Cucumis sativus* plant were collected and chemical constituents were extracted by ethanol as solvent. Wistar rats of 150-250 gram were taken as study animal into four groups. Anti-anxiety and antidepressant activity were performed by Elevated plus maze and Forced swim test in Wistar rat. The ethanolic extract of low dose (250 mg/kg) and high dose (500 mg/kg) were subjected for anti-anxiety and antidepressant activity.

RESULTS

In the elevated plus maze as animal models of anxiety, extract treated 500 mg/kg showed more effective than 250 mg/kg in both time spent and number of entry in open arm but decrease in close arm as compared to control group ($p < 0.05$). In forced swim test as an animal model of depression, extract treated 250 and 500 mg/kg produce significant reduction ($p < 0.05$ and $p < 0.001$ respectively) in the immobility period. The extract 500 mg/kg was found to be similar to that of standard drug imipramine ($p < 0.001$).

CONCLUSION

The present study concludes that the extract possesses potent and sustained anti-anxiety and antidepressant activity of *Cucumis sativus* fruits.

KEYWORDS

Anti-anxiety, Antidepressant, *Curcumis sativus*

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INTRODUCTION

Depression is a mental health disorder characterized by persistent feelings of sadness, hopelessness and a lack of interest in activities. Anxiety is a feeling of worry, nervousness, or unease about something with an uncertain outcome. It is a normal response to stress but can become a disorder when the feelings become chronic and interfere with daily life. Depression and anxiety can be caused by a variety of factors, including genetics, environment, and life events. They can also occur as a result of other medical conditions or as a side effect of certain medications. These disorders can affect people of all ages and can lead to significant impairments in daily functioning if left untreated.¹ According to the World Health Organization (WHO), suicide is one of the most frequent consequences of depression, with an estimated prevalence of 5% in the general population.²

It is expected that depression will overtake cardiovascular disease as the second-leading cause of premature mortality or disability worldwide in near future.³ For a long time, benzodiazepines were the medicine of choice for treating acute anxiety episodes, while amine uptake inhibitors and monoamine oxidase inhibitors were used to treat depression. Anxiety is influenced by the gamma amino butyric acid (GABA) system and serotonergic neurotransmission. Selected serotonin reuptake inhibitors are also well known for their potent antidepressant effects.⁴ The allopathic treatment process also gives the range of psychological side effects & resistance to prolonged medication. In this situation, we require medications with fewer adverse effects. As a result, research into herbal medications has been expanded in an effort to find new and better tolerated compounds derived from plant sources.⁵

According to the World Health Organization (WHO), “a large proportion of the global population, particularly in developing countries, relies on traditional remedies such as herbs for healthcare.⁶ Indigenous remedies have been used by many traditional healers for hundreds of years.⁷ Only a small portion of the indigenous knowledge was recorded in books and numerous other religious scripts, with the majority being passed down verbally from generation to generation as inherited culture.⁸ Therefore, patients in developed countries may perceive herbal therapies as being more natural and safer compared to synthetic drugs, while in developing countries, access to herbal therapies may be more feasible due to cultural, traditional, and economic factors.⁹

Cucumber is the edible fruits belong to family Cucurbitaceae. Fruits contain different chemical constituents which have been reported presence of glycosides, vitamins, sitosterol, bitter principle cucurbitacins A, B, C, D, E, and I, lactic acid, tannic acid, ascorbic acid, agmatine, polyamines, triterpenoids, tannins, polyphenols, flavonoids, saponin, many amino acids have been isolated.^{10,11}

The *Cucumis sativus* comprise by an active substance that is in charge of producing a certain pharmacological effect. Recent study suggested that it is employed in conventional medicine to treat a variety of diseases. The fruit of the plant is an astringent and is used to treat laxative, anthelmintic, and antipyretic.¹² The fruit's pulp has beneficial properties for leprosy, piles, dysentery, hypoglycemia, antioxidants, and diabetes.¹³

The present study was conducted to examine the antianxiety and antidepressant activities of the ethanolic extract of *Cucumis sativus* fruit in experimental rats by using the elevated plus maze and forced swim test.

