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Research Article

Preclinical evaluation of *Boswellia serrata* for anxiolytic activity

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ABSTRACT

Background: *Boswellia serrata* (BS) has been described in the ancient Ayurvedic texts Sushruta Samhita and Charaka Samhita. It possesses anti-inflammatory, analgesic, anti-arthritic and antioxidant properties. It is found that BS helps in surging of GABA levels in mice brain. The aim of this study was to evaluate the possible anxiolytic activity of BS in *Swiss albino* mice by light and dark arena (LDA) and elevated plus maze (EPM) models.
Methods: In this study, BS (50 mg/kg, 100 mg/kg and 200 mg/kg; p.o) was evaluated for anxiolytic action and compared with standard drug (diazepam) and control (normal saline) in mice by LDA and EPM models. In LDA, number of entries and time spent in light and dark boxes were noted for individual mouse. Similarly, number of entries and time spent in open and closed arms were recorded for EPM model.
Results: One-way Analysis of Variance (ANOVA) followed by Dunnett's *post-hoc* test was used to analyze the data. BS in a dose of 50 mg/kg has shown significant increase in time spent in light box ($p<0.05$) and decrease in time spent in dark box ($p<0.05$) when compared to control group in LDA model. Similarly, in EPM model 200 mg/kg of BS significantly increased time spent in open arm ($p<0.001$) and decrease in time spent in closed arm ($p<0.001$) when compared to control group.
Conclusion: BS in dose of 50 mg/kg and 200 mg/kg has significant anxiolytic action in animal models.

Keywords: Anxiolytic activity, *Boswellia serrata*, Elevated plus maze, Light and dark arena

INTRODUCTION

Anxiety disorders are the most common psychiatric disorders in the general population. Around 15-20% patients in general practice present with anxiety disorders. It is characterized by excessive worrying, apprehension, uneasiness and fear about uncertainties in future. Recent studies have shown that as many as 18% of Americans, 20.7% of Indians and 14% of Europeans are affected by anxiety.[1,2]

The DSM-IV (American Psychiatric Association) covers the following major categories of anxiety disorders: panic disorder (with or without agoraphobia), social phobia (social anxiety disorder), specific phobia, generalized anxiety disorder, acute stress disorder, post-traumatic stress disorder, obsessive-compulsive disorder and anxiety disorder not otherwise specified.[3]

Decreased levels of GABA, an inhibitory neurotransmitter in the central nervous system is responsible for causing anxiety. Most of the anxiolytics achieve their goal by modulating the GABA receptors.[4-6] Benzodiazepines are the most commonly used, safe and effective drug in the short term management of anxiety.[7] Long-term use of benzodiazepine has psychological and physical adverse effects. They are also associated with tolerance, physical dependence and withdrawal syndrome on sudden discontinuation.[8,9] Hence, the requirement for newer, well-tolerated and efficacious treatment remains high.

Boswellia serrata (BS) is a tree of moderate height, grows mainly in hilly parts of India. The therapeutic value of resinous gum (Guggulu), has been known since a long period. Gum resin possesses anti-inflammatory, anti-arthritic, and analgesic activity.[10,11]

BS is a proven antioxidant.[12] Phytochemical analysis has shown the presence of many valuable compounds such as lignans, flavonoids, hydrolysable tannins (ellagitannins), polyphenols, triterpenes, sterols and alkaloids. *In vitro* tests also revealed that *Boswellic* acid, isolated from

the gum resin of *Boswellia*, inhibits the synthesis of pro-inflammatory 5-lipoxygenase products, including 5 hydroxyeicosatetraenoic acid and leukotriene B4, which are responsible for bronchoconstriction, chemotaxis, and increased vascular permeability.[13] *Boswellic* acid is also a specific inhibitor of 5-lipoxygenase.[14,15]

It was found that BS helps in surging of GABA levels in mice brain. Mice pretreated with BS exhibited down-regulation of xanthine oxidase enzymatic activity, showing that BS has the ability to arm the cellular components against oxidative stress.[16]

So, the present study is done to elucidate the possible anxiolytic activity of BS in *Swiss albino* mice.

METHODS

Animals

Institutional Animal Ethical Committee [IAEC/03/18-02-2013] approval was obtained before conducting this study. Male *Swiss albino* mice weighing 25-35 g were used for the study. The mice were inbred in the central animal house of Department of Pharmacology, Yenepoya Medical College, Yenepoya University, Mangalore, Karnataka, India under suitable conditions of housing, temperature, ventilation and nutrition. The study was conducted in accordance with standard Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Inclusion criteria:
- Male *Swiss albino* mice weighing between 25 g and 35 g.
- Age 3-4 months.
- Healthy with normal behavior and activity.

