ejpmr, 2016,3(4), 394-402

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

ANTIEPILEPTIC AND ANXIOLYTIC ACTIVITY OF ETHANOLIC EXTRACT OF *BRASSICA NIGRA L. KOCH* SEEDS ON WISTAR ALBINO RATS

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Article Received on 18/02/2016 **Article Revised on 09/03/2016** **Article Accepted on 30/03/2016**

ABSTRACT

The epilepsy is one of the most common neurological conditions that show a prevalence rate in 1–2% of the world population. Although a considerable number of antiepileptic drugs are available for the treatment of epilepsy and there is still an urgent need for development of new drugs as alternatives. The rich floral biodiversity of nature has provided herbal health practitioners and other traditional healers with an impressive pool of 'natural pharmacy' from which plants are selected as ingredients to prepare herbal remedies and medicines (phytomedicines) for the treatment, management and control of a variety of human ailments. One of such therapeutically useful medicinal plants is *Brassica nigra L. Koch* [family: Cruciferae] commonly known as "BLACK MUSTARD". It is a cultivated crop and has been regarded to possess various medicinal properties. Phytochemical invetigation and Pharmacological investigation was carried out on the ethanolic extract of *Brassica nigra L. Koch* seed. The phytochemical investigation revealed the presence of carbohydrate, alkaloid, flavonoid, fixed oil and glycosides. In Maximum electroshock induced seizure (MES) induced convulsions model ethanolic extract of *Brassica nigra L. Koch* seeds (100 and 200 mg/kg) has significantly ($p<0.01$) decreased the duration in various phases of epileptic seizure and increased the percentage protection when compare to the control group and in pentylenetetrazole induced convulsions model the ethanolic extract of *Brassica nigra L. Koch* seeds (100 and 200 mg/kg) has significantly ($p<0.01$) increased the onset of clonus, onset of tonus and percentage recovery when compare to control group. It was effective against MES induced seizures, since inhibition of the MES test predicts activity against generalized tonic-clonic and cortical focal seizures. In our study the ethanolic extract of *Brassica nigra L. Koch* seed at 100 and 200 mg/kg doses has significantly ($p<0.01$) increased the time spent and number of entries into the open arm indicating the test drugs could reduce the fear and anxiety in the rat. The ethanolic extract of *Brassica nigra L. Koch* seed at 100 and 200 mg/kg doses also showed significantly ($p<0.01$) decrease in locomotor function of the rat when compared with the control tested at different time interval after treatment by using digital actophotometer. Therefore, the results obtained from the study suggest that the ethanolic extract of *Brassica nigra L. Koch* seeds possess antiepileptic and anxiolytic potential possibly due to the phytoconstituents of extract. The extract significantly decreased epileptic seizure and anxiety when compared to control group animals.

KEYWORDS: *Brassica nigra L*, Epilepsy, antiepileptic, anxiolytic.

1. INTRODUCTION

An epilepsy is a chronic disorder of the brain, characterized by recurrent seizures, which are brief episodes of involuntary movement that may involve a part of the body (partial) or the entire body (generalized), and are sometimes accompanied by loss of consciousness and control of bowel or bladder function. Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures, and by the neurobiological, cognitive, psychological, and social consequences of this condition. An epileptic seizure is the clinical manifestation of an abnormal and excessive discharge of a set of neurons in the brain. The definition of epilepsy requires the occurrence of at least one epileptic seizure. Therefore, a seizure is the event and epilepsy is the disorder. By definition, one seizure does not make epilepsy, nor does a small series of seizures that have an immediate precipitating factor, for example, alcohol withdrawal seizures. The seizures must be spontaneous and recurrent to represent epilepsy. Seizures result from an electrochemical disorder in the brain. Brain cells use chemical reactions to produce electrical discharges. Each brain cell either excites or inhibits other brain cells with its discharges. When the balance of excitation and inhibition in a region of brain is moved too far in the direction of excitation, then a seizure can result. Approximately 50 million people worldwide have epilepsy, making it one of the most common neurological diseases globally.[1-4]

Herbal medicine derived from plant extracts increasingly utilized to treat a wide variety of clinical diseases. There is a growing interest in the pharmacological evaluation of various plants used in traditional system of medicine. Many researchers have been directed towards the provision of empirical proof to back up the use of many plants in traditional medical practices. Scientific evaluation of medicinal plants is important for the discovery of novel drugs and also helps to assess the risks of toxicity associated with the use of either herbal preparations or conventional drugs of plants origin.

