Comparison of neuropharmacological activities of methanolic extracts of *Cuminum nigrum* (Linn.) and *Centratherum anthelminticum* (Linn.) in mice

Raana Mahmood[1]*, Rahila Ikram[2], Ghazala H Rizwani[3] and Humera Khatoon[4]
[1]Karachi Medical & Dental College, Pharmacology Department, Karachi, Pakistan
[2]Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan
[3]Department of Pharmacognosy, Faculty of Pharmacy, Hamdard University, Karachi, Pakistan
[4]Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan

Abstract: The study is conducted to observe and investigate the effects of oral dosing of methanolic extracts of *Cuminum nigrum* (L) and *Centratherum anthelminticum* (L) on neuropharmacological activities of mice. Methanolic extracts of *Cuminum nigrum* (L) and *Centratherum anthelminticum* (L) were soluble in Dimethyl sulphoxide (DMSO) i.e. an organic solvent, so it is used in this study. Screening for anxiolytic and antidepressant effects were performed using open field test, head dip test, stationary rod test, cage crossing test, light and dark box and swimming– induced depression test. Thirty animals were divided into three groups of 10 animals each and numbered as 1 (control, on DMSO), 2(on methanolic extract of *Cuminum nigrum* (L), 3 (on methanolic extract of *Centratherum anthelminticum* (L). The extracts and DMSO were administered orally for 60 days. Any possible change in animal behavior was evaluated on day 15, 30 and 60 of dosing. The groups 2 and 3 showed significant increase ($p<0.001$, $p<0.01$) in open field activity and light and dark box test respectively, while significantly decreased activity was observed in head dip and cage crossing activity ($p<0.01$) after 60 days of dosing. Based on above findings, it is suggested that the extracts of *Centratherum anthelminticum* (L) and *Cuminum nigrum* (L) have antidepressant and anxiolytic potential with sedative effects.

Keywords: Antidepressant, anxiolytic, *Centratherum anthelminticum* (L), *Cuminum nigrum* (L), neuropharmacological, sedative.

INTRODUCTION

Persistent increase in the use of herbs and drugs originated from plant sources for the prevention and treatment of various ailments has been found in the past few years. People have now been switching their therapy from conventional towards herbal drugs, especially for treating chronic illnesses such as diabetes, chronic pain and inflammatory disorders and neuropsychiatric diseases (Pan *et al*., 2013). Medicinal herbs are the potential source of drugs. The active compounds are isolated from these herbs and used to constitute allopathic medicines. The biological properties of these medicinal herbs are investigated and proven scientifically (Newman and Cragg, 2012). The scientific advancement in isolating active compounds is playing a vital role in the development of new drugs. Literature review revealed that herbal drugs are not only potent, economical and effective but also possess fewer side effects than available traditional medicines (Zafar, 2010). Today approximately 75-80% of developing population depends on traditional medicines among which use of plant extracts is most common (Savithramma *et al*., 2011).

The *Cuminum nigrum* (L) and *Centratherum anthelminticum* (L) are having established and proven role as analgesic, anti-inflammatory and anti-oxidant (Thippeswamy & Nidu, 2005). The culinary herbs *Cuminum nigrum* (L) and *Centratherum anthelminticum (L)* are used in South East Asian foods for aroma and flavor. These herbs are also used in old Indian medicines for treating different diseases of GIT (Roug and Jiang, 2004). It has been proved through number of studies that phytochemical, phenolic content of the herbs has antioxidant and disease curing potential (Alliwell and Gutteridge, 1989; Tsao and Akhtar, 2000).

Cuminum nigrum (L) is a flowering plant of Apiaceae family, common name in India is Kalazera or Siyah zera. Its medicinal use in Ayurvedic system is for enhancing appetite, taste perception and digestion (Roug and Jiang, 2004). Ahmad *et al.* (2000) reported hypoglycemic activity of flavonoid fraction of *C. nigrum* seeds in alloxan-induced diabetic rabbits. However, no such activity was reported of its alkaloid fraction. As far as its other medicinal properties, black zeera is reported to have carminative and stimulant activity. It is also used for the treatment of GI disorders such as dyspepsia and diarrhea (Baser *et al.* 1997). Furthermore, literature review showed some recent studies related to antimicrobial and antioxidant activities of essential oil of this plant species (Oroojalian *et al.*, 2010; Mazidi *et al.*, 2012).

