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Research Article

Preclinical Evaluation of Combination Effect of Boswellia serrata and Buspirone as Anxiolytic

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Abstract

Introduction: The Boswellia serrata, belonging to family Burseraceae is a tree which imparts many medicinal properties such as anti-inflammatory, anti-cancer, etc. Recent studies have shown that it helps to surge GABA levels in mice brain as a result, it can be an anxiolytic. This study has been designed to evaluate the anxiolytic effect of Boswellia serrata along with the combination of Buspirone in swiss albino mice.

Materials and Methods: In this study, Boswellia serrata was evaluated for its anti-anxiety effect in alone and in combination with Buspirone with the help of various models and was compared with the standard drug.

Result and Conclusion: It was found that the combination effect of Boswellia serrata and Buspirone has shown significant anti-anxiety effect when tested in different models i.e., Elevated Plus Maze, The light and dark box, Marble-Burying Behavior, The Hole-Board Test.

Keywords: *Anxiety; Anti-Anxiety activity; Boswellia serrata; Buspirone; Elevated Plus Maze; The light and dark box; Marble-Burying Behavior; The Hole-Board Test*

Introduction

Ayurveda is an ancient method for treating the health issues i.e., diseases. Its root has been found in Vedas which are also considered as the oldest written scriptures or literature in the world through which many philosophies and theories got spread [1,2]. In Ayurveda, mostly herbs, shrubs, trees and many sea materials has been using in treatment of disease. The main benefit of the Ayurveda is the medicines used to treat disease do not impose or impose minimal side effect.

In Sushruta Samhita, Sushruta (an ancient Indian physician) himself wrote that the Dhanvantri (Hindu god of medicine) incarnate himself as king of Varanasi and provide all the information about the medicines to the group of physicians including Sushruta [3].

India has more than 50,000 plants, diversely spread in 16 different agro-climatic zones, 25 biotic provinces, 426 habitats and 10 vegetation zones. It includes 18,000 flowering plants, 23,000 fungi, 2500 algae, 1800 bryophytes and 1600 varieties of lichens. Out of this quantum around 15,000-20,000 contains medicinal value and out of these only 7000 - 7500 plants are used in the traditional system [4].

Boswellia serrata is a species of Burseraceae family occurs in dry mixed deciduous forests or in very dry teak forests with other species like *Acacia leucophloea, Anogeissus latifolia* and *Terminalia* species. Characteristically, it is mostly found on the ridges and slopes of hills as well as on flat terrain which attains a larger size on fertile soils. It resists fire better than various other species occurs in its zone as well as it resists drought [5]. BS serves as nurse tree for other plant species and it is also frosty hardy.

The plant BS has been documented in the list of threatened species of India [6]. The plant *Boswellia serrata* is exploited indiscriminately because of its high economical and medicinal value. It is identified by govt. of India for its genetic improvement.

The viability of the seeds of BS is very low and its percentage of germination is very low such as 10 - 15% only. For germination, it requires high humid conditions.

Plant contains resin (50%), essential oil (8 - 9%), and gum (20 - 23%). Its essential oil is a mixture of diterpenes, monoterpenes and sesquiterpenes. Phenolic compounds and Serratol (diterpene alcohol) are also found in essential oil of *Boswellia serrata*. Gum of BS contains pentose and hexose sugars (D-arabinose, D-galactose, D-xylose and D-mannose) along with some digestive and oxidizing enzymes. Resins of BS consists triterpene, tetracyclic triterpene and pentacyclic triterpene in which Boswellic acid is the main active constituents [7].

Boswellic acid is also of six different types:

1. α-Boswellic acid
2. β-Boswellic acid
3. Acetylated α-Boswellic acid
4. Acetylated β-Boswellic acid
5. 11-keto-β-Boswellic acid
6. 3-O-acetyl-11-keto-β-Boswellic acid.