Several Botanists, Pharmacologist and Microbiologist are looking in different parts of the globe to find natural chemicals that can be used in the treatment of numerous diseases. Two third of all plants in the world have medicinal properties. Around 7000 compounds in Pharmacopeia are herbal components. Regular use of herbs improves the strength of the body and boosts body Immunity.

2. METHODOLOGY

2.1. Collection of Plant material and Authentication

The seeds were collected from Bhangarotu, Mandy District, Himachal Pradesh. It was authenticated by Jayaraman P., Ph. D., Director, Institute of Herbal Botany, Plant Anatomy Research Centre, Chennai with Reg. No: PARC/1300/2012**.** The plant material was identified as *Brassica nigra L. Koch* belongs to the family Brassicaceae.

2.2. IAEC Approval

The study was conducted after obtaining the approval from Institutional Animal Ethical Committee constituted as per the direction of the CPCSEA, Proposal number NCP/IAEC/NO: 01/2012-13.

2.3. Preparation of Plant Extract

The dried seeds were collected and pulverized into coarse powder. The coarse powder was packed tightly in the Soxhlet apparatus and extracted with 500 ml of 70% ethanol at 55 ˚C for 72 hours by continuous hot percolation method. The extract was concentrated to 1/4th of its original volume by evaporating at room temperature.[5] The ethanolic extract of *Brassica nigra L. Koch* seed was subjected to phytochemical study.

2.4. Phytochemical Investigation[6,7]

I. Chemical Test for Carbohydrates: Monosaccharides are soluble in water and practically insoluble in organic solvents like chloroform, ether and in absolute alcohols. These are optically active compounds and respond to various color reactions and identification tests.

Iodine test: It is specific for polysaccharides. Few drops of Iodine solution was added to aqueous solution of drug/polysaccharide. Formation of blue color, which disappears on heating and reappears on cooling, indicates the presence of starch.

Barford test: This test is used to distinguish between monosaccharide and disaccharides. Two ml of Barford reagent (Cupric acetate, acetic acid and water) was added to 1ml aqueous solution of drug and boil. Formation of brick red precipitate in 5 minutes indicates presence of monosaccharide while in 7 minutes indicates disaccharide.

II. Chemical Test For Proteins And Amino Acids

Proteins are high molecular weight polymers of amino acids. Amino acids are colorless ionic compounds, more or less soluble in water and present in acid hydrolysis of plant and animal proteins. The presence of proteins and amino acids can be detected by following chemical tests.

Biuret test: To the aqueous solution of protein in hot water, few drops of Biuret reagent (KOH, $CuSO_4$ and sodium potassium tartarate) is added, which turns blue reagent to violet. In laboratory, it is usually done by adding few drops of 0.5% $CuSO_4$ solution to the alkaline aqueous protein solution. At least one peptide linkage is necessary for this test; individual amino acids do not produce violet color.

Millons test: Any compound containing a phenolic hydroxyl group gives Millon's test positive. Consequently, any protein containing phenolic hydroxyl group (like tyrosine and phenyl alanine) will give a positive test of a pink to dark-red color due to formation of a mercury salt of nitrated amino acid. The Millon's reagent is a solution of mercuric and mercurous ions in nitric and nitrous acids. Take 1 ml of protein solution in a test tube and add few drops of Millons reagent. White precipitate is produced, which turns red after heating for 5 minutes on water bath.

