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Neuropharmacological Profile of Novel Benzopyran-2-One Derivatives in Experimental Mice

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Abstract

The present study was aimed to assess the neuropharmacological properties of the novel Benzopyran-2-one (coumarin) derivatives i.e., 7-(2-(m-nitro)-phenyl thiazolidinyl)-4-methyl benzopyran-2-one (comp-I), 7-(2-(o,p-dichloro)-phenyl thiazolidinyl)-4-methyl-benzopyran-2-one (comp-II) and 7-(2-(p-methyl)-phenyl thiazolidinyl)-4-methyl-benzopyran-2-one (comp-III) by using the various pharmacological activities i.e., anticonvulsant, anxiolytic, spontaneous motor activity, skeletal muscle relaxant and analgesic activities along with assay of serum gamma amino butyric acid (GABA) levels. ***Methods:*** *Investigation of all neuropharmacological profiles were evaluated by performing the various pharmacological activities using the different screening models, i.e., anticonvulsant activity by pentylenetetrazole (PTZ) induced seizure model, anxiolytic activity (exploratory behaviour) by evasion test, assay of GABA levels after anticonvulsant model was performed by using chromatographic technique, spontaneous motor activity (SMA) by actophotometer test, skeletal muscle relaxation by rotarod test and analgesic activity by Eddy's hot plate and Haffner's tail clip methods in Wistar albino mice.* ***Results:*** *All the Benzopyran-2-one derivatives showed significant anticonvulsant activity by prolonging the onset of seizures and reducing the duration of seizures and mortality rate when compared with control group. The test compounds showed significant anxiolytic effect. The serum GABA levels were significantly elevated in the treated groups, when compared with that of control group. The test compounds showed significant decrease in the spontaneous motor activity. The test compounds significantly showed a skeletal muscle relaxation after 30 min of dosing. The test compounds showed significant increase in paw licking time to heat stimuli in hot plate method and increase in the reaction time of mice to dislodge the clip.* ***Conclusion:*** *The present study has revealed that all the Benzopyran-2-one derivatives possess significant neuropharmacological properties confirmed by anticonvulsant, anxiolytic, skeletal muscle relaxant, analgesic effects and decrease in spontaneous motor activity at a dose of 20 mg/kg p.o. in swiss albino mice.*

Keywords: *Benzopyran-2-one derivatives, CNS disorders, GABA, neuropharmacology*

INTRODUCTION

One of the foremost problems in the treatment of central nervous system (CNS) disorders is the selective delivery of the therapeutic agent to the affected cells [1]. The management of neurological and psychiatric disorders is a vast and evolving area for researchers, primary care physicians and specialists [2]. These disease states, including depression, epilepsy, neurodegenerative disorders, neuropathic pain, multiple sclerosis (MS) and schizophrenia are complicated disorders affecting mood, memory and mobility: the true essence of being [3]. The availability and use of drugs with demonstrable efficacy in psychotic disorders has grown since the late 1950s to the point that 10% to 15% of prescriptions written in the U.S are of medications intended to affect mental processes, to sedate, stimulate or otherwise modify

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mood, thinking or behavior [4]. Schizophrenia is a devastating psychiatric illness that affects ~ 1% of the population worldwide [5]. Alzheimer disease (AD) is a major public health issue with a prediction of 12 millions Americans being affected by 2025 [6]. Depression is currently one of the leading causes of disability on the global burden of disease list worldwide and is predicted to rank second by the year 2020 [7]. The development of medications for the treatment of CNS disorders is based on careful assessment of adverse effects as well as efficacy [8]. Besides addiction liabilities, benzodiazepines adversely affect the respiratory, digestive and immune system of body and the chronic treatment with benzodiazepines often prove more harmful in the longer run [9]. To achieve the substantial improvement in the treatment of CNS illness, two factors need to be considered: (i) Identification of the underlying mechanism of the CNS defects and, (ii) Selective drug delivery to limit potential side-effects. Neuropharmacology is understanding the actions of drugs on the functions of the brain, whether it be on single cells or behavior, is a multilevel, multifaceted process that begins with and builds upon the concept of molecular interactions [10]. These drugs may originate from plants, minerals or synthetic derivatives. Changes in mood, thinking and behavior may be mediated by interaction of these drugs with particular target sites or receptors found in the nervous system [11]. Recently coumarins have evoked a great interest in neuropharmacological research and have been found effective in CNS. Review of literature shows that natural plants containing coumarins as an active constituent such as *Petiveria alliacea,* [12] *Torresea cearensis ,Eclipta alba, Pterodon polygaliflorous and Hybanthus ipecacuanha* a group of Brazilian medicinal plants, [13] *Leucas inflate*, [14] *Angelica gigas*, [15] *Ficus platyphylla*, [16] *Careya arborea*, [17] *Morus alba L* (mulberry) [18] and prenyloxycoumarins [19] have been reported for CNS activities. Chemically synthesized benzodiazepine derivatives from coumarins have been reported for antianxiety activity [20]. Thiosemicarbazido derivatives of coumarin for a potential anticonvulsant and analgesic activity have been reported earlier [21]. Some bi-heterocyclic coumarin derivatives are reported to have analgesic and anti-inflammatory activities [22]. Some substituted coumarins are also reported for anticonvulsant activity [23]. Therefore, we can conclude that any plant extract or chemically synthesized derivatives containing coumarin may show neuropharmacological effects i.e., action on CNS. Hence, the present study deals with the investigation of the neuropharmacological effects such as analgesic effect, spontaneous motor activity (SMA), anxiolytic, skeletal muscle relaxant and anticonvulsant activities of some novel Benzopyran-2-one (coumarin) derivatives i.e., 7-(2-(m-nitro)-phenyl thiazolidinyl)-4-methyl benzopyran-2-one (comp-I), 7-(2-(o,p-dichloro)-phenyl thiazolidinyl)-4-methyl-benzopyran-2-one (comp-II) and 7-(2-(p-methyl)-phenyl thiazolidinyl)-4-methyl-benzopyran-2-one (comp-III) in mice.

REVIEW OF LITERATURE

Coumarin, the simplest member of the group of oxygen heterocycle called benzopyran-2-one, occur naturally in plants, microorganisms and naturally occurring phenolic substance. These are composed of fused benzene and pyrone rings which may be considered, to be lactones of the 2-hydroxy-Z-cinnamic acid. Coumarins owe their class name to ‘coumarou’ the vernacular name of tonka bean (*Dipteryx odorata* Wild. Fabaceae), from which coumarin itself was isolated in 1820 [24, 25]. Coumarin is present in wide variety of plants including cassia, lavender, yellow sweet clover, fruits (e.g. bilberry and cloudberry) green tea and chicory [26]. Over one thousand coumarins have been described and the

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simplest among them are widely distributed in all of the vegetable kingdom. With MW: 146.14 and Formula: C9H6O2. Physical properties [27] include Physical state: colourless flakes, with characteristic odour, Melting point: 68-70 oC, Density: 0.94 g/cm3 and poor Solubility in water.

