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Evaluation of anxiolytic and sedative effects of 80% ethanolic *Carica papaya* L. (*Caricaceae*) pulp extract in mice ☆

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ABSTRACT

Ethnopharmacological relevance: *Carica papaya* has been used in the Ethiopian traditional medicine to relieve stress and other disease conditions.
Aim of the study: The present study was undertaken to evaluate the anxiolytic and sedative effects of 80% ethanolic *Carica papaya* (*Caricaceae*) pulp extract in mice.
Materials and methods: *Carica papaya* pulp extract was screened for anxiolytic effect by using elevated plus maze, staircase and open field tests, and ketamine-induced sleeping time test for sedation at doses of 50, 100, 200, 400 mg/kg. Distilled water and Diazepam were employed as negative and positive control groups, respectively.
Results: *Carica papaya* pulp extract 100 mg/kg significantly increased the percentage of open arm time and entry, and reduced the percentage of entry and time spent in closed arm in elevated plus maze test; reduced the number of rearing in the staircase test; and increased the time spent and entries in the central squares while the total number of entries into the open field were not significantly affected, suggesting anxiolytic activity without altering locomotor and sedative effects. A synergistic reduction in the number of rearing and an inverted U-shaped dose response curves were obtained with important parameters of anxiety
Conclusions: The results of this study established a support for the traditional usage of *Carica papaya* as anxiolytic medicinal plant.

1. Introduction

Anxiety is a highly prevalent psychological and physiological state characterized by cognitive, somatic, emotional, and behavioral components, and affecting one eighth of the total population of the world and became a very important area of research interest in psychopharmacology (Barlow et al., 1996; Dhawan et al., 2001). Synthetic anxiolytic drugs are available for treating anxiety, but they are burdened with adverse effects, and constraints on resources and time often render therapies such as psychologic interventions impracticable. Thus, an effective medication with few adverse effects would be a welcome addition to the therapeutic repertoire.

Different parts of *Carica papaya* have been used traditionally to treat various ailments in humans across the world. Since last three decades, data are accumulating to systemically evaluate the traditional claims of its application in different cases. Its male contraceptive effect has been widely evaluated and study of the alkaloid extract of *Carica papaya* seeds prevented ovum fertilization, reduced sperm cell counts, sperm cell degeneration and induced testicular cell lesion, changes that induce reversible male infertility and a potential for a pharmaceutical male contraceptive (Lohiya et al., 2000; Goyal et al., 2010). While in female, unripe or semi-ripe papaya that contains high concentration of latex produces marked uterine contraction and may be unsafe during pregnancy as evidenced in animal studies (Anuar et al., 2008; Abdulazeez et al., 2009). Different works have supported the wound healing effect of *Carica papaya*. Additionally, sufficient data are available for the following effects: anthelmintic, CNS activities, anti-tumor, antimicrobial, antimalarial, and antioxidant (Gupta et al., 1990; Stepek et al., 2005; Gurung and Skalko, 2009; Otsuki et al., 2010) and others.

There is a traditional claim for the use of *Carica papaya* pulp for anxiety and a published ethnobotanical survey report on sedative effect of *Carica papaya* (Abate, 1989; Krishna et al., 2008). Additionally, some of the phytochemicals that have been reported to present in the extracts of *Carica papaya* were demonstrated to elicit such pharmacological activities in different literatures (Kuribara et al.,

☆ Chemical compounds studied in this article; Diazepam (PubChem CID: 3016); Ketamine (PubChem CID: 3821); Ethanol (PubChem CID: 702).

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1999; Goutman et al., 2003; Yuan et al., 2004; Wang et al., 2007). No scientific investigations have been conducted till date to verify the use of *Carica papaya* as anxiolytic remedies. The present study therefore, focuses on delineation of the anxiolytic activities of extracts of *Carica papaya* pulp by different models of anxiety and its effects on sedation by the ketamine-induced sleeping time test.

2. Materials and methods

2.1. Chemicals and drugs

The hydroalcoholic extract of *Carica papaya* pulp was used as experimental extract, Diazepam (Intas Pharmaceuticals, India) as anxiolytic drug (standard), Ketamine (Trittau, German) as sedative agent, Ethanol (Uni.chem., India) as an extraction solvent, Distilled water, Dragendrof's reagent, 10% ethanolic ferric chloride, dilute ammonia, acetic anhydride, concentrated sulfuric acid, 5% ethanolic ferric chloride, 1% aqueous hydrochloric acid, and chloroform (BDH, Poole England) were purchased from the respective sources and were of analytical grade.

2.2. Preparation of the plant material

The fresh matured fruit was purchased from local market in Addis Ababa. The *Carica papaya* was identified by a taxonomist and a voucher specimen (ZK/001) was deposited at the National Herbarium, School of biological sciences, Addis Ababa University.