Ninhydrin test: The Ninhydrin test is used to detect the presence of alpha-amino acids and proteins containing free amino groups. Protein solution when heated with ninhydrin molecules, it gives characteristic deep blue or pale yellow color due to formation of complex between two ninhydrin molecule and nitrogen of free amino acid.

III. Chemical Tests For Lipids

Solubility in Polar and Nonpolar Solvents: Lipids are insoluble in polar solvents like water and soluble in non-polar solvents like petroleum ether, benzene and mineral oil.

Emulsification test: If emulsifiers like bile salts, tween or soap solution is mixed with lipids and water; the lipids broken down into smaller fragments, which remained suspended for long periods of time in water.

IV. Chemical Test for Alkaloids: The chemical tests are performed from neutral or slightly acidic solution of drug following type of chemical test given by alkaloids is.

Dragendorff's Test: Drug solution + Dragendorff's reagent (Potassium Bismuth Iodide), formation of Orange red color.

Mayer's Test: Drug solution + few drops of Mayer's reagent (K_2HgI_4), formation of creamy white precipitant.

Wagner's Test: Drug solution + few drops of Wagner's reagent (dilute Iodine solution), formation of reddish-brown precipitate.

V. Chemical Test for Sterols and or Triterpenes

The extracts were refluxed with alcoholic potassium hydroxide until the saponification was complete. The saponification mixture was diluted with distilled water and extracted with diethyl ether. The ethereal extract was evaporated and the unsaponifiable matter was subjected to following tests.

Libermann-Burchard's test: The ether soluble residue was dissolved in chloroform and few drops acetic anhydride was added followed by few drops of concentrated sulphuric acid from the sides of the test tube and observed for the formation of blue to blue-red color.

Salkowski's reaction: To the ether soluble residue, 2 ml of concentrated sulphuric acid was added and observed for the formation of yellow ring at the junction, which turns red after one minute.

VI. Chemical Tests For Tannins: Tannins show specific chemical reaction like solution of tannins precipitate gelatin, alkaloids, salt of Copper, Lead and Tin etc. and shows color reaction with $K_2Cr_2O_7$, chromic acid and iron salts.

Test with Iron salts: It show color reaction with iron salt like $FeCl_3$ and potassium ferrocyanide $K_4Fe(CN)_6$ in presence of ammonia. Addition of $FeCl_3$ solution to the solutions of hydrolysable tannins forms bluish black precipitate whereas with condensed tannins it forms greenish brown colored precipitate.

Vanniline hydrochloric acid test: Small amount of the extract is mixed with few drops of vanillin hydrochloric acid, appearance of pink color showing the presence of tannins in both aqueous and ethanolic extract.

VII. Test for Saponins

Foam test: A small amount of various extracts were extracted with petroleum ether and acetone. To the insoluble residue left after extraction, a few ml of water was added and shaken vigorously for 15 minutes and observed for formation of honeycomb froth that persisted for at least 30 minutes.

VIII. Chemical tests for Flavonoids

Alkaline reagent test: Take test solution in a test tube and add few drops of Sodium hydroxide solution, intense yellow color is formed which turns to colorless on addition of few drops of dilute acid indicates presence of flavonoids.

Zinc hydrochloride test: To the test solution add a mixture of zinc and concentrated hydrochloric acid.

Shinoda test: Take test solution and add to it few magnesium turnings and concentrated hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.[8]

2.5. Anticonvulsant Activity[9, 10, 11]

A.**Maximum electroshock induced seizure (MES) Model:** For inducing convulsion by electro shock, a rectangular pulse current of high voltage (150 mA; 60 Hz) is employed. The electro shock was given to each rat for 0.2 seconds with the help of convulsion meter through pinna electrodes. Drugs likely to be effective in Grandmal epilepsy usually confer protection against electrically induced convulsion in animals. The MES convulsions are divided into five phases such as Tonic flexion, Tonic extension, Clonic convulsion, Stupor, Recovery or death.

A substance is known to possess anticonvulsant property, if it reduces or abolishes the extensor or recovery phase of MES convulsion.