**Corresponding author:* e-mail: drraanazafar@gmail.com

Centratherum anthelminticum (L) belongs to family Astraceae. It is also used in Ayurvedic medicine to cure ulcers and as bowel astringent, it can eradicate worm infestation e.g. earthworm and tapeworm infestations (Purnima *et al*., 2009). The whole plant has been reported to have antimicrobial and contraceptive activity (Gulian *et al*., 2010). Recently, Arya *et al.* (2012) reported its anti-diabetic activity in type-2 diabetic rat models via stimulation of insulin release. Other recently reported pharmacological activities include melanogenesis (Zhou *et al.*, 2012) and wound healing activities (Sahoo *et al*., 2012). Looi *et al.* (2013) identified vernodalin as active constituent isolated from chloroform fraction of *C. anthelminticum(L)* seeds which showed cytotoxic and apoptotic activity in human breast cell cancer line.

So far, many scientific studies have been carried out on *Cuminum nigrum* (L) and *Centratherum anthelminticum*, but to the best of our knowledge, this is the first report on the neuropharmacological activity of these plant species. Some neuropsychiatric diseases, such as anxiety and depression leading to cognitive decline, have become quite common in recent years. Therefore, most of the research work globally has been focusing on mental health today. In the present study, we investigated the effects of methanolic extracts of *C. nigrum* (L) and *C. anthelminticum* (L) in animal models for their possible antidepressant and anxiolytic effects which have not been explored until now.

MATERIALS AND METHODS

Collection of seeds

The seeds were collected from herbal market of Karachi, Pakistan. The seeds were identified and authenticated by Prof. Dr. Ghazala H. Rizwani, Dean of Pharmacy, Hamdard University, Karachi, Pakistan. The specimens of seeds are deposited in Pharmacognosy Herbal Museum for future reference with voucher # 00111 for *Cuminum nigrum* (L) and voucher # 00112 for *Centratherum anthelminticum* (L).

Preparation of extract

The seeds of *Cuminum nigrum* (L) and *Centratherum anthelminticum* (L) were soaked in methanol for fifteen days. The extracts were collected, filtered and concentrated under reduced pressure in a rotary evaporator and stored in an airtight container at 4°C (Galani and Panchal, 2014)**.**

Grouping and housing of animals

Healthy albino mice weighing from 25-30gm were selected for the study. All animals were randomly distributed into three groups of ten mice each. Group 1 served as control, group 2 was on *Cuminum nigrum* and group 3 on *Centratherum anthelminticum* . Animals were housed at 26 ± 2^{o}C room temperature with 12/12 hours light/dark cycle i.e. light on from 08.00 am to 08.00 p.m. All animals had free access to food and water *ad libitum*. They were housed under standard conditions and kept for one week before starting the dosing to acclimatize with the surroundings. All animals were handled as per Helsinki's Resolution 1964. This study was approved by Board of Advanced Studies and Research, University of Karachi vide resolution # 10(P) 11 dated: 21-02-2014 & 03-03-2014.

Dosing

Methanolic extracts were insoluble in water so it was dissolved in 10% DMSO. The methanolic extract of *Cuminum nigrum* (L) was administered to group 2 at a dose of 500 mg/kg p.o (Ahmad *et al.*, 2000). Each mice in the group 3 was administered methanolic extract of *Centratherum anthelminticum* (L) orally at a dose of 200 mg/kg (Purnima *et al.*, 2009). These doses were administered through feeding tube one hour before tests. Animals of control group (group 1) were administered 0.25 ml of 10% DMSO orally. The dosing was done for a period of 60 days.

Screening tests

Open field test

Open field test is used for observation of locomotor and behavioral activity in rodents exposed to novel and bright environment. It is a commonly used model to assess anxiety-like behavior in animals. The apparatus consisted of perpex cage (76cm length x 76cm width x 42cm height) floor arena was divided into 25 even squares. Every mouse individually placed and observed in the apparatus for 10 minutes (Seibenhener and Wooten., 2015; Perveen *et al*., 2009). The number of squares both central and peripheral crossed by animal were counted. The test was performed on day 0, 15, 30 and 60 of dosing.

