ORIGINAL ARTICLE

Anxiolytic and hypnotic effects of ethanolic root extract of *Carica papaya* in mice

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Abstract

Aim: The aim of the present study is to evaluate the anxiolytic and hypnotic effects of ethanolic root extract of *Carica papaya* in mice. **Materials and Methods**: Anxiolytic activity was observed using behavior paradigms such as elevated plus maze, Ymaze, open field test, and hole board apparatus, while the hypnotic activity was assessed by sodium pentobarbital-induced hypnosis in mice. In elevated plus maze and Y maze, the animals were treated with the extract at dose (75 and 150 mg/kg) po for 7 days and were observed on 7th day of treatment. Anxiolytic activity using open-field apparatus and in sodium pentobarbital-induced hypnosis, the effects were observed on day 1, 7, 15, and 30 days of treatment. **Results and Discussion**: In the elevated plus maze, there was an increase in the number of entries and time spent in the open arm. In Y maze decrease in the number of visits to three arms was observed. In the hole board test observed increase in the number of head dippings. In sodium pentobarbital-induced hypnosis, there is a significant increase in the duration of sleep and a decrease in the latency of sleep. The anxiolytic and hypnotic effects were substantially greater at dose 150 mg/kg in comparison to dose 75 mg/kg. **Conclusion:** The results obtained indicate that ethanolic *C. papaya* root extract might have significant anxiolytic and hypnotic activity.

Key words: Anxiolytic, *Carica papaya*, hypnotic

INTRODUCTION

Anxiety is one of the most common mental disorders. One-eighth of the world population is affected.[1] In children, different types of anxiety symptoms are panic disorder, social phobia, separation anxiety disorders, and generalized anxiety disorder.[2] Anxiety is a combination of fear, anxiousness, and nervousness. The most commonly used drugs are benzodiazepines, selective reuptake inhibitors, tricyclic antidepressants, β blockers, barbiturates, and azapirones. Among these drugs such as barbiturates, benzodiadepines, and azapirones are mainly act on the gamma-aminobutyric acid (GABA) which plays an important role in the pathophysiology of anxiety and some of the anticonvulsants pregabalin, valproate, and gabapentin are mainly acts on the metabolic pathway of GABA and increase its levels.[1,3]

Sleep plays an important role in the central nervous system to regulate mental functions and physiological parameters. Insomnia is defined as lack of sleep; it is one of the disorder which is seen on anxiety where the glutamate and GABA are play a role in the pathophysiology of insomnia. The drugs used to treat insomnia are barbiturates, benzodiazepine hypnotics, monoamine oxidase inhibitors, zolpidem, antihistamines,[3] However, most of the drugs which are used to treat anxiety produces undesirable side effects such as drowsiness, muscle relaxation, sedation, hepatotoxicity, and insomnia. It was investigated on medical herbs to cure anxiety with less side effects such as Ginseng, *Bacopa monnieri* Linn., *Ginkgo biloba* Linn., *Salvia officinalis* Linn, and *Piper methysticum*.[1]

Carica papaya is a traditionally medical and nutritional value plant belonging to the family *Caricaceae* and it is a polygamous species; it is present in female, male, and hermaphrodite. The stems are marked with scars and fallen off leaves with long

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Received: 11-08-2020
Revised: 05-10-2020
Accepted: 18-10-2020

petioles; it consists of fragrant flowers, large berry fruits, black seeds, and the leaves contains a milky juice.[4]

The medicinal properties of *C. papaya* are antimicrobial, anthelmintic, antimalarial, antifungal, antiamoebic, diuretic, hepatoprotective, male antifertility, female antifertility, histaminergic, immunomodulatory, antioxidant, gastroprotective, and wound healing.[4]

However, previously not much pharmacological work has not been reported on the anxiolytic and hypnotic activity of *C. papaya* roots. The roots of the plant were not explored for these activities. Hence, based on the evidence of possible central nervous system activity of plant roots, we have carried out the effect of ethanolic root extract of *C. papaya* for its potential anxiolytic and hypnotic effects.

MATERIALS AND METHODS

Plant Collection and Authentication

The roots of the *C. papaya* plant were collected in the month of September 2019 at Gandepalli, East Godavari, Andhra Pradesh, India. The plant was authenticated by Dr. T. Raghuram, Associate Professor, Maharani College, Peddapuram and voucher specimen number given is 23251.