Literature survey reveals that natural plants containing coumarins show various biological activities such as antimicrobial, [28] cytotoxic, [29, 30] antibacterial, [31] antiulcerogenic, [32] antitumor promoting effect, [33] antifungal, [34] antioxidant, [35, 36] anti proliferative effect, [37] anti-inflammatory and analgesic, [38, 39] antispasmodic and bronchodilator, [40] hypotensive, [41] antidiabetic, [42] vasodilator, [43] antiedema, [44] photosensitizing, [45] antiamoebic, [46] hepatoprotective, [47] sedative and hypnotic, [48] anxiolytic [49] and antimalarial activity [50]. Coumarins are also used in many cosmetic products [51]. Chemically synthesized derivatives of coumarin have also been reported to show CNS activities such as anxiolytic, [20] anticonvulsant [21] and analgesic activities [22]. Literature survey reveals that along with the biological activities on CNS, coumarin derivatives with diverse structural features show versatile pharmacological effects such as antimicrobial, [52] anti-cancer, [53] anti-tubercular, [54] anti-inflammatory, [55] anti-HIV, [56] anti-mycobacterial, [57] anti-fungal, [58] anti-filarial, [59] anti-coagulant [60] and anti-ulcer [61] activities. Chemically synthesized derivatives of coumarin have also been reported to show various other biological activities such as antifungal, [62] microtubule inhibitor with antimitotic activity in multidrug resistant cancer cells, [63] antiinflammatory, [64] anticandida, [65] MAO-B inhibitor, [66] antiprotozoal, [67] antioxidant, [68, 69] Gyrase B-inhibitor, [70] 5-lipoxygenase inhibitor, [71] inhibitor of heat shock protein 90 (HSP 90), [72] inhibitor of multi-drug transporter P-glycoprotien, [73] allosteric MEK1 inhibitor [74] and xanthine oxidase inhibitors [75]. Along with this, synthetic coumarin derivatives are also used as acidichromic colorant [76].

MATERIALS AND METHODS

Animals (Wistar albino mice) weighing 20-25gm were procured from the animal house of K.L.E.Society's College Of Pharmacy, Hubli, Karnataka and were used for the study. The animals were housed under standard 12-h light/dark cycle and were kept for one week to acclimatize to laboratory conditions before starting the experiment. They were given free access to water and standard feed, 12h prior to an experiment; the mice were deprived of food but not water.

The test compounds used for the present study were synthesized according to the method of Ronad et.al. [77], i.e.,7-(2-(m-nitro)-phenyl thiazolidinyl)-4-methyl benzopyran-2-one (comp-I), 7-(2-(o, p-dichloro)-phenyl thiazolidinyl)-4-methyl-benzopyran-2-one (comp-II) and 7-(2-(p-methyl)-phenyl thiazolidinyl)-4-methyl-benzopyran-2-one (comp-III).

Toxicity Studies

Acute oral toxicity – Acute toxic class method

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), [78] revised draft guidelines 423, revised from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Approval of the Institutional Animal Ethical Committee was obtained prior to the experimentation on animals. Acute toxicity studies were performed on female Wistar mice weighing between 20-25gm. Mice were fasted overnight prior to the experimental up and down procedure. Animals were observed individually after dosing at least once during first 30 min, periodically during first 24 h with special attention given during first 4 h for toxic

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symptoms such as salivation, lacrymation, tremors, respiratory distress, convulsions, death etc. The maximum nonlethal dose was found to be 200 mg/kg body weight, hence 1/10 th dose was taken as effective dose (i.e., 20 mg/kg) for all three benzopyran-2-one (coumarin) derivatives i.e, 7-(2-(m-nitro)-phenyl thiazolidinyl)-4-methyl benzopyran-2-one (comp-I), 7-(2-(o,p-dichloro)-phenyl thiazolidinyl)-4-methyl-benzopyran-2-one (comp-II) and 7-(2-(p-methyl)-phenyl thiazolidinyl)-4-methyl-benzopyran-2-one (comp-III) for investigation of neuropharmacological activities such as spontaneous motor activity, anti-anxiety activity, skeletal muscle relaxant activity, anticonvulsant activity and analgesic activity.

Anticonvulsant Activity

Pentylenetetrazole (PTZ) induced siezures

Wistar albino mice of either sex weighing between 20-25 g were divided into five groups, i.e., Group-I: Control – 1 % sodium CMC + PTZ (5 ml/kg p.o. + 90 mg/kg body weight i.p.),Group-II: Standard - Diazepam (1 mg/ kg body weight, p.o.),Group-III: Compound-I (20 mg/kg body weight, p.o.),Group-IV: Compound-II (20 mg/kg body weight, p.o.),Group-V: Compound-III (20 mg/kg body weight, p.o.). Mice were treated with either Benzopyran-2-one derivatives i.e. comp-I, comp-II and comp-III (20 mg/kg p.o.) or normal (1% sodium CMC, 5 ml/kg p.o.) or standard drug Diazepam (1mg/kg p.o.).They were all given PTZ (90 mg/kg i.p.) 60 min later and observed for the seizures. The time taken for the onset of seizures and the duration of seizures was noted. The percentage of animals protected (not showing convulsion) within 60 min after PTZ administration was recorded [79, 80, 81].

ANXIOLYTIC EFFECT

Evasion test

Wistar albino mice of either sex weighing between 20-25 g were divided into five groups, i.e., Group-I: Control – 1 % sodium CMC (5 ml/kg p.o.),Group-II: Standard - Diazepam (1 mg/ kg body weight, p.o.),Group-III: Compound-I (20 mg/kg body weight, p.o.),Group-IV: Compound-II (20 mg/kg body weight, p.o.),Group-V: Compound-III (20 mg/kg body weight, p.o.). The animals were introduced into a rectangular box with an inclined plane by which the mice can escape from the box and the mice that escaped within 5 min from the rectangular box were selected for this test. 30, 60, 90 and 120 minutes after administration of normal saline or diazepam (standard drug) or Benzopyran-2-one derivatives, the animals (n=6) were placed in the box and the number of mice remaining in the box after 5 min of test duration in each group was noted [82, 83].