Procedure: Albino rats of either sex weighing between 150-200 gm were weighed, marked and divided into four groups containing 6 rats each. The animals were fasted for 18 hours prior to the experiment with water *ad* libitum.

Table 2.1: Group and corresponding treatment for MES model study for anticonvulsant activity

S.No.	Group	Treatment
1	Group I (control)	0.2ml of 0.5% CMC solution p.o.
2	Group II (standard)	Phenytoin sodium in normal saline solution at a dose of 25 mg/kg body weight i.p
3	Group III (Test 1)	70% ethanolic extract of *Brassica nigra L. Koch* seed in 0.5% CMC solution at a dose of 100 mg/kg body weight p.o.
4	Group IV (Test 2)	70% ethanolic extract of *Brassica nigra L. Koch* seed in 0.5% CMC solution at a dose of 200 mg/kg body weight p.o.

Thirty min after i.p. administration of the standard drug and sixty min after p.o. administration of the test drug,

the electro shock was given to each rat for 0.2 seconds (150 mA; 60 Hz) with the help of Electroconvulsion meter through pinna electrode and the effects were observed.

B. PTZ-induced convulsions model

Inducing agent: Pentylenetetrazole in normal saline 80 mg/kg body weight i.p.

Procedure: Wistar Albino rats of either sex weighing between 200-300 gm were weighed, marked and divided into three groups containing 6 rats each. The animals were fasted overnight prior to the experiment with access water *ad* libitum.

Table 2. 2. Group and corresponding treatment for PTZ-induced convulsion model study

S.No.	Group	Treatment
1	Group I (control)	0.2ml of 0.5% CMC solution p.o.
2	Group II (standard)	Diazepam in normal saline at a dose of 5 mg/kg body weight i.p
3	Group III (Test 1)	70% ethanolic extract of *Brassica nigra L. Koch* seed in 0.5% CMC solution at a dose of 100 mg/kg body weight p.o.
4	Group IV (Test 2)	70% ethanolic extract of *Brassica nigra L. Koch* seed in 0.5% CMC solution at a dose of 200 mg/kg body weight p.o

One hour after administration of vehicle, standard and test drug to the respective groups, the animals were treated with PTZ (Pentylenetetrazole, 80 mg/kg) subcutaneously. Each animal was placed in to individual polypropylene cage and were observed initially for 30 min and later up to 24 hrs to record latency (onset of clonus), Onset of tonic convulsions, and Status of animal after 30 min, Status of animal after 24 hrs and Percentage protection.

2.6. ANXIOLYTIC ACTIVITY [9, 12]

A. Elevated plus- maze model

Out of many possibilities to modify maze tests water maze, the Y-maze, the radial maze and the elevated plus maze have found acceptance in many laboratories. The test has been proposed for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic compounds screened by decreasing anxiety with increased open arm exploration time; anxiogenic compounds have the opposite effect. The plus-maze consists of two open arms, 50 × 10 × 40 cm, and two enclosed arms, 50 × 10 × 40 cm, with an open roof, arranged so that the two open arms are opposite to each other. The maze is elevated to a height of 50 cm.

Procedure

Albino rats of either sex weighing 200–250 gm body weight marked and divided into four groups containing 6 rats each were housed for 10 days prior to testing in the apparatus. The groups and corresponding treatment were similar to as given in Table 2.2. During this time the rats were handled by the investigator on alternate days to reduce stress. The animals were fasted for 18 hours prior to the experiment with water *ad* libitum.

Thirty min after i.p. administration of the standard drug and sixty min after p.o. administration of the test drug, All groups of animal were tested on elevated plus-maze apparatus placed in the center of the maze, facing one of the enclosed arms and open arm exploration time spent by each animals were noted.