Preparation of Ethanolic Root Extract of *C. papaya*

The freshly collected roots of *C. papaya* were washed with water to remove dirt and sand particles and dried under shade for 40 days and they were grounded into powder using a mechanical grinder. The powder was extracted with 95% ethanol for 3 days followed by hot percolation for 3 h. Then, it was filtered and distilled at 80°C. Then, it was transferred into the empty china dish and evaporated to get an ethanolic extract and kept in anhydrous calcium chloride-containing desiccators.

Experimental Animals

Swiss albino male mice (20–30 g) were used for this study. All the animals are properly caged and maintained standard pellet diet and water *ad libitum*, placed in a properly air-conditioned room with 12 h light and dark cycles. The animal experiments were performed based on the Institutional Animal Ethics Committee approval and guidelines REG. No. 1269/a/10/CPCSEA.

Preliminary Phytochemical Screening

In preliminary phytochemical testing, the ethanolic root extract of *C. papaya* root phytochemical studies was performed to test the presence of the secondary metabolites such as carbohydrates, phenolic substances, tannins, glycosides, flavonoids and alkaloids, saponins, terpenoids, carotenoids, phytosteroids, proteins, and amino acids.[5]

Acute Toxicity Studies

C. papaya ethanolic root extract was administered orally in a dose of 50, 100, 200, 400, 800, and 1600 mg/kg to groups of mice (n = 6); observed for signs of behavioral, neurological toxicity, and percentage mortality was noted 24 h later. The doses were administered to the animals. As per the OECD guideline 42,018, so far, no pharmacological activities have been carried out on this plant species. Therefore, based on the other species in the same genus, doses were considered for acute toxicity studies.[6]

Drug Administration

Experimental mice were randomly divided into four groups, each group containing (n = 5) animals. One group was served as control (0.9% normal saline); two groups were treated with the extract of *C. papaya root* (75 and 150 mg/kg). Diazepam (2 mg/kg) was used as a reference for both anxiolytic and hypnotic activity. The extract was dissolved in 1 ml acacia mucilage (1%) to obtain the final concentration. All respective groups were administered orally through oral intubation.[3] The doses were selected based on the literature review.

The animals were divided into four groups and are treated with,
Group I: Vehicle.
Group II: Diazepam (2 mg/kg).
Group III: Ethanolic root extract of *C. papaya* (75 mg/kg).
Group IV: Ethanolic root extract of *C. papaya* (150 mg/kg).

Elevated Plus Maze

The apparatus elevated plus maze consisting of two open arms (50 cm × 10 cm) and two enclosed arms (50 cm × 10 cm × 40 cm) was used. The maze was elevated to a height of 60 cm from the floor.[7]

The animals were treated with ethanolic root extract of *C. papaya* (75 and 150 mg/kg) and vehicle for 7 days. On the 7th day, 1 h after the administration of extract, the study was carried out. On the test session of the 7th-day, Group 2 is treated with diazepam (2 mg/kg).

The animals were placed individually at the center of the maze head facing the open arm and the number of entries and time spent in open arm and closed arms were recorded for 5 min. For every trail, the apparatus was cleaned with 10% of the ethanolic solution.[1]

Y maze

The apparatus is made of wood; it consists three arms which are in equal diameters. Each arm is 13 cm high, 40 cm long, 3 cm at the bottom, and 10 cm wide at the top.[1]

The animals were treated with ethanolic root extract of *C. papaya* (75 and 150 mg/kg) and vehicle for 7 days. On the 7th day, 1 h after the administration of extract, the study was carried out. On the test session of the 7th-day, Group 2 is treated with diazepam (2 mg/kg).

The animals were placed on the center of the apparatus the number of visits to three arms was recorded for 5 min. For every trail, the apparatus was cleaned with 10% of the ethanolic solution.

Open Field Test

The apparatus is a rectangular box. The walls are made up of glass (37 m height) and floor is made up of wood consists 25 equal squares (16 cm × 16 cm).[8]

The animals were divided into four groups and are treated with,

The animals were treated with ethanolic root extract of *C. papaya* (75 and 150 mg/kg) and vehicle for 30 days. On the test session of 1, 7, 15, 30 days, Group II is treated with diazepam (2 mg/kg).

The animals were placed at the corner of the open field apparatus; the number of the center square and peripheral square crossed and the number of rearings was recorded on day 1, 7, 15, and 30 for 5 min.

Hole Board Test

The apparatus is made up of wood (1 cm thick and 40 cm square) and it consists of 16 holes each hole with a diameter 3 cm and is raised from the ground at 25 cm height.[9]

The animals are divided into four groups and are treated with,

One hour after the administration of extract, the study was carried out. The animals were individually placed on center of the apparatus, the number of head dippings was recorded for 5 min (Yao *et al*., 2010).