GABA assay by paper chromatography method

The GABA levels in serum were assayed by paper chromatography method as mentioned by Mishraa, et.al., with some modifications. The serum sample (100 µl) was added to 1.5 ml of absolute alcohol and centrifuged at 3000 g for 15 minutes. The upper layer was aspirated and 0.3 ml was put on Whatman's filter paper which was dipped in mobile phase solution (n-butanol, glacial acetic acid, water) until the mobile phase is sufficiently run in upward direction of the paper then subsequently the filter paper was dried in air. Thereafter, ninhydrin salt solution was sprayed on chromatography paper for spot development and heated at 65oC for 10 minutes [84]. The spot, developed due to chromatographic mobility of GABA, was cut and put in 3 ml solution of absolute alcohol for elution. The optical density of eluted sample was taken on a spectrophotometer at wavelength of 509 nm and compared with standard GABA solution.

Spontaneous Motor Activity (SMA)

Actophotometer test

Wistar albino mice of either sex weighing between 20-25 g were divided into five

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groups, i.e., Group-I: Control – 1 % sodium CMC (5 ml/kg p.o.),Group-II: Standard - Diazepam (1 mg/ kg body weight, p.o.),Group-III: Compound-I (20 mg/kg body weight, p.o.),Group-IV: Compound-II (20 mg/kg body weight, p.o.),Group-V: Compound-III (20 mg/kg body weight, p.o.).Spontaneous motor activity was evaluated using actophotometer [85]. Mice were grouped of 6 each and treated with 1% sodium CMC (5 ml/kg p.o.) or the Benzopyran-2-one test drugs i.e. comp-I, comp-II and comp-III (20 mg/kg p.o.) or received diazepam as standard drug (1 mg/kg p.o.). Activity (number of counts) was recorded 30 min after treatment for 10 min. SMA measurements (number of counts) started 30 min after the administration of the test drug and the results were compared with those of control. The experiments were repeated at an interval of 30 min, for a total of 120 mins. (i.e. at 0, 30, 60, 90 and 120 mins). Results of the treated groups were compared with those of control group at each time interval.

Skeletal Muscle Relaxant Activity

Rotarod test

Wistar albino mice of either sex weighing between 20-25 g were divided into five groups, i.e., Group-I: Control – 1 % sodium CMC (5 ml/kg p.o.),Group-II: Standard - Diazepam (1 mg/ kg body weight, p.o.),Group-III: Compound-I (20 mg/kg body weight, p.o.),Group-IV: Compound-II (20 mg/kg body weight, p.o.),Group-V: Compound-III (20 mg/kg body weight, p.o.).Spontaneous motor activity was evaluated using actophotometer. Mice were grouped of 6 each and treated with 1% sodium CMC (5 ml/kg p.o.) or the Benzopyran-2-one test drugs i.e. comp-I, comp-II and comp-III (20 mg/kg p.o.) or received diazepam as standard drug (1 mg/kg p.o.).Mice were placed on a horizontal wooden rod (32 mm diameter) rotating at a speed of 16 rpm [86, 87]. The animals remaining on the rod for 3 min or more in two successive trials were selected for the test and were divided into 5 groups of 6 animals each. The animals were treated with 1% sodium CMC (5 ml/kg p.o.) or standard drug diazepam (1 mg/kg p.o.) or Benzopyran-2-one derivatives (20 mg/kg p.o.) and after 30 min of treatment animals were placed on the rod to note the time taken for the mice to fall from the rotating rod.

Analgesic Activity

Eddy's Hot Plate method

Wistar albino mice of either sex weighing between 20-25 g were divided into five groups i.e., Group-I: Control – 1 % Sodium CMC (5 ml/kg p.o.), Group-II:Standard - Pentazocine (10 mg/kg body weight, i.p.), Group-III:Compound-I (20 mg/kg body weight, p.o.),Group-IV: Compound-II (20 mg/kg body weight, p.o.),Group-V:Compound-III (20 mg/kg body weight, p.o.). The test was carried out using Eddy's hot plate apparatus, where the temperature was set at 55 ± 10 C. Mice of either sex weighing between 20-25 g which showing cut off time below 15 sec were selected for test and divided randomly into five groups [88, 89, 90]. The mice were placed on hot plate and recorded the reaction time in second/s for licking of hind paw or jumping with cut off time of 15 seconds to avoid tissue injury. The reaction time following the administration of the test derivatives, standard drug (pentazocine) and control saline vehicle were measured at 0, 30, 60, 90 and 120 minutes respectively.

Tail clip method

Wistar albino mice of either sex weighing between 20-25 g were divided into five groups i.e., Group-I: Control – 1 % Sodium CMC (5 ml/kg p.o.), Group-II:Standard - Pentazocine (10 mg/kg body weight, i.p.), Group-III:Compound-I (20 mg/kg body weight, p.o.),Group-IV: Compound-II (20 mg/kg body weight, p.o.),Group-V:Compound-III (20 mg/kg body weight, p.o.) [91]. All the mice were screened by applying a metal artery clip to

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the base of the tail with its jaws sheated with rubber tubing. The pressure exerted by the clip was so adjusted that it was just sufficient to make all control mice respond. Those animals that did not show efforts to dislodge the clip within 15 sec were not used for the experiment. The mice showing positive response were divided into 5 groups of 6 each. The tail clip was applied 0, 30, 60, 90 and 120 min after oral administration of the Benzopyran-2-one derivatives i.e., comp-I, comp-II and comp-III at a dose of 20 mg/kg each or pentazocine (10 mg/kg). 1% Sodium CMC (5 ml/kg p.o.) was used as control. It was considered a positive analgesic response if there was no attempt to dislodge the clip within 15 sec in any of four consecutive trials.

Statistical Analysis

The results are expressed as the mean ± S.E.M. The results obtained from the present study were analyzed by using one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison tests. Data was computed for statistical analysis by using Graph Pad Prism 5.0 software and compared with the vehicle control group. P values less than 0.05 were considered statistically significant.

RESULTS

Acute Toxicity Study

The acute oral toxicity study was performed as per the OECD guidelines 423 by Following up and down method it has been found that all three Benzopyran-2-one (coumarin) derivatives i.e., comp-I, comp-II and comp-III were toxic above a dose of 200 mg/kg. Hence, 20 mg/kg dose was selected for all the neuropharmacological activities.

Anticonvulsant Activity

PTZ induced seizure model

Results of anticonvulsant activity in PTZ induced seizure model is shown in Table 1. For anticonvulsant activity diazepam (1mg/kg, p.o) was used as a standard drug which showed significant prolongation of onset of seizures to 655.3±20.49 sec and reduced the duration of seizures to 0.50±0.28 sec. Also, it showed a 100% protection from mortality when compared with the control which showed onset of seizure at 15.99±1.66 sec and duration of seizures for 18.01±0.40 sec with 100% mortality. All the test compounds were found to be statistically significant at value $P < 0.0001$ and $P < 0.001$. But anticonvulsant activity of comp-II (66.66±0.47 % mortality protection) was found to be better than comp-I and comp-III with regard to prolongation of onset of seizures, reduction of duration of seizures and % of mortality protection.