2.7 Locomotory activity

A. Digital Actophotometer

Digital Actophotometer contain photoelectric cells. A continuous beam of light from six lights was made to fall on corresponding photoelectric cells, the photoelectric cell got activated when an animal crossed the beam of light and thereby cuts off the rays of light falling on it. These cut-offs were counted automatically for a period of 10 min and the figure considered as a measure of the locomotor activity of the animal.

Procedure

Wistar albino rats of either sex weighing 200–250 gm body weight marked and divided into four groups containing 6 rats each were housed for 10 days prior to testing in the apparatus. The groups and corresponding treatment were similar to as given in Table2. 2. During this time the rats were handled by the investigator on alternate days to reduce stress. The animals were fasted overnight prior to the experiment with water *ad* libitum.
Rats were placed in the digital photoactometer 1 hr after standard and test drug administration and the cut-offs were noted to measure the locomotor activity of the animal for period of 10 min.

Statistical Analysis

The collected data was subjected to appropriate statistical tests including one way ANOVA (Analysis of Variance), followed by Dunnett's test. P values of less than 0.05, 0.01 and 0.001 were considered as less significant, significant and more significant respectively. The analysis was carried out using Graph pad prism software of version 4.

3. RESULTS

3.1. Phytochemical analysis

Ethanolic extract of *Brassica nigra L. Koch* seeds were subjected to qualitative phytochemical tests for different phytochemical constituents. From the Phytochemical analysis, the plant extract shown the presence of shown Glycosides, Flavonoids, Alkaloids, Carbohydrates, fixed oil, volatile oil.

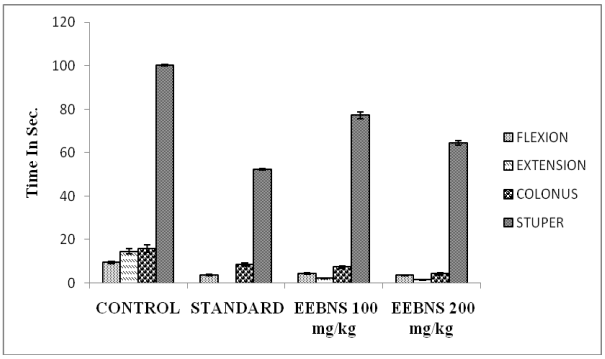
Table No. 3.1: Phytochemical Analysis of ethanolic extract of *Brassica nigra L. Koch* seeds

S.NO	PHYTOCONSTITUENTS	ETHANOLIC EXTRACT
1.	CARBOHYDRATES	+
2.	AMINO ACIDS	-
3.	LIPIDS	-
4.	ALKALOIDS	+
5.	FIXED OIL	+
6.	TANNINS	-
7.	SAPONINS	-
8.	FLAVONOIDS	+
9.	GLYCOSIDES	+

(+) Present, (-) Absent

3.2. Pharmacological Activity: *The effect of ethanolic extract of* Brassica nigra L. Koch *seeds on Maximal electro shock induced convulsions in rat is given in Annex I.*

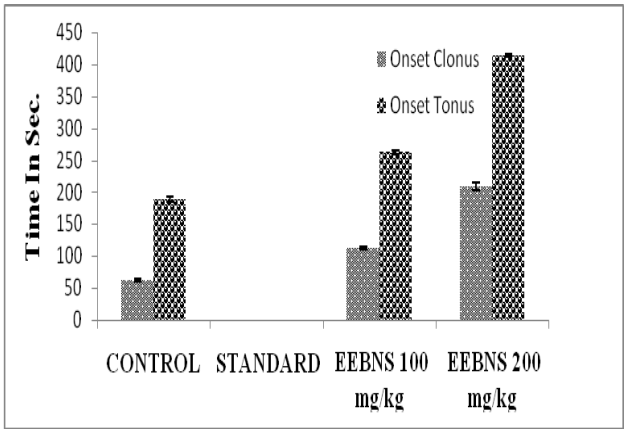
Evaluation was made by electroshock with the help of Electroconvulsio meter through pinna electrode after 1 hr of administration of vehicle, standard and test drug to the respective group of animals. The ethanolic extract of *Brassica nigra L. Koch* seeds exhibited a dose dependent significant ($p<0.01$) reduction in various phases of epileptic seizure on comparison with the control group. It was observed that the EEBNS 100 and 200 mg/kg were showed 66.66% ($p<0.01$) and 83.33% ($p<0.01$) inhibition of convulsion produced by MES, respectively. The phenytoin used as a standard drug inhibited 100% of convulsion. Extract at both the doses significantly prolonged the onset of convulsions in the extract treated group compared to vehicle treated control group.