Sodium Pentobarbital-induced Hypnosis

In sodium pentobarbital-induced hypnosis, sleeping time and the onset of sleep were observed by the change in the time of drug administration and time of loss of righting reflex, while the duration of sleep was represented by time to recover from righting reflex[10] (Nugroho *et al*., 2012). On the day of test session, mice of respective groups were administered with normal saline (10 mL/kg), diazepam, 2 mg/kg, and ethanolic extract of *C. papaya* (75 and 150 mg/kg) through oral intubation. After 30 min of administration, mice were injected with sodium pentobarbital 40 mg/kg intraperitoneally and placed in separate cages. The onset of sleep (loss of righting reflex) and the duration of sleep were the observed parameters.[11] Test sessions were conducted on day 1, 7, 15, and 30 of treatment.

Statistical Analysis

Data were analyzed by GraphPad INSTAT® version 3.0 software and presented as mean±SEM values. The statistical tests used were one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The levels of statistical significance ranged from $P < 0.05$ to $P < 0.001$.

RESULTS

Phytochemical Screening

The phytochemical screening of ethanolic root extract of *C. papaya* has shown the presence of carbohydrates, phenolic substances, tannins, glycosides, flavonoids, alkaloids, phytosteroids, proteins, and amino acids.

Acute Toxicity Studies

There are no toxic symptoms and signs of mortality were observed after a single oral dose of up to 1600 mg/kg of extract. The behavioral patterns of mice were observed for an initial 4 h, followed by 24 h after administration in the control and treated groups. The treated groups did not show any significant change from the control in behavior patterns, skin and eye coloration, body temperature, food intake, and no sign of mortality were observed.

Elevated Plus Maze

The effect of 7 days treatment of ethanolic root extract of *C. papaya* (75 mg/kg and 150 mg/kg) po has shown that time spent in open arm and number of entries in the open arm are increased and decreased in time spent in closed arm and number of entries in the closed arm which were compared to the control group, whereas in diazepam 2 mg/kg also shows same results [Table 1].

Y Maze

The effect of 7 days of treatment of ethanolic root extract of *C. papaya* (75 mg/kg and 150 mg/kg) po has shown that decreased in the number of visits to the three arms of Y maze which were compared to the control group, whereas in diazepam 2 mg/kg also shows same results [Table 2].

Open Field Test

The effect of 30 days treatment of ethanolic root extract of *C. papaya* (75 mg/kg and 150 mg/kg) po on the test sessions of day 1, 7, 15, and 30 has shown that increased in the center

Table 1: Effect of ethanolic root extract of *C. papaya* on mice in the elevated plus maze

Treatment	Time spent in open arm (S)	Time spent in closed arm (S)	No of entries in open arm	No of entries in closed arm
Vehicle	40.50±11.89	234.5±12.61	1.33±0.61	11.33±0.25
Diazepam (2 mg/kg)	115±6.84	169.7±5.65	8.00±1.06	9.00±1.06
ERECP (75 mg/kg)	107.66±0.50	184.±0.25	8.66±0.25	7.33±0.54
ERECP (150 mg/kg)	116.66±0.25*	170.66±0.25*	11.66±0.66*	6.33±0.25*

All the values are expressed as Mean±SEM, n=5, *P<0.001 when compared with standard values. *C. papaya: Carica papaya*

Table 2: Effect of ethanolic root extract of *C. papaya* on mice in Y maze

Treatment	No of Visits
Vehicle	44.17±3.28
Diazepam (2 mg/kg)	26.83±2.07
ERECP (75 mg/kg)	38.66±1.39
ERECP (150 mg/kg)	27±1.15*

All the values are expressed as mean±SEM, n=5, *P<0.001 when compared with standard values. *C. papaya: Carica papaya*

square crossings, whereas the peripheral square crossings and the number of rearings are also increased which were compared to the control group, the best activity was observed on day 30, whereas in diazepam 1 mg/kg also shows same results [Table 3a–c].

Hole Board Test

The effect of ethanolic root extract of *C. papaya* (75 mg/kg and 150 mg/kg) po has shown that an increased in the number of head dippings which were compared to the control group, whereas in diazepam 2 mg/kg also shows same results [Table 4].

Sodium Pentobarbital-induced Hypnosis

The effect of 30 days treatment of ethanolic root extract of *C. papaya* (75 mg/kg and 150 mg/kg) po on the test sessions of day 1, 7, 15, and 30 has shown that increased in the duration of sleep and decreased in latency of sleep which were compared to the control group, whereas in diazepam 2 mg/kg also shows the same results (Table 5a and b).