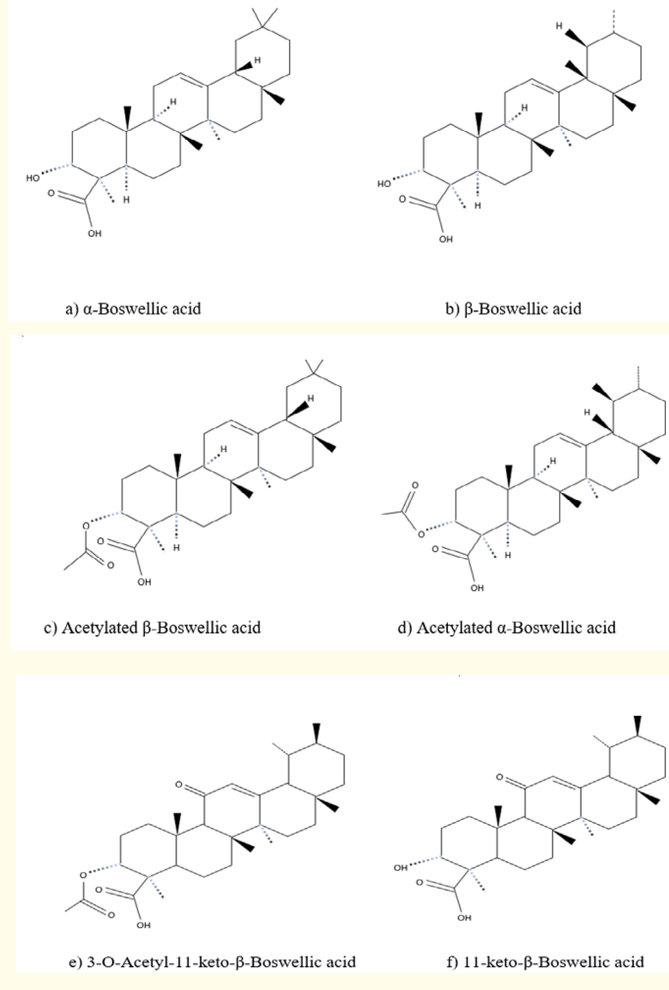

Figure 1: *Structures of different types of Boswellic acid.*

Pharmacological profile

Boswellia serrata is known for its wide range of pharmacological value [8]. BS is used for medicinal purpose from ancient time.

Boswellia serrata is used for various activities such as:

a. Anti-inflammatory activity
b. Antioxidant activity
c. Anti-ulcer activity
d. Anti-arthritic activity
e. Anti-asthmatic activity
f. Anti-atherosclerotic activity
g. Anti-cancer activity
h. Anti-diarrhoeal activity
i. Hepato-protective activity
j. Anti-microbial activity
k. Wound healing activity
l. Diuretic activity
m. Analgesic activity
n. Anti-hyperglycemic activity.

According to WHO, 65% population of India use Ayurvedic and Herbal medicines to enhance their health status in rural areas. In developed countries, use of herbal medicines becoming more popular nowadays. Ayurvedic medicines are generally used to treat or prevent diseases and to improve quality of life.

An anxiety is a medical state related to our psychological as well as physiological behavior having numerous characters like cognitive, emotional, behavioral and somatic. The term anxiety came from a Latin word "Ango" which means "to vex or torment" may be in the absence or presence of any psychological stress. It can also create a feeling of worry, feeling of fear, feeling of uneasiness. Sometimes, it is considered a normal reaction for a stressor. Mostly, the effect of anxiety is lived for the short time period when we encounter something unpleasant or outside of our comfort level but when the symptoms are severe and remains for longer duration and affects negatively on the personality, ability to work or socially which is considered as a problem [9]. Sometimes, anxiety becomes beneficial as it alerts us towards possible hazard [10].

When people encounter with anxiety, they generally feel upset, uncomfortable and uneasiness. In normal, anxiety does not last long. It can be only one illness or a group of illness. Diagnosis should be done when people face extreme level of anxiety which interfere with their daily life and create disturbance in to-do list. Anxiety is form of mental illness which is mostly common. Females are more likely affected by anxiety disorder than males [11].

There are many sign and symptoms to detect if a person is affected from anxiety disorder or not. Physical sign and symptoms as well as psychological symptoms both can be considered under diagnosis of anxiety disorder.

Recent data (2017) of anxiety shows that 284 million people are affected by this disorder worldwide which is 3.8% share of global population [12] while it was 273 million in 2010.

There are 4 types of anxiety: Generalized anxiety disorder, Specific phobias, Social anxiety, and Panic disorder. There are two other conditions where anxiety disorder also present i.e. Obsessive-compulsive disorder (OCD) and Post-traumatic stress disorder (PTSD).