Light & dark box test

The light/dark box test is based on the rodents' innate aversion to brightly illuminated area and on the spontaneous exploratory behavior of the animals (Michel and Martin, 2003). The light and dark test equipment used is consisted of a little dark safe cubical and larger bright area cubical. The dimensions of the box are 46cm ×27cm ×30cm. The two cubicles are connected by an opening in the centre of the partition at the floor level. The dimensions of the opening are 7.5cm × 7.5cm.The division of box is 2/5 and 3/5 (Michel and Martin, 2003). The percentage of time spent in light compartment and numbers of transitions were noted for the duration of 10 minutes. Each mouse was placed separately on day 0, 15, 30 and 60 days of dosing.

Head dip test

For exploration of behavioral activity, the head dip test was used (Kliethermes and Crabbe, 2006). The apparatus consisted of a wooden Box (35cm x 45cm x 45cm) with 12 holes evenly spaced (2.5cm diameter). Albino mice of control and treated groups were placed in an exploratory

box for 10 minutes and the numbers of head dips were counted. Test repeated at day 0, 15, 30 and 60th of dosing.

Home cage crossing activity test

In behavioral neuroscience the home cage activity parameter is widely used to observe drug effects on basal locomotion activity (Perveen *et al.,* 2006). The equipment for cage crossing activity was consisted of a transparent cage (26cm x 26cm x 26cm). Control and treated groups were placed in the transparent cage separately for 10 minutes and the numbers of crossings were counted on day 0, 15, 30 and 60th of dosing.

Stationary rod test

The stationary rod test is used for observing learning ability of mice (Kishioka *et al.,* 2009). It consisted of stainless steel rods with the platforms on both sides. A brief training period before the start of the experiment was conducted. Control and treated groups were placed in the centre of the rod and allowed to walk. The time of crossing the rod to reach platform was noted on day 0, 15, 30 and 60th of dosing.

Forced swimming test

For assessment of swimming-induced depression in rodents, forced swimming test was used (Drugan *et al.,* 2010). It is consisted of an acrylic glass cylinder (50cm in height, 15cm in diameter) filled with water at temperature (27±2^{o}C) to a specific level (35cm high). Control and treated groups were placed in the cylinder separately and struggling time was noted for 05 minutes. The test was done on day 0, 15, 30 and 60th of dosing.

STATISTICAL ANALYSIS

The data obtained from the present study was analyzed through SPSS version 19. All results were expressed as mean ± S.D (Standard deviation). The significance of difference between mean were calculated by applying two way ANOVA, Post hoc analysis by LSD. An effect was defined as significant $p < 0.05$, very significant < 0.01 and considered highly significant $p < 0.001$.

RESULTS

All parameters were measured manually. The data are expressed as mean ± SD of observations of tests. In open field test the average peripheral squares crossed by animals receiving extracts of *Cuminum nigrum* and *Centratherum anthelminticum* showed highly significant ($p<0.001$) increased activity as compared to animals of control group at 60th day of dosing (table 1).

In light / dark box activity the duration of time spent in light compartment by groups of animals of *Cuminum nigrum* and *Centratherum anthelminticum* showed very significant ($p<0.01$) increased activity in light compartment as compared to control group of animals (table 2).

In head dip and home cage crossing test, the activity was also very significantly ($p<0.01$) decreased as compared to control group of animals (table 3 and table 4 respectively). While non-significant change was observed in stationary rod crossing time which showed that the cognitive ability is not altered with 60 days dosing of the extracts (table 5).

In forced swimming test the struggling time is very significantly decreased ($p<0.01$) in animals of *Cuminum nigrum* and *Centratherum anthelminticum* as compared to control due to decreased fear of water (table 6).

DISCUSSION

The incidence of anxiety and depression is very high and is associated with morbidity. Although several drugs are available, but due to economic burden, adverse effects and limitations of their use people are now moving towards alternative medications.

In this study, it was demonstrated that methanolic extracts of *Cuminum nigrum* (L) and *Centratherum anthelminticum* (L) have antidepressant and anxiolytic potential. Review of previous research studies indicated that long-term exposure to any physical or psychological stress induces morphological and functional changes, especially in amygdala nuclei in the brain, hippocampus and other regions including prefrontal cortex are observed. These changes lead to increase in anxiety-like behavior, cognitive changes and mood alterations (Roozendaal *et al*., 2009). It has been documented that prolonged stress stimulates brain cortex and hypothalamus excessive release of acetylcholine from the sympathetic nerves, which in turn results in hypersecretion of adrenaline and noradrenaline from the adrenal medulla. These may cause various deleterious symptoms including panic attacks, phobia and other stress responses (Yanagihara *et al.,* 2014). In the present study, exploratory and locomotor activities of mice were assessed in the open field, head dip, home cage crossing activity and light and dark box test to determine anxiolytic and anti-depressant effects of the drugs by measuring changes in their spontaneous activities.