DISCUSSION

There are no toxic symptoms and signs of mortality were observed after a single oral dose of up to 1600 mg/kg of extract. The behavioral patterns of mice were observed for an initial 4 h, followed by 24 h after administration in the control and treated groups. The treated groups did not show any significant change from the control in behavior patterns, skin and eye coloration, body temperature, food intake, and no sign of mortality were observed. The maximum dose of 1600 mg/kg did not show any considerable behavioral changes, except, drowsiness and sedation but these effects were diminished later.

In the elevated plus maze, 7 days treatment of ethanolic root extract of *C. papaya* (75 mg/kg and 150 mg/kg) po has shown that time spent in open arm and number of entries in the open arm are increased and decreased in time spent in closed arm and number of entries in the closed arm which were compared to the control group. The results are comparable with that of standard diazepam (2 mg/kg). According to the previous published studies, the number of entries and time spent in the open arm were sensitive to agents that act through GABA receptor A (GABA-A) receptor complex, while diazepam is among the benzodiazepines that exert anxiolytic properties by directly targeting the GABA-A receptor.[12]

In Y maze, 7 days treatment of ethanolic root extract of *C. papaya* (75 mg/kg and 150 mg/kg) po has shown that decreased in the number of visits to the three arms of Y maze which were compared to the control group and the results are comparable with that of standard diazepam (2 mg/kg). There is a decrease in the number of visits to the three arms of Y maze indicating that the extract possesses anxiolytic activity.[1]

In open field test, 30 days treatment of ethanolic root extract of *C. papaya* (75 mg/kg and 150 mg/kg) po on the test sessions of day 1, 7, 15, and 30 has shown that increase in the center square crossings, whereas the peripheral square crossings and the number of rearings are also increased and the results are comparable with that of standard diazepam 2 mg/kg. Increased in the center square crossings, peripheral square crossings, and number of rearings indicates that anxiolytic activity.

In addition to this, a significant reduction in latency to first head dip, with augmentation in head dipping behavior by ethanolic root extract of *C. papaya* (75 mg/kg and 150 mg/kg) po. The increase in the number of head dips narrates the release of anxiety and anxiolytic effect of drug substance.[13]

The result from the above tests reveals that the ethanolic root extract of *C. papaya* 150 mg/kg is the more potent anxiolytic dose. The mechanism of anxiolytic activity of the extract might be due to the presence of flavonoids since

Table 3a: Effect of ethanolic root extract of *C. papaya* on mice in open field test by measuring center square crossings

Treatment	1st day	7th day	15th day	30th day
Vehicle	20.33±0.6	25.33±0.25	26.66±0.9	34±1.15
Diazepam (2 mg/kg)	48.66±0.25	47.33±0.66	48±0.43	48.66±0.25
ERECP (75 mg/kg)	24.66±0.66	28±0.4	30.33±0.6	36±0.66
ERECP (150 mg/kg)	31±1.15*	33.33±0.66*	37±0.75*	43.66±0.66*

All the values are expressed as mean±SEM, n=5, *P<0.001 when compared with standard values. *C. papaya: Carica papaya*

Table 3b: Effect of ethanolic root extract of *C. papaya* on mice in the open field test by measuring peripheral square crossings

Treatment	1st day	7th day	15th day	30th day
Vehicle	46.66±0.5	48.33±0.25	47.66±0.5	50±0.8
Diazepam (2 mg/kg)	94±0.43	93.33±0.66	94.33±0.5	94±0.43
ERECP (75 mg/kg)	52.33±1.09	59.66±0.9	64±0.75	75.66±0.25
ERECP (150 mg/kg)	64.33±0.66*	76±1.15*	76.33±0.5*	85.33±0.66*

All the values are expressed as mean±SEM, n=5, *P<0.001 when compared with standard values. *C. papaya: Carica papaya*

Table 3c: Effect of ethanolic root extract of *C. papaya* on mice in open field test by measuring no. of rearings

Treatment	1st day	7th day	15th day	30th day
Vehicle	5.66±0.25	8±0.43	9.33±0.25	11.33±0.66
Diazepam (2 mg/kg)	15.66±0.5	18±0.43	15.66±0.25	19±0.43
ERECP (75 mg/kg)	8.33±0.25	12.33±1.09	13±0.43	18.33±0.25
ERECP (150 mg/kg)	12.66±0.66*	16.66±0.25*	17.33±0.66*	20.6±0.5*

All the values are expressed as Mean±SEM, n=5, *P<0.001 when compared with standard values. *C. papaya: Carica papaya*