Exclusion criteria:
- Mice <25 g and >35 g; age <3 months and >4 months.
- Animals previously used in other experiments.

A total of 60 mice (n=60) were used. They were divided into ten groups of six mice in each.

Drugs

The test drug, BS was purchased from Natural Remedies, Bangalore, India. The standard antianxiety drug - diazepam and control - normal saline (NS) were purchased from our institutional pharmacy.

Acute oral toxicity test

Acute oral toxicity was carried out in male *Swiss albino* mice according to Organization of Economic Co-operation and Development (OECD) guidelines, ANNEX-423.[17] Animals were administered BS in a dose of 5, 50, 300 and 2000 mg/kg per orally to find out safe dose range in animals. Animals were observed for 48 hrs from the time of drug administration. Mice were looked for general behavior and mortality. As the highest dose of BS (2000 mg/kg) did not cause mortality in mice, the dose range of 50, 100, 200 mg/kg of BS were used in the present study.

Anxiolytic activity of BS was evaluated by using two models - light and dark arena (LDA) and elevated plus maze (EPM). The experiment was conducted in Ethanopharmacology laboratory of the Department of Pharmacology, Yenepoya Medical College, Yenepoya University, between 8:00 A.M. and 2:00 P.M. The food but not water was withdrawn 6 hrs prior to study period. Animals were weighed and appropriate dose of the drug was administered per orally (p.o.) to the different groups of animals. The standard drug - diazepam and test drug BS were dissolved in NS before administration to animals. The experiment was conducted 60 mins after the oral administration of the drug.

Light and dark arena

The apparatus consists of a rectangular box (45 cm × 27 cm × 27 cm), partitioned into two equal compartments, which are connected by a 7.5 cm × 7.5 cm opening in the wall between compartments. One light compartment painted white on all four sides and illuminated with 40 W white light source, which was placed 25 cm above the open compartment. The dark compartment painted black on all four sides and top covered with black plywood. Mouse was placed in the center of the light compartment and observed for 5 mins for the time spent and number of entries in light and dark boxes.[18]

The animals were divided as follows for LDA model:
- Group I: 10 ml/kg of NS
- Group II: 1.0 mg/kg of diazepam
- Group III: 50 mg/kg of BS
- Group IV: 100 mg/kg of BS
- Group V: 200 mg/kg of BS.

Elevated plus maze

It is a novel test used for evaluating the anxiolytic action of a drug in rodents. The wooden plus maze consists of two open arms (length 16 cm × breadth 5 cm) and two closed arms of the same size (height 12 cm). The arms of the same type are placed opposite to each other, with a central square of 5 cm. The maze is in a height of 25 cm above the floor. The individual mouse was placed at the center of the elevated maze with their head facing towards the open arm. Number of entries and time spent in the open and closed arms were recorded for 5 mins for each mouse. The preference of the mouse for first entry into closed arm indicates the relative safety of closed arms as compared with the relative fearfulness of open arms. Mouse being rodent feel safe in dark. Hence normal rodents prefer dark

arm first. Anxiolytics would be expected to increase the proportion of entries and time spent in open arms.[18]

The animals for EPM model were divided as follows:
- Group VI: 10 ml/kg of NS
- Group VII: 1.0 mg/kg of diazepam
- Group VIII: 50 mg/kg of BS
- Group IX: 100 mg/kg of BS
- Group X: 200 mg/kg of BS.

Data analysis

Results are represented as mean ± standard deviation. Statistical analysis was performed by using one-way Analysis of Variance and Dunnett's test for multiple comparisons.

RESULTS

The experiment was conducted in 60 (n=60) male *Swiss albino* mice after 60 mins of oral drug administration. Mice were divided into ten groups of six mice in each. Mice in the first five groups were evaluated for anxiolytic action by LDA model. Table 1 shows the number of entries and time spent in light and dark boxes. Remaining five groups of mice were evaluated for anxiolytic action by EPM model. Table 2 shows the number of entries and time spent in open and closed arms. Mice treated with BS in a dose of 50 mg/kg have shown significant increase in the time spent in light box and decrease in time spent in dark box when compared to control group ($p<0.05$) in LDA model. Similarly, mice treated with 200 mg/kg of BS have shown significant increase in time spent in open arm and decrease in time spent in closed arm ($p<0.001$) when compared to control group in EPM model.