Anxiolytic Activity

Evasion test

Number of animals (mice, n=6) remaining in the box during exploratory period of 5 min after drug administration were counted. Here mean of two values were taken. Results of evasion test to test the exploratory behaviour is shown in Table 2. For Evasion test, diazepam (1 mg/kg, p.o.) was used as a standard drug which showed a significant anxiolytic effect after 30 min (no of mice remaining in box – 4.0±1.0 i.e mean of two values 4 and 4), 60 min (06±0.0 mice remaining in box – i.e. mean of 6 and 6), 90 min (06±0.0 mice remaining in box – i.e. mean of 6 and 6)) and 120 min (4.5±0.50 mice remaining in box – i.e. mean of 4 and 5)) of drug administration when compared with control (0 mice remaining in box) throughout the test period. It was found that all three derivatives i.e., comp-I, comp-II and comp-III possesses the anti-anxiety activity in mice. All the test compounds were found to be statistically significant at $P < 0.0001$ and $P < 0.001$ values. comp-II was found to be better than comp-I and comp-III.

GABA Assay

Table 3 showing the GABA levels in all the treated groups with Benzopyran-2-one

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derivatives were assayed and compared to that of standard GABA solution. GABA levels were significantly increased in the treated group as compared to that of control (Table 5). GABA levels in the groups treated with test compounds were found to be less than that of the standard GABA solution (0.1 ug/ml). GABA levels in Diazepam treated animals were near to standard GABA solution (769.23 pmol/ml). The GABA levels was found to be 307.69, 615.38, 461.53 pmol/ml in groups treated with comp-I, comp-II and comp-III respectively. Thus, GABA levels of group treated with comp-II was found to be higher than that of comp-I and comp-III.

Spontaneous Motor Activity (SMA)
Actophotometer Test

Results of SMA are shown in Table 4 for SMA diazepam (1 mg/kg, p.o) was used as a standard drug. Diazepam showed a significant decrease in locomotor activity after 30 min (37.25±2.28 counts) and continued its effect at 60 min (12.25±1.65 counts), 90 min (17.25±2.72 counts) and 120 min (22.75±2.46 counts) when compared with control at 30 min (305.3±7.95 counts), 60 min (260.0±25.46 counts), 90 min (277.5±20.87 counts) and 120 min (307.8±7.25 counts). It was found that all three Benzopyran-2-one derivatives i.e., comp-I, comp-II and comp-III decreased the spontaneous motor activity in mice and all the test compounds were found to be statistically significant with a P values less than 0.0001 ($P<0.0001$) where comp-II was found to be better than comp-I and comp-III.

Skeletal Muscle Relaxant Activity
Rotarod test

Time taken by the mice in sec to fall from the rod in 180 sec (3 min) of trial period after 60 min of drug administration. Results of rotarod test to test the skeletal muscle relaxation is shown in Table 5, for skeletal muscle relaxation diazepam (1 mg/kg, p.o) was used as a standard drug, which showed a significant relaxation of skeletal muscles after 30 min (9.858±0.58 sec required for mice to fall from rod) of drug administration when compared with the control (165.2±6.12 sec required for mice to fall from rod). It was found that all three derivatives i.e., comp-I, comp-II and comp-III possesses the skeletal muscle relaxant activity in mice, where comp-II was found to be better ($P<0.0001$) than comp-I and comp-III.

Analgesic Activity
Eddy's Hot Plate Method

The reaction time of the mice is shown in Table 6. at different time interval. In hot plate method pentazocine was used as standard drug which showed significant increase in reaction time to the heat stimulus. The values were found to be significant at 30 min (4.54±0.82 sec) 60 min (7.39±0.93 sec), 90 min (7.94±0.61 sec) and 120 min (5.30±0.46 sec) after treatment, when compared with control (1.61±0.24, 1.62±0.37, 1.66±0.27, and 1.62±0.30 sec after 30, 60, 90 and 120 min respectively). The test Benzopyran-2-one derivatives i.e., comp-I, comp- II and comp-III showed significant increase in the reaction time at 60 min and 90 min after drug administration. These entire test compounds were found to be statistically significant at value $P < 0.0001$. It was found that all three Benzopyran-2-one derivatives possesses analgesic activity where comp-II was found to be better than comp-I and comp-III.

Tail Clip Method

The reaction time of the mice is shown in Table 7 at different time interval. In tail clip method pentazocine was used as standard drug which showed significant increase in reaction time to the pain stimulus. The values were found to be significant at 60 min after treatment (9.54±0.63 sec) and 90 min (10.90±0.68 sec) when compared with control (1.51±0.14 and 1.58±0.025 sec at 60 and 90 min). These entire test compounds were

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found to be statistically significant at value P < 0.0001. It was found that all three Benzopyran-2-one derivatives possess analgesic activity where comp-II was found to be better than comp-I and comp-III.

Results (Tables and Figures)

Table 1: *Effect of Benzopyran-2-one derivatives on pentylenetetrazole (PTZ) - induced seizure in mice.*

Sl. No.	Treatment	Dose (mg/kg p.o.)	Onset of Seizures (sec)	Duration of seizure(sec)	Percentage of mortality protection
1	Control (1 % sodium CMC) + PTZ	5 ml/kg+ 90 mg/kg	15.99 ± 1.66	18.01± 0.40	0.00 ± 0.00
2	Diazepam	1	655.3 ± 20.49***	0.50 ± 0.28***	100.0 ± 0.00***
3	Compound-I	20	54.59 ± 2.96*	9.08 ± 0.65***	0.00 ± 0.00
4	Compound-II	20	46.08 ± 2.71	7.69 ± 0.47***	66.66 ± 0.47***
5	Compound-III	20	50.09 ± 3.95	8.52 ± 0.68***	0.00 ± 0.00
		P value	0.0001	0.0001	0.0001

All values are expressed as mean ± S.E.M. (n=6).
** p <0.01, ** p < 0.001 and *** p < 0.0001 as compared to control (ANOVA followed by Dunnett's test)*

Table 2: *Effect of Benzopyran-2-one derivatives on exploratory behavior in mice by Evasion test.*

Sl. No.	Treatment	Dose (mg/kg p.o)	Number of animals (n=6) remaining in the box after 5 min (mean of two values)			
			30 min	60 min	90 min	120 min
1	Control (1 % sodium CMC)	5 ml/kg	0	0	0	0
2	Diazepam	1	4.0 ± 1.0*	6.0 ± 0.0***	6.0 ± 0.0***	4.5 ± 0.50**
3	Compound-I	20	1.5 ± 0.50	3.5 ± 0.50**	2.5 ± 0.50**	1.5 ± 0.50
4	Compound-II	20	2.5 ± 0.5	4.0 ± 0.0***	6.0 ± 0.0***	2.5 ± 0.50*
5	Compound-III	20	2.0 ± 0.0	3.5 ± 0.50**	2.5 ± 0.50**	2.0 ± 0.0*
	P value		0.0272	0.0004	0.0002	0.0037