GABA (Gamma Amino Butyric Acid) is the important inhibitory neurotransmitter present in the central nervous system. GABA is generally known as centre for the regulation of anxiety disorder for most of the drugs such as benzodiazepines and another analogue. Influence of GABA may lead to relief in anxiety as shown in figure 2.

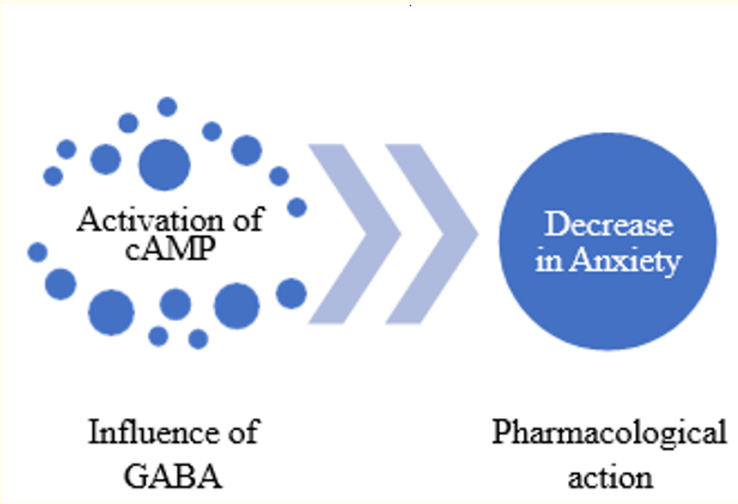

Figure 2: *Action of GABA towards anxiety.*

Treatment and management of anxiety

There are various drugs available in the market which help to reduce anxiety. Most of the drugs are helpful to reduce anxiety in daily life but because of the side effects of available allopathic drugs people are shifting towards using herbal drugs to reduce anxiety as herbal drugs as it causes very fewer side effects or adverse effects [13].

Selective serotonin reuptake inhibitors (Paroxetine, Fluoxetine, Sertraline, Escitalopram, Citalopram, Fluvoxamine) and selective serotonin norepinephrine reuptake inhibitors (Venlafaxine, Duloxetine) are the first line of drugs to treat anxiety. Withdrawal reactions may occur after stopping treatment with SSRIs.

Benzodiazepines are the most effective anxiolytic drugs which starts showing their effect soon after administration. These are the drugs generally used in 50 - 90% cases of anxiety. Along with the efficacy, benzodiazepines do have various adverse effects such as cognitive impairment, Impaired driving skills, dizziness, fatigue and others.

Other group of anxiolytics i.e. Tricyclic depressant such as Imipramine and Clomipramine are effective anti-anxiety drugs whereas their adverse effect frequencies are high.

Buspirone is recently found new anxiolytic drug which has been termed as Anxio-selective as it does not cause sedation, hypnosis, muscle relaxant and anti-convulsant property when given [14].

As we already know with the help of many reviews, that traditional/Ayurvedic medicines (from herbs, shrubs, and trees etc.) cause negligible or very less amount of adverse effect than allopathic medicines. So, to enhance the quality of medication we need to move towards the herbal plant rather than chemically synthesized drugs. Therefore, gum resins of the herbal plant have been taken to cure anxiety.

As in recent preclinical studies, it is observed that *Boswellia serrata* influence GABA i.e. surging of GABA in mice brain which shows anti-anxiety effect [15], so the primary objective is to confirm the anti-anxiety effect of *Boswellia serrata* in combination with buspirone. the present study done to evaluate combination effect of *Boswellia serrata* and Buspirone in Swiss albino mice.

Materials and Methods

Authentication of drug sample

The drug sample was authenticated by CSIR- Central Institute of Medicinal and Aromatic Plants, Kukrail Picnic Spot Road, Lucknow, U.P -226015. Certification number: CIMAP/Bot- Pharm./2020/02.

Complex formation of *Boswellia serrata* with Phosphatidyl choline

Phosphatidyl choline has been reported to enhance absorption of *Boswellia serrata* when complex was made of both i.e. *Boswellia serrata*-phosphatidyl choline complex [16]. Amphiphilic nature of *Boswellia serrata*-phosphatidyl choline complex enhance bioavailability of *Boswellia serrata* which is because of its solubility increased in both water and lipid.