In open field test, the number of squares crossed is used as measurement of exploratory and locomotor activity (Ajibade *et al.,* 2011, Choleris *et al*., 2001). The results of our study suggested antidepressant and anxiolytic behavior in animals treated with *Cuminum nigrum* (L) and *Centratherum anthelminticum* (L) in comparison to control group as evident by increase in number of total squares crossed by animals on 60th days of dosing of extract.

Table 1: Open Field Test

Central squares crossed in open field				
Groups				
	Day 0	Day 15	Day 30	Day 60
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
01	16.83±3.43	16.50 ± 3.39	17.17±3.66	17.00±3.69
02	16.20±2.39	16.40±2.30	18.60±4.22	20.00±2.83
03	16.40±2.30	16.00±2.24	17.60±2.61	18.80±2.59
Peripheral squares crossed in open field				
Groups				
	Day 0	Day 15	Day 30	Day 60
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
01	131.67±13.70	131.83±13.69	132.20±13.68	133.50±14.01
02	132.00±4.86	131.33±4.86	148.83±4.91*	168.83±4.40***
03	131.83±13.90	145.50±6.92	147.67±13.22*	154.33±13.04***

Table 2: Light/Dark Box (LDB) Test

Percentage of time in light compartment				
Groups				
	Day 0	Day 15	Day 30	Day 60
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
01	38.00±6.69	37.50±4.46	38.83±5.52	38.17±4.49
02	38.83±2.71	39.67±1.37	41.67±2.87	58.17±2.48**
03	39.17±2.71	43.33±3.72	45.67±6.12	59.50±4.55**
Number of Transitions				
Groups				
	Day 0	Day 15	Day 30	Day 60
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
01	15.50±3.01	13.33±3.66	11.66±4.27	11.00±2.0
02	16.50±1.38	16.67±1.75	17.33±1.03	18.00±2.19
03	16.50±2.81	15.83±1.17	16.33±2.69	17.67±2.06

Table 3: Head Dip Test

Groups	Head Dips			
	Day 0	Day 15	Day 30	Day 60
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
01	28.83±4.12	27.83±4.49	28.00±4.33	27.50±4.89
02	28.17±3.92	27.17±4.26	21.83±6.64*	19.16±4.99**
03	28.33±2.42	26.17±4.31	20.83±1.83*	17.00±3.52**

Table 4: Cage Crossing Test

Groups	Cage Crossings			
	Day 0	Day 15	Day 30	Day 60
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
01	25.83±3.54	25.17±4.26	25.17±4.26	24.67±4.80
02	26.33±1.86	25.00±4.09	22.16±2.04	17.33±2.66**
03	26.17±3.71	25.00±4.33	21.83±2.93	19.16±0.98**

Values are presented as mean ±S.D. n=10, *p<0.05 is considered significant as compared to control group, **p<0.01 is considered more significant, ***p<0.001 is considered highly significant as compared to control group

Table 5: Stationary Rod Test

Groups	Time to Reach Plateform			
	Day 0	Day 15	Day 30	Day 60
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
01	12.83±2.31	13.66±2.42	14.00±2.90	14.33±3.50
02	12.67±2.34	12.67±1.37	13.33±2.73	14.33±2.16
03	13.00±2.00	13.33±1.12	14.83±1.17	15.00±3.03

Table 6: Forced Swimming Test (struggling time)

Groups				
	Day 0	Day 15	Day 30	Day 60
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
01	151.0±31.30	152.10±31.01	156.00±27.37	152.60±29.95
02	150.83±3.60	150.00±5.97	146.00±7.37	134.00±4.69**
03	151.50±4.08	150.00±4.85	143.33±6.77	131.00±7.10**

Values are presented as mean ±S.D. n=10, *p<0.05 is considered significant as compared to control group, **p<0.01 is considered more significant, ***p<0.001 is considered highly significant as compared to control group

In the light and dark box test, anxiolytics have been found to increase locomotion and time spent in the light zone, whereas anxiogenics increase the time in dark zone (Imaizumi *et al*., 1994; Bourin and Hascoet, 2002). In this study the animals receiving the extracts of *Cuminum nigrum (L)* and *Centratherum anthelminticum (L)* spent more time in light zone as compared to control due to anxiolytic and antidepressant activity.