DISCUSSION

Feel worried and anxious are the common symptoms in day today life. However, some persons fail to control their worries and ultimately their routine life is affected due to excessive anxiety. Lifestyle modifications like yoga, meditation and dietary alterations can be helpful in the early part of anxiety disorder. However, most of the anxiety patients are benefited by pharmacotherapy. Benzodiazepines even though established efficacy for many anxiety disorders, associated with many adverse effects.[8,9]

Oxidative stress mechanisms underlying anxiety disorder have been in existence since long time. It was claimed that nitric oxide and peroxynitrite might play a major role in setting up a vicious etiological cycle involving free radicals and inflammatory cytokines in post-traumatic stress disorder.[19,20]

Association of vitamin E depletion, increased oxidative stress markers and anxiety behaviors in phospholipid transfer protein knock-out mice has further suggested an oxidative role in the pathogenesis of anxiety.[21] Even clinical studies have reported elevated lipid peroxidation byproducts in obsessive - compulsive disorder, panic disorder and social phobia.[22-25]

Thus, anti-oxidants could have anxiolytic potential by preventing oxidative pathway. Green tea, polyphenol (–) epigallocatechin gallate and chlorogenic acid, a dietary polyphenol have shown anxiolytic effects in experimental models due to their potent antioxidant property.[10,26] Around 50-65% of anxiety patients are benefited by herbal medications with conventional medications.[27,28]

Table 1: Effect of BS on mice behaviour in LDA model.

Group	Entry into light box	Entry into dark box	Time spent in light box	Time spent in dark box
Group I: NS 10 ml/kg	10.33±1.9	10.17±2.4	88.00±7.8	212.00±7.8
Group II: diazepam 1 mg/kg	10.83±5.6	10.67±5.8	142.00±5.1	158.00±5.1
Group III: BS 50 mg/kg	14.00±2.6	14.00±2.6	162.83±63.4*	137.17±63.4*
Group IV: BS 100 mg/kg	13.17±1.7	13.17±1.7	130.33±26.8	169.67±26.8
Group V: BS 200 mg/kg	10.17±4.3	10.00±4.3	138.50±48.8	161.50±50.8

Values are expressed as mean±SD (n=6). One-way ANOVA followed by Dunnett's test, *$p<0.05$ as compared to normal control, NS: Normal saline, BS: *Boswellia serrata*, ANOVA: Analysis of Variance, SD: Standard deviation

Table 2: Effect of BS on mice behaviour in EPM model.

Group	Entry into open arm	Entry into closed arm	Time spent in open arm	Time spent in closed arm
Group I: NS 10 ml/kg	9.33±3.1	9.83±4.1	86.50±13.1	213.50±13.1
Group II: diazepam 1 mg/kg	21.33±5.3**	7.00±1.7	194.33±8.5**	105.67±8.5**
Group III: BS 50 mg/kg	14.00±2.6	14.00±2.6	162.83±63.4*	137.17±63.4*
Group IV: BS 100 mg/kg	13.17±1.7	13.17±1.7	130.33±26.8	169.67±26.8
Group V: BS 200 mg/kg	10.17±4.3	10.00±4.3	138.50±48.8	161.50±50.8

Values are expressed as mean±SD (n=6). One-way ANOVA followed by Dunnett's test, **$p<0.001$ as compared to normal control, NS: Normal saline, BS: *Boswellia serrata*, ANOVA: Analysis of Variance, EPM: Elevated plus maze

In this study, BS has shown significant anxiolytic activity in preclinical models. BS in a dose of 50 mg/kg significantly increased the time spent in light box and decrease in the time spent in dark box ($p<0.05$) when compared to control group in LDA model (Table 1). Whereas, in EPM model 200 mg/kg of BS has shown significant anxiolytic action ($p<0.001$) when compared to control group (Table 2). Although, EPM and LDA models are used to assess the anxiolytic activity of drugs, the results are not always consistent between them. This difference in the dose-response in EPM and LDA models is due to different aspects of anxiety behavior, such as bright light anxiety behavior in LDA model and open-space-anxiety like behavior in EPM model.[29] Exact mechanism of BS as an anxiolytic is yet to be elucidated in further studies. BS may protect the cellular components against oxidative stress and increase GABA levels in the brain.[16] Thus, BS can be an alternative anti-anxiety drug in near future. However, further studies are required to elucidate its exact mechanism of action, efficacy and adverse effects in humans.

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