Effect of ethanolic extract of *Brassica nigra L. Koch* seeds on Maximal electro shock induced convulsions in rat.

The Effect of ethanolic extract of *Brassica nigra* (L.) Koch seeds on PTZ (Pentylenetetrazole) induced convulsion in rat is given in Annex II.

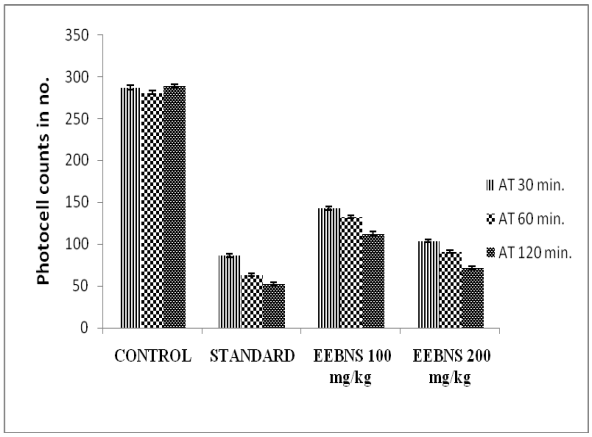
Intraperitoneal administration of PTZ induced tonic-clonic convulsions with 100% mortality in the control group. The ethanolic extract of *Brassica nigra L. Koch* seeds at dose of 100 mg/kg p.o. and at dose of 200 mg/kg p.o. significantly ($p < 0.01$) increased the onset of clonus (112±2.082 and 209.16±5.606) and onset of tonus (262.33±3.084 and 413.5±2.460) in rats compared with the control group (62.16±2.626 and 189.67±4.216). Extract at dose of 100 mg/kg p.o. offered 83.33% and at dose of 200 mg/kg p.o. offered 100 % protection against PTZ- induced convulsion in Wistar albino rats.



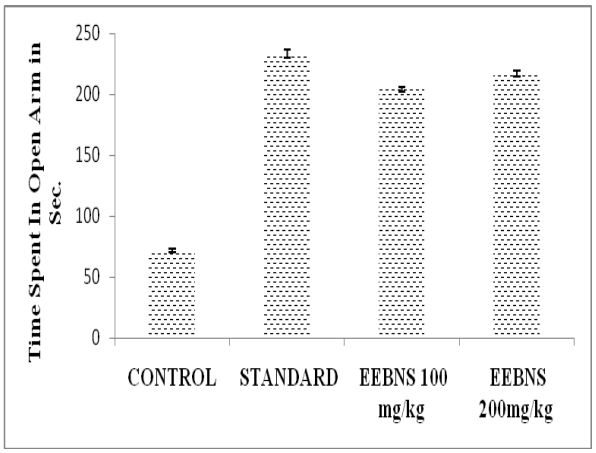
Effect of ethanolic extract of *Brassica nigra L. Koch* seeds on PTZ (Pentylenetetrazole) induced convulsion in rat.

The effect of ethanolic extract of *Brassica nigra L. Koch* on number of entries (open arm) in elevated plus-maze model is given in Annex III.