MATERIAL AND METHODS

Animals and Consent

In this investigation, experimental Wistar rats of either sex weighing between 150 and 250 g were employed. Standard housing conditions (25°C, 55% relative humidity, and 12-hour light/dark cycles) were used for the animals' cages. The 24 rats were divided into four group having six rats in each group. Prior to conducting behavioural investigations, the animals spent five days becoming used to the laboratory environment and were given free access to tap water and normal laboratory rat food. All of the readings were taken between the hours of 10 a.m. and 4 p.m. on the same day. The protocol was approved by our Institutional Review Committee, Universal College of Medical Science (UCMS), Bhairahawa, Nepal.

Collection and Authentication of Plant Material

Plant materials were collected from Nayagau-1, Bhairahawa, Nepal. Herbarium was prepared with fresh plant material and was submitted for identification and certificate of plant. Certificate of plant identification was issued by Assistant Professor Subodh Khanal, Department of Soil and Environment Science (Institute of Agriculture and Animal Science, Paklihawa Campus).

Extraction¹⁴

C. sativus fruits sliced to small pieces and dried completely under the controlled temperature of 25°C and ground with an electric grinder. About 250 gm of the powder was extracted with ethanol in the ratio of 5:8 by soxhlet extractor at 50-65°C. The extract was then concentrated to viscous semisolid mass under reduced pressure by rotary evaporator at 40°C and dryness at 32±30°C in hot air oven. The resulting dry extract was weighed and percentage yield were calculated. The dried extract was stored in refrigerator at 4°C.

Acute Toxicity Study¹⁵

The toxicity was investigated in accordance with Organisation for Economic Co-operation and Development (OECD) guideline. The first test was, limit test employing female Wistar rats weighing between 150 and 250 grams. The animals were acclimated to laboratory conditions before being used in experiments. According to the recommendation in the guideline, three rats received a dose of extract produced in 1% dimethyl sulfoxide (DMSO) in increments of 500 mg/kg, 2000 mg/kg, and 5000 mg/kg. After receiving the dose, each animal was monitored every hour. Following then, toxicity and mortality up to 14 days were monitored daily.

Dose and Dosage form Preparation^{16, 17}

Drug and extract dose selection and dosage form preparation were done on the following basis.

- Diazepam: 1mg/kg body weight was taken as per the earlier studies.
- Imipramine: 30 mg/kg body weight was taken as per the earlier studies.
- Extract: 250 mg/kg (low dose) and 500 mg/kg (high dose) were taken as effective doses.

Anti-Anxiety Activity Elevated plus maze¹⁸

The standard test duration was 5 minutes. The rats were randomly assigned to the following groups:

- Control: Vehicle (1% DMSO) 10 ml/kg, p. o.
- Standard: Diazepam 1 mg/kg
- High dose: Extract 500 mg/kg, p.o.
- Low dose: Extract 250 mg/kg, p.o.

The frequency and length of arm visits, independently for open and closed arms, were typically measured in this test. When a rat's four paws were on an arm, it was said to have entered that arm.

Antidepressant Activity Forced Swim Test¹⁹

Rats of either sex were individually made to swim in an open, 15-cm-high, 25-cm-long tube filled with fresh water and kept at 26°C. All the rats were divided into four groups.

Control: -vehicle (1% DMSO) 10 ml/kg, p. o

Standard: -imipramine 30 mg/kg (i.p)

Low dose: -250 mg/kg p.o.

High dose: -500 mg/kg p.o.

The final four minutes of the six-minute session, which made up the overall immobility time were recorded. When each rat was deemed to be immobile, it stopped struggling and stayed still in the water, only making the movements required to maintain its head above water. An antidepressant-like effect is shown by a reduction in the amount of time spent immobile.

Statistical Analysis

All the data in the tables and figures were represented as mean \pm standard error of mean of each group (n=6) and plotted with Microsoft excel, 2010 statistical analysis were evaluated by one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test between different groups (* P <0.05), ** P <0.001) using GraphPad Prism software version 8.02. The P values less than or equal to 0.05 were considered statistically significant.