All values are expressed as mean ± S.E.M. (n=6).
** p <0.01, ** p < 0.001 and *** p < 0.0001 as compared to control (ANOVA followed by Dunnett's test)*

Table 3: *Effect of Benzopyran-2-one derivatives on serum GABA levels in mice.*

Sl.No.	Treatment	Optical Density (OD) at 509 nm	GABA levels (pmol/ml)
1	Control	0.03	214.28
2	Diazepam	0.10	769.23
3	Compound-I	0.04	307.69
4	Compound-II	0.08	615.38
5	Compound-III	0.06	461.53
6	Std. GABA	0.13	1000.00

$\frac{\text{O.D. of test}}{\text{O.D. of std}} \times 1000 = \text{pmol/ml}$[67]

Note: 1ug = 1000 pg. Hence, to convert values into picogram (pg), values must be multiplied by 1000.

O.D. of standard GABA solution (0.1ug/ml) = 0.13

Concentration of standard GABA solution = 0.1ug/ml

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GABA levels of all Benzopyran-2-one derivatives was assayed and compared with the level of standard GABA solution (0.1ug/ml)

Table 4: *Effect of Benzopyran-2-one derivatives on spontaneous motor activity in mice by Actophotometer test.*

Sl. No.	Treatment	Dose (mg/kg p.o)	0 min	30 min	60 min	90 min	120 min
1	Control (1 % sodium CMC)	5 ml/kg	306.0 ± 3.16	305.3± 7.95	260.0± 25.46	277.5 ±20.87	307.8± 7.25
2	Diazepam	1	302.8 ± 2.49	37.25 ± 2.28***	12.25 ± 1.65***	17.25 ± 2.72***	22.75 ± 2.46***
3	Compound-I	20	307.5 ± 2.90	162.3 ± 4.23***	54.25 ± 3.32***	47.75 ± 1.25***	64.50 ± 2.39***
4	Compound-II	20	310.8 ± 2.25	159.8 ± 4.55***	41.50 ± 2.21***	39.00 ± 2.48***	54.00 ± 3.48***
5	Compound-III	20	309.3 ± 3.52	160.5 ± 8.65***	49.75 ± 0.85***	43.50 ± 2.72***	59.25 ± 1.79***
	P value		0.3788	< 0. 0001	< 0.0001	< 0.0001	< 0.0001

All values are expressed as mean ± S.E.M. (n=6).
*** *p < 0.0001 as compared to control (ANOVA followed by Dunnett's test).*

Table 5: *Effect of Benzopyran-2-one derivatives on skeletal muscle relaxation in mice (Rotarod test).*

Sl.No.	Treatment	Dose (mg/kg p.o.)	Time in seconds (To fall from rotarod)
1	Control (1 % sodium CMC)	5 ml/kg	165.2 ± 6.12
2	Diazepam	1	9.858 ± 0.58***
3	Compound-I	20	143.9 ± 5.05**
4	Compound-II	20	105.8 ± 1.95***
5	Compound-III	20	121.6 ± 3.79***
	P value		< 0.0001

All values are expressed as mean ± S.E.M. (n=6).
* *p <0.01,* ** *p < 0.001 and* *** *p < 0.0001 as compared to control (ANOVA followed by Dunnett's test)*

Table 6: *Effect of Benzopyran-2-one derivatives in mice by Eddy's hot plate test.*

Sl. No.	Treatment	Dose mg/kg (p.o.)	0 min	30 min	60 min	90 min	120 min
1	Control (1 % sodium CMC)	5 ml/kg	1.570 ± 0.24	1.613 ± 0.24	1.623 ± 0.37	1.665 ± 0.27	1.620 ± 0.30
2	Pentazocine	10	1.458 ± 0.23	4.545 ± 0.82***	7.390 ± 0.93***	7.943 ± 0.61***	5.300 ± 0.46***
3	Compound-I	20	1.445 ± 0.21	1.630 ± 0.18	5.963 ± 0.64**	5.840 ± 0.55***	4.453 ± 0.49**
4	Compound-II	20	1.445 ± 0.22	1.838 ± 0.19	6.265 ± 0.59***	6.245 ± 0.56***	4.795 ± 0.54***
5	Compound-III	20	1.435 ± 0.20	1.520 ± 0.19	5.613 ± 0.66**	5.620 ± 0.54***	4.728 ± 0.33***
	P Value		0.9921	0.0004	0.0002	< 0.0001	0.0002

All values are expressed as mean ± S.E.M. (n=6).
*** *p* < 0.0001 as compared to control (ANOVA followed by Dunnett's test)

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Table 7: *Effect of Benzopyran-2-one derivatives in mice by Haffner's tail clip test.*

Sl. No.	Treatment	Dose mg/kg (p.o.)	0 min	30 min	60 min	90 min	120 min
1	Control (1 % sodium CMC)	5 ml/kg	1.423 ± 0.10	1.548 ± 0.61	1.510 ± 0.14	1.580 ± 0.25	1.568 ± 0.19
2	Pentazocine	10	1.523 ± 0.16	6.220 ± 1.26***	9.540 ± 0.63***	10.90 ± 0.68***	10.22 ± 1.10***
3	Compound-I	20	1.468 ± 0.08	1.640 ± 0.17	6.063 ± 0.58***	6.470 ± 0.87***	5.075 ± 0.43**
4	Compound-II	20	1.630 ± 0.18	2.140 ± 0.21	8.190 ± 0.39***	9.605 ± 0.18***	5.828 ± 0.26***
5	Compound-III	20	1.670 ± 0.18	1.920 ± 0.16	6.688 ± 0.29***	9.183 ± 0.41***	5.170 ± 0.29**
	P Value		0.7479	0.0002	< 0.0001	< 0.0001	< 0.0001

All values are expressed as mean ± S.E.M. (n=6).
*** *p < 0.0001 as compared to control (ANOVA followed by Dunnett's test)*