Head dip activity is used as a useful tool to assess various emotional states of animal. In head dip activity of our study decrease in number of head-dips in groups of *Cuminum nigrum (L)* and *Centratherum anthelminticum (L)* could be related to its anxiolytic effect since the animals is comfortable in the head dip apparatus (Solangi and Najam, 2013).

Decrease in number of cage crossings in animal treated with the extracts in comparison to control group animals indicates animals' passive behavior. It could be due to sedative effects of *Cuminum nigrum* (L) and *Centratherum anthelminticum* (L) extract reflected as decreased exploration in animals.

The time spent on stationary rod is not decreased which could be due to anxiolytic activity of the extracts because the animal is not having fear of falling. Decrease in fear and anxiety response probably related to decreased level of 5HT in amygdala nuclei in brain (Moya *et al*., 2011).

In Forced swimming test, the swimming or struggling time of the animals were reduced. It could be due to anxiolytic response of the extracts or could be due to that animal is not having fear of water and take floating attitude in the swimming tank (Solangi and Najam, 2013; Sakakibara *et al.,* 2005). Further research is required to evaluate the exact mechanism of action of *Cuminum nigrum (L)* and *Centratherum anthelminticum (L)* extract.

Literature review suggested number of mechanisms in the induction and inhibition of anxiety states. Moya *et al* (2011) suggested 5-HT_{2c} receptor activation in amygdala region causes anxiogenic behaviors while involvement of relative balance of conventional neurotransmitters, such as gamma-amino-butyric acid (GABA), noradrenaline, dopamine and glutamate (Durant *et al*., 2010) including other modulators corticotropin-releasing hormone, neuropeptide Y also play role in inhibition and stimulation of depression and anxiety states (Wu *et al.,* 2011). Although the drugs that control the psychological stress shows effect by multiple mechanisms. $GABA_{A,B}$ receptor modulators (benzodiazepines and related drugs), serotonin (5-HT_{1A} receptor agonists) and 5-HT_{2C} inhibitors are currently the principal drugs employed in the management of anxiety disorders (Millan, 2003).

From the results of the current study it has been suggested that behavioral profiles of methanolic extract of *Cuminum nigrum* and *Centratherum anthelminticum* are having established anxiolytic effects. Benzodiazepines (BZDs) are the most widely prescribed class of drugs all over the world since many decades to treat several forms of anxiety; however, they have prominent side effects such as sedation, ataxia and amnesia, and cause pharmacological dependence (Lader and Morton, 1991). GABA is the most abundant inhibitory neurotransmitter in the central nervous system. Results of our present study suggested that extract of *Cuminum nigrum* and *Centratherum anthelminticum* probably acted as positive allosteric modulators (PAMs) of the $GABA_A$ receptor, bind to a site other than GABA and potentiate its effect by making the chloride-ion channel open more frequently or

for longer period of time, thus exerting their anxiolytic, and antidepressant effects (Griffin *et al.,* 2013). The probable mechanism needs to be further evaluated.

CONCLUSION

The *Cuminum nigrum (L)* and *Centratherum anthelminticum (L)* have anti-depressant and anxiolytic profile. Further studies will be required to investigate neurobiological mechanisms of action and possible interactions of *Cuminum nigrum* (L) and *Centratherum anthelminticum* (L) with neurotransmitters. It is also required to isolate and identify the phytoconstituents responsible for the observed central effects. The results of our study could also stimulate further research that could lead to the development of safer and economical alternative medicines from *C. nigrum* and *C. anthelminticum* for the treatment of anxiety and depression.

REFERENCES

Ahmad M, Akhtar MS, Malik T and Gilani AH (2000). Hypoglycaemic action of the flavonoid fraction of Cuminum nigrum seeds. *Phytother. Res.*, **14**(2): 103-6.

Ajibade AJ, Adenowo TK, Akintunde OW, Fakunle PB, Oyewo OO, Ashamu EA and Onaolapo AY (2011). Suppression of exploration and locomotion in adult Wistar rats following quinine administration. *J. Neurosci. Behav. Health*, **3**: 32-37.