20 ml of dichloromethane was taken in round bottom flask and used to dissolve equal molar of gum resin powder of *Boswellia serrata*.

At room temperature, mixture was stirred with the help of magnetic stirrer for 2 hours. In a rotary evaporator, the solvent was then removed at 30°C. As a result, complex was formed of *Boswellia serrata* and Phosphatidyl choline i.e., *Boswellia serrata*-phosphatidyl choline complex which was then stored in amber colour glass bottle at room temperature. Phosphatidyl choline is inert and hence does not contain any adverse effect.

In-vitro studies

Various *in-vitro* studies have been conducted to authenticate drug sample. Qualitative phytochemical screening tests were performed to identify the presence of various types of compound such as alkaloids, flavonoids, glycosides, saponins, tannins, phenolic compounds etc. as per the standard methods [17]. All these tests were performed with the methanolic solution of powder of oleo gum resin of *Boswellia serrata*.

Detection of alkaloids

Drug sample was dissolved in the 90% methanol and filtered. Then filtrate was used to perform the alkaloids test.

Mayer's test

Filtrate of drug sample was treated with Mayer's reagent (potassium mercuric iodide). Yellow coloured precipitate formation indicated the presence of alkaloids.

Hager's test

Filtrate of drug sample was treated with Hager's reagent (saturate picric acid solution). Yellow coloured precipitate formation indicated presence of alkaloids.

Detection of carbohydrates

Drug sample was dissolved in the 90% methanol and filtered. Then filtrate was used to perform the carbohydrate test.

Molisch's test

Filtrate of drug sample was treated with 2 drops of Molisch's reagent (alcoholic solution of α-naphthol). Violet ring formation at the junction indicated presence of carbohydrates.

Fehling's test

Filtrate of drug sample was hydrolysed with diluted hydrochloride acid and then neutralized with alkali and then heated with fehling's solution A and B. Red precipitate formation indicated presence of carbohydrates.

Detection of glycosides

Drug sample was dissolved in the 90% methanol and filtered. Then filtrate was used to perform the glycosides test.

Keller Killiani's test

Filtrate of drug sample was dissolved in the mixture of 1% solution of ferric sulphate in 5% glacial acetic acid. Then 2 drops of concentrated sulphuric acid. Blue colour development indicated the presence of glycosides.

Detection of saponins

Drug sample was dissolved in the 90% methanol and filtered. Then filtrate was used to perform the saponins test.

Froth test

Filtrate of drug sample was added in 20 ml of distilled water and then shaken till 15 minutes. 1 cm layer of foam development indicated the presence of saponins.

Foam test

Filtrate of drug sample was added in 2 ml of distilled water. Presence of saponins was confirmed by produced foam which remained for 10 minutes.

Detection of phenols

Drug sample was dissolved in the 90% methanol and filtered. Then filtrate was used to perform the phenols test.

Ferric chloride test

Filtrate of drug sample was treated with few drops of ferric chloride solution. Bluish-black colour formation indicated the presence of phenolic compounds.

Detection of tannins

Drug sample was dissolved in the 90% methanol and filtered. Then filtrate was used to perform the tannins test.

Gelatin test

Filtrate of drug sample was treated with 1% gelatin solution containing sodium chloride. White precipitate formation indicates presence of tannins.

Detection of flavonoids

Drug sample was dissolved in the 90% methanol and filtered. Then filtrate was used to perform the flavonoids test.

Lead acetate test

Filtrate of drug sample was treated with few drops of lead acetate solution. Yellow colourprecipitate formation indicates presence of flavonoids.

Detection of diterpenes

Drug sample was dissolved in the 90% methanol and filtered. Then filtrate was used to perform the diterpenes test.

Copper acetate test

Filtrate of drug sample was dissolved in distilled water and then treated with few drops of copper acetate solution. Emerald green colour formation indicates presence of diterpenes.

Detection of protein and amino acid

Drug sample was dissolved in the 90% methanol and filtered. Then filtrate was used to perform the protein and amino acid test.

Xanthoproteic test

Filtrate of drug sample was treated with few drops of concentrated nitric acid. Yellow colour formation indicates presence of proteins.