Results of the present study showed that ethanolic extract of *Brassica nigra L. Koch* seeds at dose of 100 mg/kg p.o. and 200 mg/kg p.o. significant change (P>0.01) in mean number of entries into open arms (41.5±1.910 and 62.5±0.7188) and in mean time spent on open arm (204±2.049 and 217.33±2.445) where as diazepam (5 mg/kg i.p) showed significant change (P>0.01) in mean number of entries into open arms and in mean time spent on open arm (68.83±1.641 and 233.5±3.128) as compared to control (21.33±1.145, 71.66±1.606). The ethanolic extract of *Brassica nigra L. Koch* seeds at dose of 100 mg/kg p.o. and 200 mg/kg p.o. showed increase in percentage time spent on open arm by 68 and 72.3 respectively. Anxiolytic activities of ethanolic extract of *Brassica nigra L. Koch* seeds (100 and 200 mg/ kg) were dose dependent and significant (P<0.01) as compared to control.



Effect of ethanolic extract of *Brassica nigra L. Koch* seeds on locomotors activity (Actophotometer) in rats at different time intervals (min).



Effect of ethanolic extract of *Brassica nigra L. Koch* seeds on number of entries (open arm) in elevated plus-maze model.

The effect of ethanolic extract of *Brassica nigra L. Koch* seeds on locomotors activity (Actophotometer) in rats at different time intervals (min) is given in Anex IV.

The ethanolic extract of *Brassica nigra L. Koch* seeds at dose of 100 mg/kg p.o. and 200 mg/kg p.o. showed a significant change (P<0.01) in the locomotor function of rat at different time interval when compared to the control.

Annex I. Effect of ethanolic extract of *Brassica nigra L. Koch* seeds on MES induced convulsions in rat.

GROUP	TREATMENT	DURATION IN VARIOUS PHASES (Time in Sec.) MEAN±SEM				
		FLEXION	EXTENSION	COLONUS	STUPER	% RECOVERY
GROUP I	Control (0.5%CMC)	9.5±0.4282	14.67±1.145	15.83±1.778	100.33±0.4216	_
GROUP II	STANDARD (Phenytoin sodium 25mg/kg)	3.83±0.4773**	_	8.67±0.7149**	52.33±0.4216**	100
GROUP III	TEST 1 (EEBNS100mg/kg)	4.5±0.4282**	2.33±0.4216**	7.5±0.4282**	77.16±1.493ns	66.66
GROUP IV	TEST 2 (EEBNS 200mg/kg)	3.67±0.2108**	1.67±0.2108**	4.33±0.4216**	64.5±1.118**	83.33

Annex II. Effect of ethanolic extract of *Brassica nigra* (L.) Koch seeds on PTZ (Pentylenetetrazole) induced convulsion in rat.

GROUP	TREATMENT	ONSET OF CLONUS	ONSET OF TONUS	% RECOVERY
GROUP 1	Control (0.5%CMC)	62.16±2.626	189.67±4.216	0
GROUP 2	STANDARD (Diazepam5mg/kg)	–	–	100
GROUP 3	TEST 1 (EEBNS100mg/kg)	112±2.082**	262.33±3.084**	83.33
GROUP 4	TEST 2 (EEBNS200mg/kg)	209.16±5.606**	413.5±2.460**	100

Annex III. Effect of ethanolic extract of *Brassica nigra L. Koch* on number of entries (open arm) in elevated plus-maze model.

GROUPS	TREATMENT	Number of Entries in OA	Time Spent in OA	Time in OA (%)
GROUP 1	Control (0.5%CMC)	21.33±1.145	71.66±1.606	23.6
GROUP 2	STANDARD (Diazepam5mg/kg)	68.83±1.641**	233.5±3.128**	77.6
GROUP 3	TEST 1 (EEBNS100mg/kg)	41.5±1.910**	204±2.049**	68
GROUP 4	TEST 2 (EEBNS200mg/kg)	62.5±0.7188**	217.33±2.445**	72.3

Annex IV. Effect of ethanolic extract of *Brassica nigra L. Koch* seeds on locomotors activity (Actophotometer) in rats at different time intervals (min).