RESULTS

Acute Toxicity Study

All the animals survived at a dose of 5000 mg/kg of body weight. No major behavioural changes were observed during the period of study. Acute toxicity study showed no mortality at a dose of 5000 mg/kg. It was taken that 1/10th and 1/20th of the same dose of extract as therapeutic dose i.e., 500 mg/kg high dose and 250 mg/kg as low dose.

Table 1. Change in Behavior Profile of Extract Administered Rat in Acute Toxicity Study

Behavior type	Extract 500mg/kg, P.O.	2000mg/kg, P.O.	5000mg/kg, P.O.	1% DMSO
Alertness	+++	+++	++	+++
Awareness	+++	+++	++	+++
Sound response	+++	+++	++	+++
Touch response	+++	+++	++	+++

+++ = present, ++ = Behavioral Response with Mild Sedation, P.O. = Per Oral

Antianxiety Activity Elevated plus maze Open arm:

Standard treated group (Diazepam 1mg/kg) showed significant increase in time spent and number of entries in open arm (** p <0.05). (279.0 \pm 6.6, 18.83 \pm 1.014) as compared to control group (49.67 \pm 3.342, 5.000 \pm 0.7303). Which indicate reduction in anxiety. Extract treated group Low dose (250 mg/kg) and

High dose (500 mg/kg) showed increase in time spent and number of entries in open arm in comparison with the control group. Extract treated group of high doses (500 mg/kg) was found to be less effective (179.3 \pm 12.24, 15.33 \pm 1.33) than standard group (279.0 \pm 6.6, 18.83 \pm 1.014) but more effective than (250 mg/kg) low dose (120 \pm 12.62, 8.00 \pm 0.84).

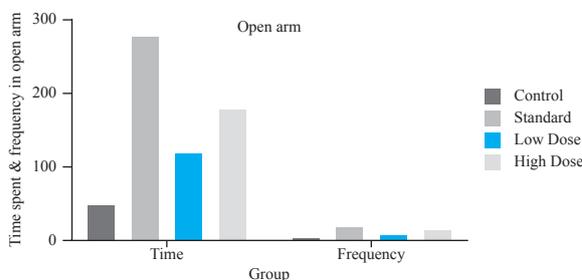


Figure 1. Effects of Cucumis sativus on open arm in elevated plus maze

Elevated plus maze Close arm:

Standard treated group (Diazepam 1mg/kg) showed significant decrease in time spent and number of entries in close arm (** p <0.05) (21.00 \pm 6.62, 10.67 \pm 1.668) as compared to control group (250.3 \pm 3.343, 13.167 \pm 2.056) which indicate reduction in anxiety. Extract of High dose (500 mg/kg) and Low dose (250 mg/kg) showed decrease in time spent and number of entries in close arm in comparison with the control group. Extract treated group of high doses (500 mg/kg) was found to be less effective (106.7 \pm 14.2, 9.833 \pm 0.609) than standard group but more effective than low dose (250 mg/kg) (180.5 \pm 11.23, 8.40 \pm 1.68).

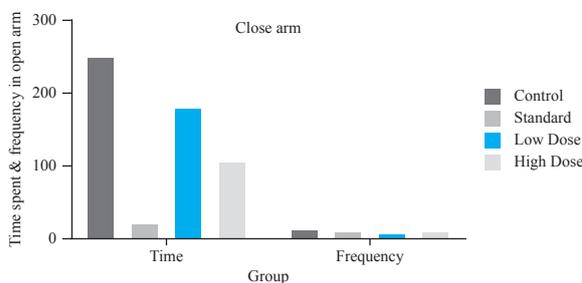


Figure 2. Effects of Cucumis sativus on close arm in elevated plus maze

Antidepressant Activity Forced Swim Test

A forced swim test was used to study the antidepressant effects of Cucumis sativus 250 mg/kg, 500 mg/kg and imipramine while tracking changes in the length of immobility. When compared to the control group, animals treated with the conventional medication imipramine 30 mg/kg showed a considerable reduction in the immobility period. In this test, the immobility duration is significantly reduced by 250 and 500 mg/kg p.o. (p <0.05 and p <0.001 respectively) compared to the control group. It was discovered that the extract at a dose of 500 mg/kg was efficient and showed behaviour similar to that of the medication imipramine (p <0.001).