Ninhydrin test

Filtrate of drug sample was treated with 0.25% w/v ninhydrin reagent and then boiled for few minutes. Blue colour formation indicates presence of amino acid.

Detection of steroids

Drug sample was dissolved in the 90% methanol and filtered. Then filtrate was used to perform the steroids test. 2 ml of chloroform and concentrated Sulphuric acid were added in filtrate. In the chloroform layer, red colour appearance indicates presence of steroids.

Solubilities study

Solubility study were performed with different solvent such as distilled water, methanol, n- hexane, and petroleum ether.

Detection of melting point

Determination of melting point allows to identify the unknown drug sample on the basis of melting point.

Melting point was determined by "Open Capillary Method". The drug was placed in a thin walled glass capillary tube closed at one end. The capillary containing the drug sample was placed in melting point apparatus and heated slowly and evenly. The temperature at which sample started to melt was taken as a melting point of the drug. The procedure was repeated thrice.

Animals

Animals approval from Institutional Animal Ethical Committee (IAEC), HIMT College of Pharmacy, Greater Noida, as per the guidelines set by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) was obtained before conducting the preclinical evaluation in swiss albino mice. Registration number: 1377/PO/Re/S/10/CPCSEA.

Inclusion criteria

- Male Swiss albino mice weighing among 25g and 35g.
- Age 3 - 4 months.
- Healthy with ordinary conduct and activity.

Exclusion criteria

- Mice 35g; age four months.
- Animals formerly used in different experiments.

Housing condition

Animals were placed in the clean cages made from non-absorbable and non-toxic material and bedding was provided. Room temperature: 20 to 25°C. Humidity level: 60%. The light/dark cycle were maintained at 12/12 hours respectively.

Protocol design

25 swiss albino mice were used to evaluate anti-anxiety effect of *Boswellia serrata* in combination with Buspirone. 5 groups were taken which consist of 5 mice in each group as mentioned in table 1-4. The animals were reused for each model after 3 days as half- life of drug is approx. 6 hours and no dissection were needed.

Groups	Number of animals used	Models	Treatment	Dose	Parameters of observations
Group I	5	Elevated Plus Maze Model	Control Group	Normal Saline	Time spent and Number of entries into open and closedarm
Group II	5		*Boswellia serrata*	200 mg/kg	
Group III	5		Buspirone	2.5 mg/kg	
Group IV	5		*Boswellia serrata* + Buspirone	200 mg/kg + 2.5 mg/kg	
Group V	5		Diazepam	1 mg/kg	

Table 1: *Protocol design for study in elevated plus maze model.*

Groups	Number of animals used	Models	Treatment	Dose	Parameters of observations
Group I	5		Control Group	Normal Saline	Number and time of head dipping and locomotion activity was recorded
Group II	5		*Boswellia serrata*	200 mg/kg	
Group III	5	Hole-Board Test	Buspirone	2.5 mg/kg	
Group IV	5		*Boswellia serrata* + Buspirone	200 mg/kg + 2.5 mg/kg	
Group V	5		Diazepam	1 mg/kg	

Table 2: *Protocol design for study in the Hole-Board test.*

Groups	Number of animals used	Models	Treatment	Dose	Parameters of observations
Group I	5	Marble-Burying Behaviour	Control Group	Normal Saline	Number of marbles buried
Group II	5		*Boswellia serrata*	200 mg/kg	
Group III	5		Buspirone	2.5 mg/kg	
Group IV	5		*Boswellia serrata* + Buspirone	200 mg/kg + 2.5 mg/kg	
Group V	5		Diazepam	1 mg/kg	

Table 3: *Protocol design for study in Marble-Burying behaviour.*

Groups	Number of animals used	Models	Treatment	Dose	Parameters of observations
Group I	5	The Light and Dark box	Control Group	Normal Saline	Time spent and Number of entries into light and dark box
Group II	5		*Boswellia serrata*	200 mg/kg	
Group III	5		Buspirone	2.5 mg/kg	
Group IV	5		*Boswellia serrata* + Buspirone	200 mg/kg + 2.5 mg/kg	
Group V	5		Diazepam	1 mg/kg	

Table 4: *Protocol design for study in The Light and Dark box.*

Drugs

Boswellia serrata was taken as test drug to identify its anti-anxiety effect. Buspirone was taken in combination with *Boswellia serrata* to study the enhanced anti-anxiety effect for effective treatment of anxiety. Diazepam was taken as standard drug for comparative study. Normal saline was taken into consideration for control group.