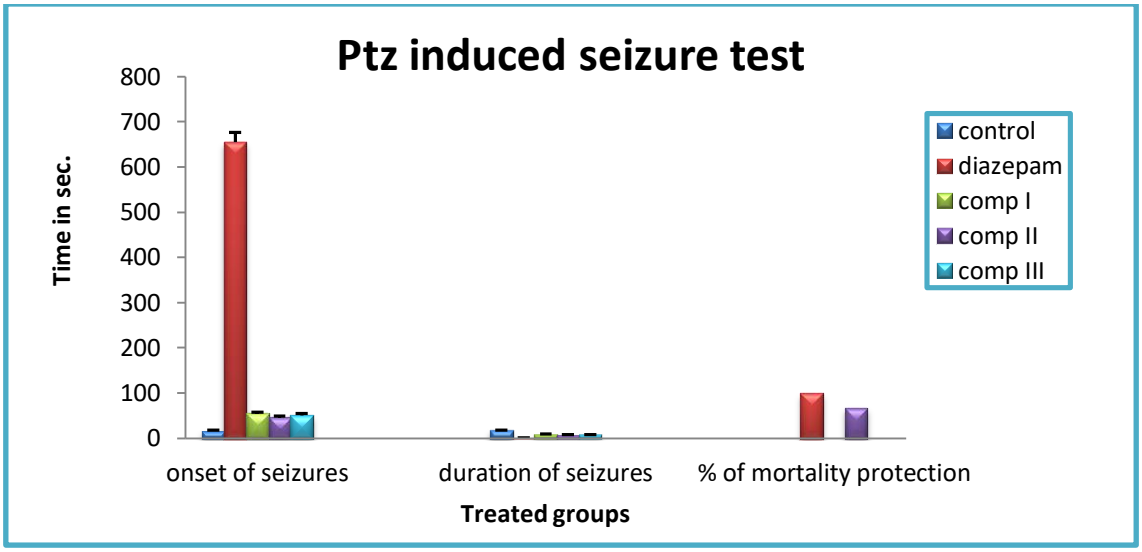

Figure 1: *Effect of Benzopyran-2-one derivatives on pentylenetetrazole (PTZ), induced seizure in mice.*

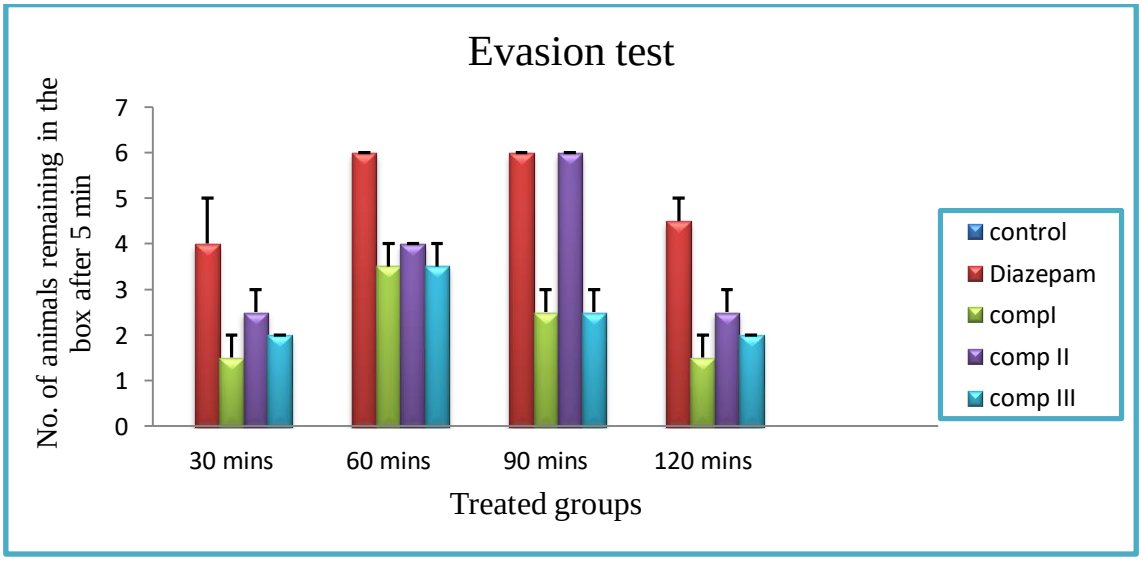

Figure 2: *Effect of Benzopyran-2-one derivatives on exploratory behavior in mice by Evasion test.*

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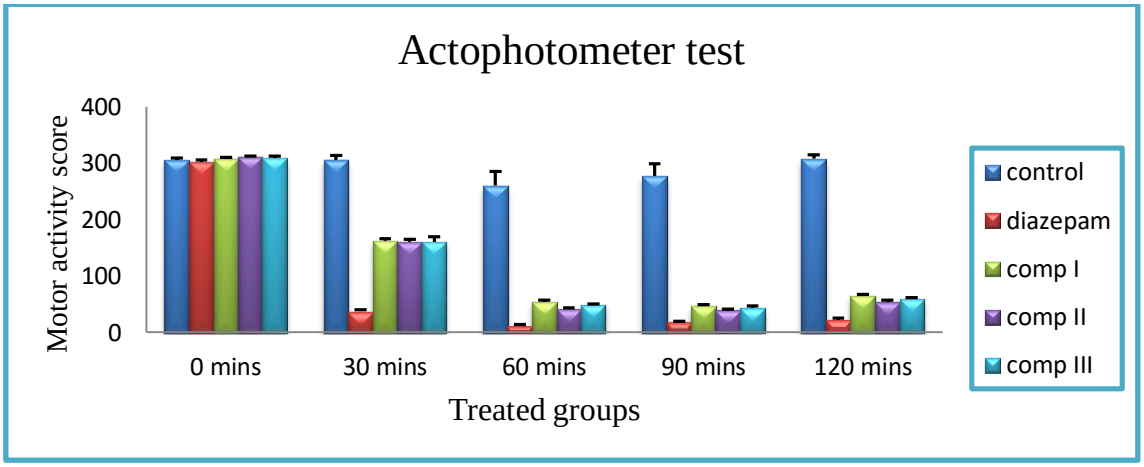

Figure 3: *Effect of Benzopyran-2-one derivatives on spontaneous motor activity in mice by Actophotometer test.*

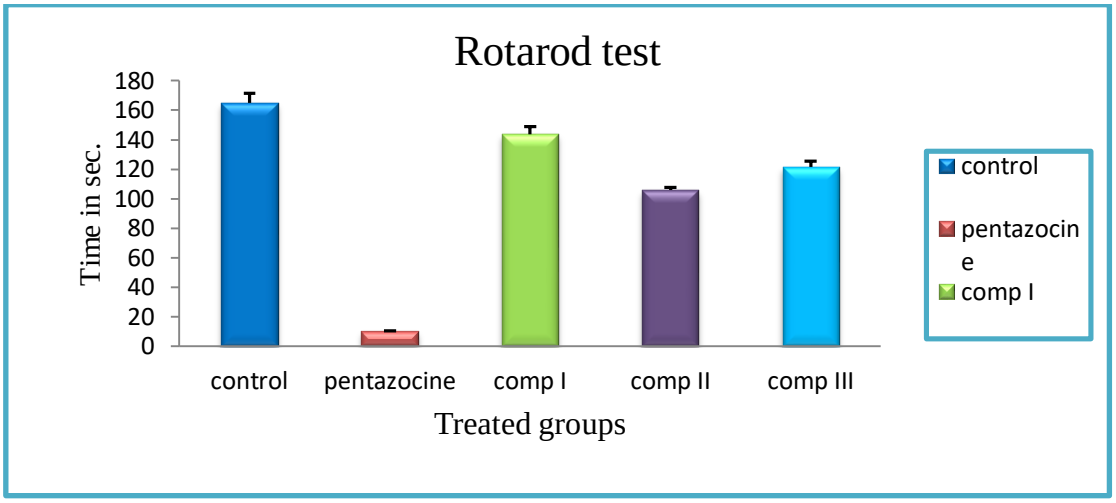

Figure 4: *Effect of Benzopyran-2-one derivatives on skeletal muscle relaxation in mice by Rotarod test.*