Table 4: Effect of ethanolic root extract of *C. papaya* in hole board test

Treatment	No. of head dippings
Vehicle	4.3±0.66
Diazepam (2 mg/kg)	10.66±0.25
ERECP (75 mg/kg)	8±0.4
ERECP (150 mg/kg)	9.33±0.25*

All the values are expressed as mean±SEM, n=5, * P<0.001 when compared with standard values

flavonoids exert their activity through GABA receptors. Earlier many traditional plants such as *Morus alba* leaves[14] (Yadav *et al*., 2008), *Cissus sicyoides*,[15] *Cocculus hirsutus*[16] (Marya and Bothara), and *Hibiscus rosa-sinensis*[17] (Begum and Younus, 2018) have exhibited anxiolytic effect for benzodiazepine receptors because of having more affinity toward flavonoids.[18] There might be serotonergic pathway involvement in producing the anxiolytic effect, as serotonin has been implicated in anxiety-like behavior in rodents.[19] Plants such as *Citrus aurantium*,[20] *Nigella sativa*,[21] and *Cinnamomum cassia*[22] are proved to produce anxiolytic effect through acting on the serotonin pathway. Moreover, the contributing alkaloids have also been reported to produce anxiolytic activity through acting on dopaminergic,[23] as dopamine receptors play an important role in mediating anxiolytic activity.[24]

Table 5a: Effect of ethanolic root extract of *C. papaya* in sodium pentobarbital-induced hypnosis by measuring latency of sleep

Treatment	1st day	7th day	15th day	30th day
Vehicle	7.3±0.3	8.1±0.6	9.9±0.5	9.7±0.5
Diazepam (2 mg/kg)	4.6±0.2	5.3±0.4	4.9±0.4	5±0.3
ERECP (75 mg/kg)	6.8±0.2	6.5±0.025	6.3±0.05	6.2±0.02
ERECP (150 mg/kg)	5.7±0.06*	5.7±0.04*	5.7±0.06*	5.3±0.06*

C. papaya: Carica papaya

Hence, it can be assumed that the anxiolytic effect produces ethanolic root extract of *C. papaya* might be exhibited due to its possible involvement with the noradrenergic pathway, as noradrenergic receptors play a significant role in anxiety.[25] However, further studies are needed to justify this assumption as well as to identify the specific phytochemical constituent and along with endogenous mediators involved responsible to produce anxiolytic activity.

In sodium pentobarbital-induced hypnosis, 30 days treatment of ethanolic root extract of *C. papaya* (75 mg/kg and 150 mg/kg) po on the test sessions of day 1, 7, 15, and 30 has shown that increased in the duration of sleep and decreased

Table 5b: Effect of ethanolic root extract of *C. papaya* in sodium pentobarbital-induced hypnosis by measuring the duration of sleep

Treatment	1st day	7th day	15th day	30th day
Vehicle	28.2±0.4	27.5±0.9	24.8±0.8	26±0.5
Diazepam (2 mg/kg)	42.7±0.7	42.9±1.1	41.4±1.2	44.4±1.4
ERECP (75 mg/kg)	28.6±0.2	31.6±0.25	32.33±0.5	33±0.4
ERECP (150 mg/kg)	32±0.4*	35.3±0.24*	36.6±0.5*	38.33±0.25*

All the values are expressed as mean±SEM, n=5, *P<0.001 when compared with standard values. *C. papaya: Carica papaya*

in latency of sleep which were compared to the control group. A similar effect was observed on diazepam 2 mg/kg. In this study, the sodium pentobarbital is mainly act on the central nervous system and activates the inhibitory GABA pathway. It has been reported that prolongation of sleep may be due to hepatic enzyme metabolism and the effect of pentobarbital on the central nervous system involves activation of inhibitory GABAergic system[26] (Aguirre-Hernández *et al*., 2007). The prolongation in the duration of sleep by the extract might be due to the involvement of GABAergic system.

Furthermore, behavioral testing on different doses of ethanolic root extract of *C. papaya* is needed to enlighten the therapeutic significance of this plant in the treatment of anxiety and insomnia. Moreover, it is also important to study the involvement of neuronal pathways (GABAergic, serotonergic, or dopaminergic pathways) by which the ethanolic root extract of *C. papaya* produces anxiolytic and hypnotic effect.

CONCLUSION

The present study indicates that the ethanolic root extract of *C. papaya* has anxiolytic and hypnotic activity. The results from the present study indicate that the ethanolic root extract of *C. papaya* is effective in the treatment of anxiety and insomnia. However, there is a need for further investigation on the pharmacological activity of these active principals of the plant.

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Source of Support: Nil. **Conflicts of Interest:** None declared.