Same animals were used after 4 days as half life of drug is approx. 6 hours and as no dissection were needed. Each group test was performed on one model in a single day.

Various models were used to evaluate the anti-anxiety effect such as Elevated plus maze models, Marble-Burying Behaviour, The Light and Dark box, and The Hole-Board Test [18].

The animals were kept on fasting overnight to avoid any food interaction with drug which may cause error in result. Drug was administered orally with the help of gavage via pharyngeal tube.

Elevated plus maze

This is a novel test used to evaluate the anti-anxiety effect of anxiolytics in rodents. It is made up of wood consisting two open arms and two closed arms (16 cm X 5 cm), placed same arms in opposite direction. All the arms connect to the centre making 5cm square. It is elevated to the height of 15 cm from the ground. Temperature and lightning were kept normal. Individual swiss albino mice were placed at the centre of elevated plus maze model with their head was facing towards open arm and was observed for 5 minutes. Number of entries in open and closed arm were recorded. 70% ethanol should be used to clean the maze after each and every trail and at the end of the day it should be cleaned with 10% bleach [19,20].

The animals were divided in groups as follows for elevated plus maze model:

- Group I: 10 ml/kg of Normal saline
- Group II: 200 mg/kg of *Boswellia serrata*
- Group III: 2.5 mg/kg of Buspirone
- Group IV: 200 mg/kg of *Boswellia serrata* + 2.5 mg/kg of Buspirone
- Group V: 1.0 mg/kg of diazepam.

The hole-board test

The hole-board model is also used to evaluate anxiolytic effect. The hole- board model consists of 16 holes which were evenly distributed on the floor of a box (40 x 40 x 25 cm). Infrared beam falling on photocells were available below the floor. Squares were drawn on the apparatus surface to count or observe locomotion activity of mice [21,22].

Individual swiss albino mice were placed at the floor of hole-board and was observed for 5 minutes. Number and time of head dipping and locomotion activity was recorded.

The animals were divided in groups as follows for the hole-board test:

- Group I: 10 ml/kg of Normal saline
- Group II: 200 mg/kg of *Boswellia serrata*
- Group III: 2.5 mg/kg of Buspirone
- Group IV: 200 mg/kg of *Boswellia serrata* + 2.5 mg/kg of Buspirone
- Group V: 1.0 mg/kg of diazepam.

Marble burying behaviour

Marble-burying behaviour is another model to evaluate the anti-anxiety effect or Obsessive-Compulsive Disorder behavior i.e. This model is able to detect phenotypes related to anxiety disorder and Obsessive-compulsive disorders. Standard polycarbonate cages (26 cm x 48 cm x 20 cm) were used with the fitted filter-top covers. Unscented bedding material was added to the cage to a depth of 5 cm [23,24]. 24 Marbles were evenly distributed across the bedding and all the marbles buried 2/3rd or more was counted where less than 2/3rd buried marbles was not counted.

Individual swiss albino mice were placed in the cage of marble burying behaviour model and observed for 30 minutes. Number of marbles buried were recorded.

The animals were divided in groups as follows for marble burying behaviour model:

- Group I: 10 ml/kg of Normal saline
- Group II: 200 mg/kg of *Boswellia serrata*
- Group III: 2.5 mg/kg of Buspirone
- Group IV: 200 mg/kg of *Boswellia serrata* + 2.5 mg/kg of Buspirone
- Group V: 1.0 mg/kg of diazepam.

The light and dark box

This is a model used to evaluate anti-anxiety effect of anxiolytics. This model consists of a rectangular box (45 cm X 27 cm X 27 cm), divided into two equal halves. The first half was a light box and the other one was dark box. The first half was painted with white colour. An illumination lamp (40-watt white light bulb) was placed 25cm above the white box to make it light box i.e., which consists lightning. It was an open box which means has no roof. The second half was painted black. Roof was placed on the top of dark box and also painted with black colour. Both the compartment was connected with a wall consists of hole (7.5 cm X 7.5 cm) or pass way which allows to movement from one compartment to another. This light and dark box model can be used to evaluate both the anxiogenic-like and anxiolytic-like activity of drug [25,26].