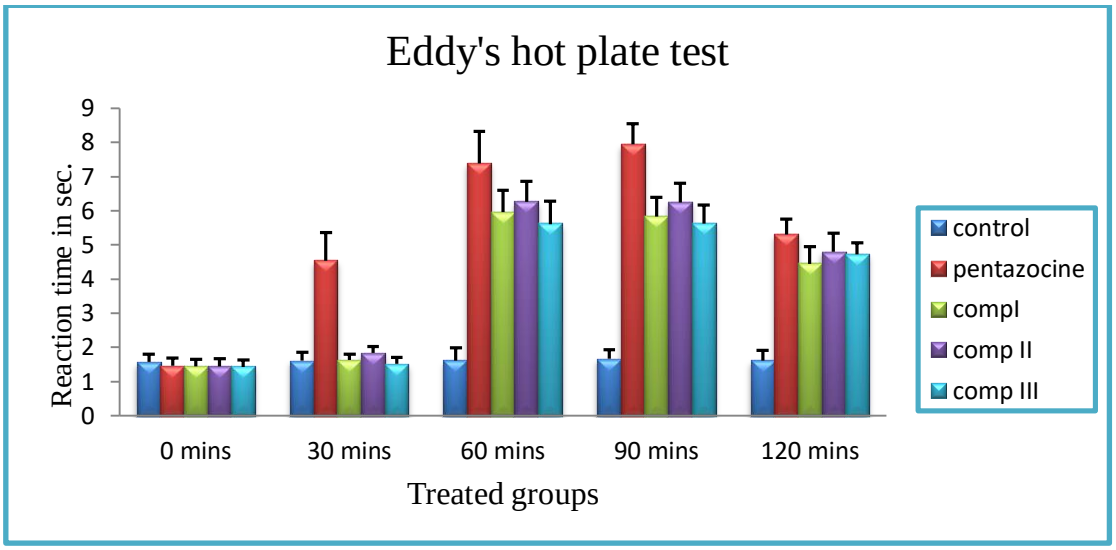

Figure 5: *Effect of Benzopyran-2-one derivatives in mice by Eddy's hot plate test.*

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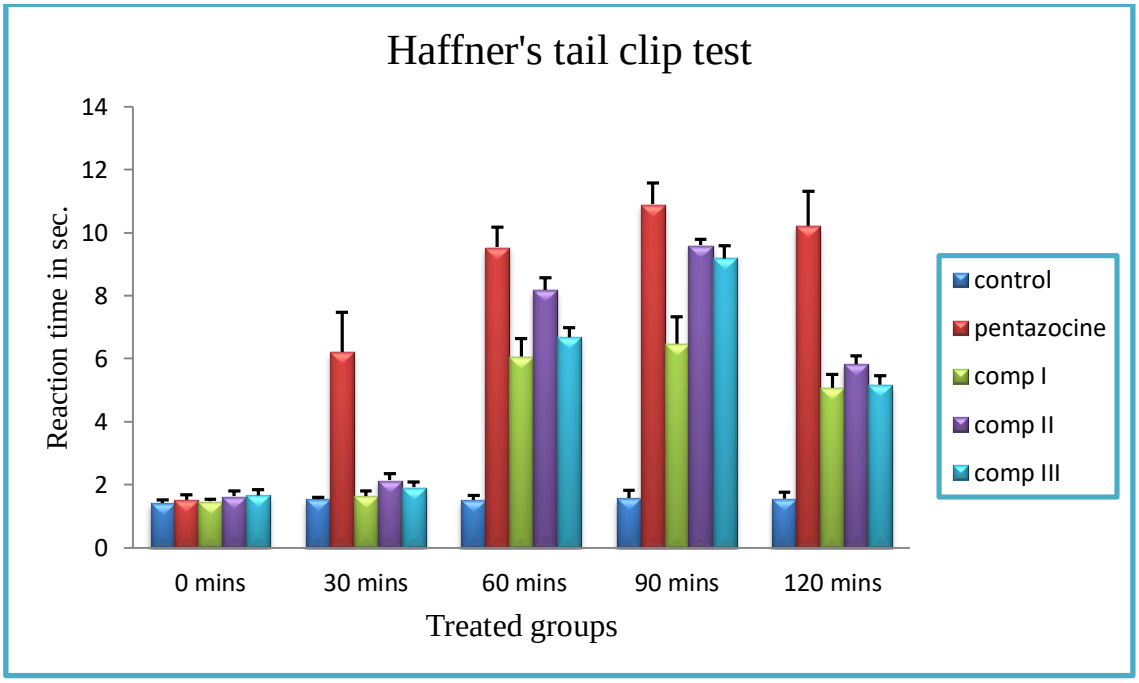

Figure 6: *Effect of Benzopyran-2-one derivatives in mice by Haffner's tail clip test.*

DISCUSSION

The present study deals with the investigation of neuropharmacological profile of newly synthesized Benzopyran-2-one derivatives. The most frequent step in evaluating drug action on the CNS is to observe the behavior of the test animals. The neuropharmacological profiles of Benzopyran-2-one derivatives (i.e., comp-I, comp-II and comp-III) were evaluated by performing the anticonvulsant activity by PTZ induced convulsion model, anxiolytic activity by evasion test, SMA by actophotometer test, motor coordination by rotarod test and analgesic activity by Eddy's hot plate method and Haffener's tail clip method.

In the present study, testing for anticonvulsant effects of Benzopyran-2-one derivatives by PTZ induced convulsant model was used. Anticonvulsant drugs those are effective against the most common forms of epileptic seizures, namely *partial* and *generalized tonic-clonic* seizures, act either by:

Reducing or limiting the sustained repetitive firing of neurons, an effect mediated by promoting or prolonging the inactivated state of voltage- activated Na^{+}-channels, thereby reducing the ability of neurons to fire at high frequencies.