Fresh matured fruits of *Carica papaya* were collected, washed, peeled and extracted as described by Nayak et al. (2007) with slight modification. The underlying epicarp was peeled and 200 g of it was blended with 50 ml of 80% ethanol to fine mixture form using a blender and macerated for 12 h. The mixture was filtered using a fine muslin cloth. Then the alcohol was allowed to evaporate with mild heating on a water bath. Further drying process was conducted in a lyophilizer for removal of water and then the extract was stored at 4 °C. The extract was brown semisolid elastic mass and the yield obtained was 16% (w/w). The doses of extract used in all models of anxiety were determined based the results of acute toxicity test conducted.

2.3. Experimental animals

Swiss albino mice of male sex were used except for acute toxicity study where female mice were used. The mice obtained from animal breeding units of School of Pharmacy, Addis Ababa University were used for the experiment. Mice of 8–12 weeks, weighing 24–30 g were used in all sets of experiments and each animal was used only once. The animals were housed in groups under controlled conditions of light (12-h light/dark cycle) and at room temperature. Food and water were available as necessary. All experiments were carried out during night cycle of light and the experiments were carried out according to the National Research Council Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). The minimum number of animals and duration of observation required to obtain consistent data were employed. The study was approved by Addis Ababa University, School of Pharmacy, Research and Ethics committee (No. PT/24/04/11) to use experimental animals according to the internationally accepted guidelines.

2.4. Acute toxicity study

Acute toxicity study was performed using the limit test dose of 2000 mg/kg as described by Organization for Economic Cooperation and Development guideline and Interagency Research Animal Committee recommendation (Organization of Economic Co-operation and Development (OECD), 2001). Six female mice were dosed sequentially and followed for any signs of toxicity and/or death within 24 h and then for 14 days thereafter.

2.5. Elevated plus maze

Elevated plus maze (EPM) is a modification of the apparatus validated for mice by Lister (1987) and comprised of two open arms ($30 \times 5 \times 0.25$ cm) and two closed arms ($30 \times 5 \times 15$ cm) that extended from a common central platform (5×5 cm) that is elevated to a height of 50 cm above floor level. Mice were given a single oral dose of the different treatments (distilled water, different doses of *Carica papaya* extract (50, 100, 200 and 400 mg/kg) and diazepam 1 mg/kg) 1 h before their placement on the EPM. The number of entries and the time spent in the open and closed arms were recorded during a 5 min test period. The percentage of arm entries in each arm (open or closed arm entry $\times$ 100/total entries) and percentage of time spent in each arm (time spent in open or closed $\times$ 100/time spent in both arms) were calculated for each mouse. During the entire experiment, the animals were allowed to socialize. All tests were taped by using a video camera and every precaution was taken to ensure that no external stimuli could evoke anxiety in the mice. After each test, the maze was carefully cleaned up with a wet tissue paper (10% ethanol solution) to eliminate the interference of the olfactory cues on the next mice.

2.6. Staircase test

The device consisted of a wooden staircase similar to the one used by Simiand et al. (1984) was used. The staircase test (SCT) is enclosed between vertical walls and had 5 identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep. The height of the walls remained constant along the length of the staircase. Each mouse received treatment (distilled water, different doses of *Carica papaya* extract (50, 100, 200 and 400 mg/kg) and diazepam 1 mg/kg) orally 1 h before the tests and was placed individually at the bottom of the staircase. The number of steps climbed and the number of rearing were video recorded for a 3 min observation period as anxiety indices. For the simplicity of the observation, the number of steps descended was not taken into account. A step was considered climbed when all four paws were placed on the step and a rear was registered when the mouse rose on its hind legs, either on the step or against the staircase wall, to sniff the air. The number of steps climbed and the rearing responses were recorded for each mouse. The apparatus was cleaned thoroughly between the recordings.

2.6.1. Co-administration of subthreshold doses of Carica papaya pulp extract and diazepam

In order to evaluate the effect of co-administration on the number of rearing in the staircase test two sub-threshold doses of CPPE (15 and 30 mg/kg) were administered individually and then in combination with 0.25 mg/kg diazepam which was also found subthreshold dose in the experimental setting employed. After 1 h of administration these treatments orally, the procedure described under Section 2.6 was followed for the number of rearing.

2.7. Open field test

The open-field arena made of a wooden box, black floor ($68 \times 68 \times 45$ cm), divided into 16 squares of equal areas as has been used by Aragao et al. (2006). The experimental room was a sound attenuated dark room. Open field apparatus, illuminated with a 40 W bulb, focusing on the field from a height of about