Individual swiss albino mice were placed at the centre of the light and dark with their head was facing towards light box and was observed for 5 minutes. Number of entries in light and dark box was recorded.

The animals were divided in groups as follows for the light and dark box model:

- Group I: 10 ml/kg of Normal saline
- Group II: 200 mg/kg of *Boswellia serrata*
- Group III: 2.5 mg/kg of Buspirone
- Group IV: 200 mg/kg of *Boswellia serrata* + 2.5 mg/kg of Buspirone
- Group V: 1.0 mg/kg of diazepam.

Data analysis

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

Result

In-vitro study Solubility studies

Solubility studies were also performed with different solvent such as water, alcohol, hexane, dichloromethane and result are shown in table 5.

S. No.	Solvent	Observation
1	Water	Insoluble
2	Alcohol	Soluble
3	Hexane	Soluble
4	Dichloromethane	Soluble

Table 5: *Observation of solubility studies of Boswellia serrata.*

Phytochemical screening

Phytochemical screening of powder form of oleo gum resin of *Boswellia serrata* is shown in below table 6.

S. No.	Phytochemicals	Inference
1	Alkaloids	Absent
2	Carbohydrates	Present
3	Glycosides	Present
4	Saponins	Present
5	Phenols	Present
6	Tannins	Present
7	Flavonoids	Present
8	Diterpenes	Present
9	Protein and amino acids	Absent
10	Steroids	Present

Table 6: *Result of phytochemical screening of powder form of oleo gum resin of Boswellia serrata.*

Determination of melting point

The melting point of test drug was determined and found in between 224 - 228°C.

In-vivo study

In-vivo studies were performed, and result are mentioned below.

Elevated plus maze

In this study, when combination of *Boswellia serrata* (200 mg/kg) and Buspirone (2.5 mg/kg) was administered, it has been observed that the number of entries and time spent in open arm has been significantly increase ($p < 0.05$) as compare to normal saline group as well as diazepam group as mentioned in table 7. Hence, it shows that it reduces anxiety most effectively.

S. No	Group	Entry into open arm	Entry into closed arm	Time spentin open arm	Time spentin closed arm
1	Normal Saline (10 ml/kg)	8.99 ± 2.9	9.72 ± 4.2	85.54 ± 12.9	211.85 ± 12.8
2	*Boswellia serrata* (200 mg/kg)	10.48 ± 4.0	10.10 ± 3.9	139.01 ± 47.79	160.25 ± 51.22
3	Buspirone (2.5 mg/kg)	9.04 ± 0.78	9.25 ± 1.95	125.51 ± 3.55	166.89 ± 4.22
4	*Boswellia serrata* (200 mg/kg) + Buspirone (2.5 mg/kg)	14.25 ± 1.25	6.85 ± 3.23	176.22 ± 5.66	128.49 ± 3.61
5	Diazepam (1 mg/kg)	21.25 ± 5.2	6.8 ± 1.5	193.69 ± 8.26	105.28 ± 7.99

Table 7: *Observation of drug on different animal group in elevated plus maze model (Values are mean ± SD, n = 5).*

Hole-board model

In this study, when combination of *Boswellia serrata* (200 mg/kg) and Buspirone (2.5 mg/kg) was administered, the locomotory activity and head dipping and rearing has been found to increase ($p < 0.001$) as compare to normal saline group and diazepam group as mentioned in table 8. Hence, it shows that it reduces anxiety most effectively.

S. No.	Group	Head dipping	Time of Head dipping (sec)	Locomotion (Number of square crossed)
1	Normal Saline	13.45 ± 3	8.65 ± 2	8.65 ± 1
2	*Boswellia serrata* (200 mg/kg)	17.25 ± 1.6	11.95 ± 1.6	8.7 ± 2.64
3	Buspirone (2.5 mg/kg)	15.08 ± 2.55	11.26 ± 2.66	8.23 ± 2.0
4	*Boswellia serrata* (200 mg/kg) + Buspirone (2.5 mg/kg)	25.85 ± 3.66	12.05 ± 1.54	13.02 ± 2.69
5	Diazepam (1 mg/kg)	17.63 ± 2	12.78 ± 3	9.0 ± 3

Table 8: *Observation of drug on different animal group in the hole-board model (Values are mean ± SD, n = 5).*

Marble-burying behaviour

In this study, when combination of *Boswellia serrata* (200 mg/kg) and Buspirone (2.5 mg/kg) was administered, it has been observed that the number of marbles buried has been found lesser ($p < 0.001$) than normal saline group and diazepam group as mentioned in table 9. Hence, it shows that it reduces anxiety most effectively.