Enhancing and facilitating GABA-mediated synaptic transmission and inhibition, an effect mediated either by a pre- or post- synaptic action. In presence of GABA, the $GABA_A$ receptor is opened, thus allowing an influx of Cl^- ions, which in turn increases membrane polarization.

Some anticonvulsant drugs also act by reducing the metabolism of GABA.

- Some act at $GABA_A$ receptors, enhancing Cl^- ion influx in response to GABA or by promoting GABA release.
- Agents that are effective against *absence seizure*, act by reducing or limiting the flow of Ca^{2+} through T-type of voltage- activated Ca^{2+} channels, thus reducing the pacemaker Ca^{2+} current.[92]

  Drugs that reduce PTZ induced seizures act by;
- Reducing T-type of Ca^{2+} currents.

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- Enhancing $GABA_A$ receptor mediated inhibitory neurotransmission.[93]

Results obtained from the PTZ induced seizure model clearly indicate significant prolongation in the onset of seizures and a significant reduction in the duration of seizures for all three Benzopyran-2-one derivatives used for present study, i.e., comp-I, comp-II and comp-III. Comp-II showed a significant reduction in the mortality rate of mice, whereas, comp-I and comp-III did not show any protection of mortality in mice.

In the present study, GABA levels were assayed to confirm the effect of test compounds on GABA. The GABA levels were significantly increased in the groups treated with test compounds and diazepam as compared to that of control. GABA levels in the treated groups with test compounds were found to be less than that of the standard GABA solution.

Therefore, we can conclude that the Benzopyran-2-one derivatives (i.e., comp-I, comp-II and comp-III) may have exerted anticonvulsant effects by enhancing GABAergic inhibitory neurotransmission similar to that of diazepam, which leads to opening of Cl^- ion channel and influx of Cl^- ions causing membrane hyperpolarisation or by decreasing the degradation of GABA [92]. But, the exact mechanism for increase in GABA content could not be reported in this study and remains to be explored.

Evasion test was used to test the effect of Benzopyran-2-one derivatives on exploratory behaviour. There was significant increase in the number of animals remaining in the box after treatment with Benzopyran-2-one derivatives. The results obtained from the evasion test of all three Benzopyran-2-one derivatives indicate that these derivatives have depressant action on the CNS [94]. Diazepam was used as a standard reference drug for the evasion test. A significant reduction in exploratory behaviour exemplified in the evasion test by all three Benzopyran-2-one derivatives (i.e., comp-I, comp-II and comp-III) indicate a CNS depressant action and a strong indication of anxiolytic action as produced by diazepam. Anxiolytics are known to exert pharmacological action by causing an increase in the GABA content in the cerebral hemisphere in mice [95]. Hence, we can conclude that all three Benzopyran-2-one derivatives (i.e., comp-I, comp-II and comp-III) used in present study act through BDZ-GABA receptors, i.e., test derivatives might involve an action on GABAergic transmission like that of diazepam.

Locomotor activity of the animals is one of the frequent step to evaluate the effect of drugs on CNS. SMA was assessed by using Actophotometer test. This model has been used in laboratory animals to evaluate the gross behavioral effects of the drugs. Locomotor activity is considered as an index of alertness and a decrease in locomotion reveals sedative effect [96]. The SMA is a measure of the levels of excitability of the CNS and this decrease may be closely related to sedation resulting from depression of the CNS [97]. The results obtained showed that the Benzopyran-2-one derivatives decreased the alertness and restlessness. There were neither tremors, twitches, convulsions nor strub tail response. No effects were noticed on alarm reaction, body posture, limb position, gait, righting reflex, muscle tone, pinna and corneal reflexes. From such observations, it is possible to conclude that the depressant effect of the Benzopyran-2-one derivatives on locomotor activity was probably not due to a peripheral neuromuscular blockage [98]. Diazepam was used as a standard reference drug for spontaneous motor activity. Hence, we can conclude that all three Benzopyran-2-one derivatives (i.e., comp-I, comp-II and comp-III) act like that of diazepam which is one of CNS depressant agent.

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From the results by Rotarod test, it is clear that all three Benzopyran-2-one derivatives (i.e., comp-I, comp-II and comp-III) used in present study showed significant relaxation of skeletal muscles as like that of diazepam. Hence, we can conclude that all the three Benzopyran-2-one derivatives exert their relaxant effect on skeletal muscle by acting through a centrally mediated GABA inhibition of polysynaptic reflexes to skeletal muscles [95].

The analgesic activity of Benzopyran-2-one derivatives (i.e., comp-I, comp-II and comp-III) was carried out by using Eddy's hot plate and Haffner's tail clip methods. The hot plate method was selected to investigate central analgesic activity, because it had several advantages, particularly the sensitivity to strong anti-nociceptives and limited tissue damage [99]. Pentazocine was used as a standard reference drug in hot plate method which acts through opoid receptors and inhibit the neurogenic pain releasing substance [90]. The results of hot plate method showed siginificant increase in the reaction time to heat stimulus in same manner as like that of pentazocine. Hence, we can conclude that all the three Benzopyran-2-one derivatives (comp-I, comp-II and comp-III) showed analgesic activity by in similar way to that of pentazocine. Haffener's tail clip method is simple and has the advantage that the reflex mechanism on which it is based involves the higher centres. The animal has to identify exactly the place where the noxious stimulus is applied and it carries out coordinated movements to remove it [100]. In Haffner's tail clip method, animals significantly prolonged the latency to dislodge the clip from their tail in treated group when compared with control group. Centrally acting analgesic drugs elevate pain threshold of animals towards heat and pressure [101]. All the three Benzopyran-2-one derivatives (i.e., comp-I, comp-II and comp-III) showed significant analgesic effect on mechanical induced pains. Hence, the results from both analgesic tests showed a clear indication of a very potent analgesic activity against different pain stimuli explored.

CONCLUSION

From the obtained results of the present study, we can conclude that the newly synthesized Benzopyran-2-one derivatives, i.e., 7-(2-(m-nitro)-phenyl thiazolidinyl)-4-methyl benzopyran-2-one (comp-I), 7-(2-(o,p-dichloro)-phenyl thiazolidinyl)-4-methyl-benzopyran-2-one (comp-II) and 7-(2-(p-methyl)-phenyl thiazolidinyl)-4-methyl-benzopyran-2-one (comp-III), possess neuropharmacological properties which includes CNS depressant activity by SMA, skeletal muscle relaxant activity, analgesic activity and anticonvulsant activity. Elevation of GABA levels in treated groups with Benzopyran-2-one derivatives further supported the CNS depressant activity. The overall effect of comp II was found to be better than that of comp I and comp III. Further evaluation of detail mechanism pathway involved in all the neuropharmacological profiles needs to be investigated.

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