GROUPS	TREATMENT	PHOTOTOCELL COUNTS		
		30 MIN	60 MIN	120 MIN
GROUP 1	Control (0.5%CMC)	287±2.966	281±2.309	289±1.483
GROUP 2	STANDARD (Diazepam5mg/kg)	86.33±2.512**	63.66±1.892**	52.33±2.390**
GROUP 3	TEST 1 (EEBNS100mg/kg)	143.16±2.007**	132.83±1.424**	112.5±2.349**
GROUP 4	TEST 2 (EEBNS200mg/kg)	103.83±1.905**	91.16±1.579**	72.16±1.682**

Values expressed as means ± SEM. n=6, ns= not significant, **p<0.001 when treated groups compared with control group analyzed by one-way ANOVA followed by Dunnett's test.

4. DISCUSSION

In present study the ethanolic extract of *Brassica nigra L. Koch* seed was studied for anxiolytic activity by experimental models namely elevated plus maze test and locomotor activity by actophotometer and anticonvulsant activity was studied by using MES induced convulsions and pentylenetetrazole induced convulsions and pre-treatment with study the ethanolic extract of *Brassica nigra L. Koch* seeds.

In MES induced convulsions model ethanolic extract of *Brassica nigra L. Koch* seeds (100 and 200 mg/kg) has significantly ($p<0.01$) decreased the duration in various phases of epileptic seizure and increased the percentage protection when compare to the control group and in pentylenetetrazole induced convulsions model the ethanolic extract of *Brassica nigra L. Koch* seeds (100 and 200 mg/kg) has significantly ($p<0.01$) increased the onset of clonus, onset of tonus and percentage recovery when compare to control group. It was effective against MES induced seizures, since inhibition of the MES test predicts activity against generalized tonic-clonic and cortical focal seizures.

PTZ induces convulsion by antagonizing the ã-aminobutyric acid (GABA)-A receptor chloride (Cl)-channel complex to attenuate GABA-dependent inhibition. Drugs protecting against tonic-clonic seizures induced by PTZ are considered useful in controlling myoclonic and absence seizures in humans. The elevated plus maze is currently one of the most widely used models of animal anxiety and the test is principally based on the exposure of animal to an elevated maze array evokes an approach-avoidance conflict that is considerably stronger than that evoked by exposure to an open maze array. The animals being exposed to the new environment tend to avoid open entries and prefer to stay in closed arm due to fear. (Vogel Vogel 2000) In our study the ethanolic extract of *Brassica nigra L. Koch* seed at 100 and 200 mg/kg doses has significantly ($p<0.01$) increased the time spent and number of entries into the open arm indicating the test drugs could reduce the fear and anxiety in the rat. The ethanolic extract of *Brassica nigra L. Koch* seed at 100 and 200 mg/kg doses also showed significantly ($p<0.01$) decrease in locomotor function of the rat when compared with the control tested at different time interval after treatment by using digital actophotometer. These results suggests that extract administration could reduce the aversion fear and produce anxiolytic activity.

5. CONCLUSION

Extract subjected for preliminary phytochemical analysis and was confirmed the presence phytoconstituents such as Glycosides, Flavonoids, Alkaloids, Carbohydrates, fixed oil, volatile oil.

The ethanolic extract of *Brassica nigra L. Koch* seeds at 100 and 200 mg/kg doses has shown significant antiepileptic in maximal electroshock induced convulsion model and PTZ induced convulsion model; anxiolytic activity in elevated plus maze model and locomotor count by actophotometer.

Therefore, the results obtained from the study suggest that the ethanolic extract of *Brassica nigra L. Koch* seeds possess antiepileptic and anxiolytic potential possibly due to the phytoconstituents of extract such as Alkaloids, glycosides, Flavonoids. The extract significantly decreased epileptic seizure and anxiety when compared to control group animals.

Further phytochemical studies are in progress to isolate, characterize and identify the specific active compounds in this plant responsible for antiepileptic and anxiolytic activity. Thus, more studies are necessary to clarify the antiepileptic and anxiolytic activity components and the mechanisms underlying in the *Brassica nigra L. Koch* seeds extract.

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