S. No.	Group	Number of marbles buried
1	Normal Saline	14 ± 2
2	*Boswellia serrata* (200 mg/kg)	6 ± 2
3	Buspirone (2.5 mg/kg)	9 ± 3
4	*Boswellia serrata* (200 mg/kg) + Buspirone (2.5 mg/kg)	3 ± 2
5	Diazepam (1 mg/kg)	4 ± 2

Table 9: *Observation of drug on different animal group in marble burying behaviour model (Values are mean ± SD, n = 5).*

The light and dark box

In this study, when combination of *Boswellia serrata* (200 mg/kg) and Buspirone (2.5 mg/kg) was administered, it has been observed that the entry and time spent in light box has been significantly increased ($p < 0.001$) as compare to normal saline group and diazepam group as mentioned in table 10. Hence, it shows that it reduces anxiety most effectively.

S. No	Group	Entry intolight box	Entry intodark box	Time spent in light box	Time spent in dark box
1	Normal Saline	10.25 ± 1.6	10.06 ± 2.1	89.26 ± 7.4	213.22 ± 7.77
2	*Boswellia serrata* (200 mg/kg)	10.19 ± 4.4	9.86 ± 4.0	139.64 ± 46.52	161.23 ± 49.7
3	Buspirone(2.5 mg/kg)	4.85 ± 1.65	7.53 ± 2.55	85.64 ± 5.66	121.14 ± 6.49
4	*Boswellia serrata* (200 mg/kg) + Buspirone (2.5 mg/kg)	12.52 ± 2.74	6.66 ± 2.39	198.35 ± 5.93	132.20 ± 8.16
5	Diazepam (1 mg/kg)	10.79 ± 5.4	10.20 ± 5.6	141.24 ± 4.9	157.11 ± 4.8

Table 10: *Observation of drug on different animal group in the light and dark box (Values are mean ± SD, n = 5).*

Discussion

In this study, phytochemical investigation showed that glycosides, tannins, saponins, carbohydrates, phenols, flavonoids, diterpenes and steroids were present whereas alkaloids, phytosterols, protein and amino acids were absent in the methanolic extract of oleo gum resin of *Boswellia serrata*.

In this study, (Group 4) when combination of *Boswellia serrata* (200 mg/kg) and Buspirone (2.5 mg/kg) was administered and experiment was conducted with the help of different models (such as elevated plus maze, hole board test, marble burying behaviour, and the light and dark box), it was found that it reduces anxiety most effectively than standard drug i.e. diazepam. In elevated plus maze model, it has been observed that the number of entries and time spent in open arm has been significantly increase as compare to normal saline group as well as diazepam group whereas in light and dark box, it has been observed that the entry and time spent in light box has been significantly increased as compare to normal saline group and diazepam group.

In marble burying behaviour model, the number of marbles buried has been found lesser than normal saline group and diazepam group. In the hole board test, the locomotory activity and head dipping and rearing has been found to increase as compare to normal saline group and diazepam group.

The combination effect of *Boswellia serrata* and Buspirone is higher than normal saline and their alone effect.

As per literature review, Diazepam is the effective drug to treat anxiety but also contains side effects or adverse effects.

Therefore, combination of *Boswellia serrata* and Buspirone can be used as an alternative medication for treatment of anxiety. Exact mechanism of action of *Boswellia serrata* and Buspirone as an anxiolytic is yet to be identified in future studies as mechanism of action of both *Boswellia serrata* and Buspirone as anxiolytic is unknown. However, further studies need to be done to identify its efficacy and adverse effects in human beings.

Conclusion

With the help of present study, it was concluded that the combination of *Boswellia serrata* and Buspirone would be a better or an alternative medication for the treatment of anxiety with standard drugs. However, further studies need to be done to identify its efficacy and adverse effects in human beings.

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Conflict of Interest

None.

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