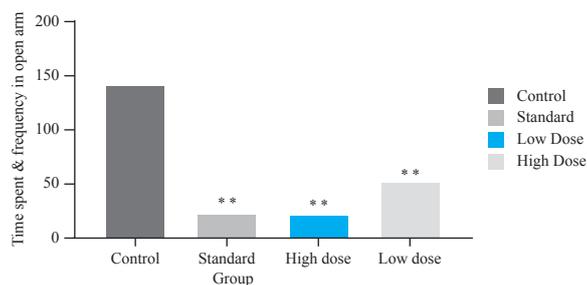


Figure 3. Antidepressant activity of *Cucumis sativus* by Forced Swim Test

DISCUSSION

This research has provided valuable information for the development of new pharmacotherapies derived from medicinal plants and isolated active phytoconstituents.²⁰ In the study the oral LD-50 of a plant extract was found to be greater than 5000 mg/kg indicating that it was largely non-toxic. The extract was also found to possess anti-anxiety, antidepressant and skeletal muscle relaxing properties. In a study, rat treated with a standard treatment of diazepam significantly increased their time spent and number of entries into the open arm and decreased their time spent and number of entries into the closed arm compared to the control group. The extract-treated groups, at doses of 500 and 250 mg/kg also showed increased time spent and number of entries into the open arm and decreased time spent and number of entries into the closed arm compared to the control group. However, the high doses of the extract were not as effective overall as the control group but were more beneficial than low doses in terms of time spent and entries into the open and closed arms. The effects of the ethanolic extract of *Cucumis sativus* fruit may be due to the presence of alkaloids, flavonoids, terpenoids, phenolics and phyosterols. It is possible that the binding of these phytochemicals to the GABA-Benzodiazepine complex is responsible for the anti-anxiety effects of the extract.²¹ In a separate study, chronically stressed rat showed a significant decrease in the number of entries into the open arm and a decrease in time spent in the open arm, suggesting a reduction in stress.²² The ethanolic extract of *Cucumis sativus* fruit demonstrated significant antidepressant effects in a study using a forced swim test. The extract at doses of 250 and 500 mg/kg significantly reduced immobility time compared to the control group ($p < 0.05$ and $p < 0.001$, respectively). The extract at a dose of 500 mg/kg was found to be effective and exhibited behavior similar to the antidepressant medication imipramine ($p < 0.001$). A preliminary phytochemical screening identified the presence of glycosides, steroids, flavonoids, and amino acids in the extract, which may enhance neurotransmitters, involved in memory and information processing and potentially be useful in the treatment of depression. *Cucumis sativus* fruit also contains flavonoids and tannins and neuroactive steroids that bind to GABA receptors in the central nervous system have been found to function similarly to benzodiazepines. These active substances in the extract that act on the GABA/benzodiazepine receptor complex may contribute to its antidepressant action. GABA is the primary inhibitory neurotransmitter in the central nervous system and various antidepressants, muscle relaxants and sedative-hypnotic medications work by inhibiting GABAergic activity, resulting in a reduction in the firing rates of key brain neurons or the direct activation of GABA

receptors by extracts.²³ It is required to further isolate and identify the bioactive component that is responsible for the antidepressant effect.

CONCLUSION

Based on the results of the present study, we conclude that the ethanolic extract of *Cucumis sativus* fruits possess significant anti-anxiety and anti-depressant activity. These results suggest that *Cucumis sativus* fruits could be of potential interest as an alternative therapeutic medication that could help the conventional pharmacotherapy of anxiety and depression.

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CONFLICT OF INTEREST

None

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