Alliwell B and Gutteridge JMC (1989). Free Radicals in Biology and Medicine, 2nd edition, Clarendon Press, Oxford, xvi+543 pp.

Arya A, Looi CY, Cheah SC, Mustafa MR and Mohd MA (2012). Anti-diabetic effects of *Centratherum anthelminticum* seeds methanolic fraction on pancreatic cells, β-TC6 and its alleviating role in type 2 diabetic rats. *J. Ethnopharmacol.*, **144**(1): 22-32.

Baser KHC, Ozek T, Abduganiv BE, Abdullaer UA and Aripov Kh N (1997). Composition of the essential oil of *Bunium persicum* (Boiss.) B. Fedtsch. from Tajikistan. *J. Essent. Oil Res*., **9**: 597-598.

Bourin M and Hascoet M (2002). The mouse light / dark box test. *Euro J. Pharmacology*, **463**: 55-65.

Choleris E, Thomas AW, Kavaliers M and Prato FS (2001). A detailed ethological analysis of the mouse open field test: Effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. *Neurosci. Biobehav. Rev.*, **25**(3): 235-260.

Drugan RC, Macomber H and Warner TA (2010). Intermittent and continuous swim stress induced behavioral depression: Sensitivity to norepinephrine- and serotonin-selective antidepressants. *Psychopharmacol.*, **212**(1): 85-91.

Durant C, Christmas D and Nutt D (2010). The pharmacology of anxiety. *Curr. Top. Behav. Neurosci*., **2**: 303-330.

Galani VJ and Panchal RR (2014). *In vitro* Evaluation of *Centratherum anthelminticum* seeds for antinephrolithiatic activity. *J. Homeop. Ayurv. Med*., **3**: 145.

Griffin III CE, Kaye AM, Bueno FR and Kaye AD (2013). Benzodiazepine pharmacology and central nervous system-mediated effects. *The Ochsner J.*, **13**(2): 214-223.

Gulian T, Ublin Z, Tianyou Z, Furqan Y and Yoichiro I (2010). Separation of flavonoids from seeds of centratherum anthelminticum (l) by high speed counter current chromatoghraphy. *J. Ethopharmacol*., **33**(2): 743.

Imaizumi M, Suzuki T, Machida H and Onodera K (1994). A fully automated apparatus for a light/dark test measuring anxiolytic or anxiogenic effects of drugs in mice. *Jpn. J. Psychopharmacol*., **14**: 83-91.

Kishioka A, Fukushima F, Ito T, Kataoka H, Mori H, Ikeda T, Itohara S, Sakimura K and Mishina M (2009). A novel form of memory for auditory fear conditioning at a low-intensity unconditioned stimulus. *PLoS One*, **4**(1): e4157.

Kliethermes CL and Crabbe JC (2006). Pharmacological and genetic influences on hole board behaviors in mice. *Pharmacol. Biochem. Behav.*, **85**(1): 57-65.

Lader M and Morton S (1991). Benzodiazepine problems. *Brit. J. Addict.*, **86**(7): 823-828.

Looi CY, Arya A, Cheah FK, Muharram B, Leong KH, Mohamad K, Wong WF, Rai N and Mustafa MR (2013). Induction of apoptosis in human breast cancer cells via caspase pathway by vernodalin isolated from *Centratherum anthelminticum* (L.) seeds. *PLoS One*; **8**(2): e56643

Mazidi S, Rezaei K, Golmakani MT, Sharifan A and Rezazadeh Sh (2012). Antioxidant activity of essential oil from black zira (*Bunium persicum* Boiss.) Obtained by microwave-assisted hydrodistillation. *J. Agr. Sci. Tech.*, **14**: 1013-1022

Michel B and Martin H (2003).The mouse light and dark box test. *Euro. J. Pharmacol.*, **463**: 55-65.

Millan MJ (2003). The neurobiology and control of anxious states. *Prog. Neurobiol*., **70**(2): 83-244.

Moya PR, Fox MA, Jensen CL, Laporte JL, French HT, Wendland JR and Murphy DL (2011). Altered 5-HT2C receptor agonist-induced responses and 5-HT2C receptor RNA editing in the amygdala of serotonin transporter knockout mice. *BMC Pharmacol.*, **11**(1): 3.

Newman D J and Cragg GM (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J. Nat. Prod.*, **75**(3): 311-335.

Oroojalian F, Kasra-Kermanshahi R, Azizi M and Bassami MR (2010). Phytochemical composition of the essential oils from three apiaceae species and their antibacterial effects on food-borne pathogens. *Food Chem.*, **120**: 765-770.

Pan SY, Zhou SF, Gao SH, Yu ZL, Zhang SF, Tang MK, Sun JN, Ma DL, Han YF, Fong WF and Ko KM

(2013). New Perspectives on how to discover drugs from herbal medicines: Cam's outstanding contribution to modern therapeutics. *Evid. Based Complement. Alternat. Med*., **2013**: 627375.

Perveen T, Haider S, Kanwal S and Haleem DJ (2009). Repeated administration of *Nigella sativa* decreases 5-HT turnover and produces anxiolytic effects in rats. *Pak. J. Pharm. Sci.*, **22**(2): 139-144.

Perveen T, Rafiq R, Haider S and Haleem DJ (2006). Increased serotonergic functions following administration of 1-(1-Naphthyl) piperazine in propranolol injected rats. *Pak. J. Pharm. Sci.*, **19**(3): 190-194.

Purnima A, Koti BC, Tikare VP, Viswanathaswamy AHM, Thippeswamy AHM and Dabadi P (2009). Evaluation of analgesic and antipyretic activities of *Centratherum anthelminticum* (L) kuntze seed. *Indian J. Pharm. Sci.*, **71**(4): 461.

Roozendaal B, McEwen BS and Chattarji S (2009). Stress, memory and the amygdala. *Nat. Rev. Neurosci.*, **10**(6): 423-433.

Roug L and Jiang ZT (2004). Chemical composition of the essential oils of *Cuminum nigrum* (L) *China Flavour Frag J*., **19**(4): 311-313.

Sahoo HB, Sagar R and Patel VK (2012). Wound healing activity of *Centratherum anthelminticum* Linn. *Mol. Clin. Pharmacol.*, **3**(1): 1-7.

Sakakibara H, Ishida K, Izawa Y, Minami Y, Saito S, Kawai Y, Butterweck V, Tamaki T, Nakaya Y and Terao J (2005). Effects of forced swimming stress on rat brain function. *J. Med. Invest*, **52**(Supplement): 300-301.

Savithramma N, Rao ML and Suhrulatha D (2011). Screening of medicinal plants for secondary metabolites. *Middle East J. Sci. Res.*, **8**(3): 579-584.

Seibenhener ML and Wooten MC (2015). Use of the open field maze to measure locomotor and anxiety-like behavior in mice. *J. Vis. Exp.*, **96**: 52434.

Solangi MA and Najam R (2013). Anxiolytic and memory enhancing activity of *Lagenaria Siceraria* in rodents. *Int. J. Biomed. Adv. Res.*, **4**:40-46.

Thippeswamy NB and Naidu KA (2005). Antioxidant potency of cumin varieties, cumin black and bitter cumin on antioxidant system. *Euro Food Res Technical*., **220**: 472-476.

Tsao R and Akhtar MH (2005). Nutraceuticals and functional foods I: Current trend in phytochemical antioxidant research. *J. Food. Agric. Environ.*, **3**(1): 10-7.

Wu G, Feder A, Wegener G, Bailey C, Saxena S, Charney D and Mathe AA (2011). Central functions of neuropeptide Y in mood and anxiety disorders. *Expert Opin. Ther. Targets.*, **15**(11): 1317-1331.

Yanagihara N, Zhang H, Toyohira Y, Takahashi K, Ueno S, Tsutsui M and Takahashi K (2014). New insights into the pharmacological potential of plant flavonoids in the catecholamine system. *J. Pharmacol. Sci.*, **124**(2): 123-128.

Zafar A (2010). A Review on Analgesic: From natural sources. *Int. J. Pharm. Biol. Sci. Arch.*, **1**(2): 95-100.

Zhou J, Shang J, Ping F and Zhao G (2012). Alcohol extract from *Vernonia anthelmintica* (L.) wild seed enhances melanin synthesis through activation of the p38 MAPK signaling pathway in B16F10 cells and primary melanocytes. *J. Ethnopharmacol.*, **143**(2): 639-647.