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Research Article

EFFECT OF A NOVEL 5-HT$_3$ RECEPTOR ANTAGONIST 3-METHOXY-*N*-*P*-TOLYLQUINOXALIN-2-CARBOXAMIDE (QCM-4) ON THE ACUTE AND CHRONIC RODENT MODELS OF DEPRESSION

YESHWANT VIJAY KURHE[1]*, MAHESH RADHAKRISHNAN[1], DEEPALI GUPTA[1], DEVODOSS THANGARAJ[1]

[1]Department of Pharmacy, Birla Institute of Technology and Science, Pilani, Rajasthan 333031, India. Email: yashkurhe@gmail.com

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ABSTRACT

Objective: 5-HT$_3$ receptor the only type of ion channel among the family of serotonergic receptors which is recognized as a potential novel therapeutic target for depression, anxiety and cognition. In the present work was designed to evaluate the antidepressant-like effect of a novel 5-HT$_3$ antagonist 3-methoxy-*N*-*p*-tolylquinoxalin-2-carboxamide (QCM-4) in a battery of behavioral acute and chronic rodent models of depression.

Methods: Behavioral models with high predictive validities like forced swim test (FST) and tail suspension test (TST) in mice along with mechanistic models including 5-hydroxytryptamine (5-HTP) induced head twich response in mice and reserpine induced hypothermia in rats were used for screening QCM-4. Chronic surgical model of olfactory balbectomy (OBX) was performed and further evaluated by sucrose preference test and open field test (OFT) for anti-depressant like effect of QCM-4.

Results: Following were the principle findings in the present research; Firstly, QCM-4 dose dependently reduced the immobility duration in FST and TST. Secondly, in the mechanistic models, QCM-4 dose dependently potentiated 5-HTP induced head twich response and attenuated the reserpine induced hypothermia. Lastely, in the surgical OBX model, QCM-4 reversed the anhedonia and behavioral alterations in animals which was evaluated by increase in the sucrose consumption and reduction in ambulation, fecal contents as well as number of rearings in OFT by QCM-4 treated OBX animals compared to OBX control.

Conclusion: The results of the present study, indicates that QCM-4, a novel 5-HT$_3$ receptor antagonist exhibit an anti-depressant like effect. Our further studied will be focused on the chronic unpredictable mild stress induced alterations and molecular mechanism involved in the anti-depressant like action of QCM-4.

Keywords: Depression, 5-HT$_3$ antagonist, Immobility duration, Hypothermia, Head twich,OBX.

INTRODUCTION

Depression is one of the most common psychiatric disorders that affect around 20% of the world population is recognized as a serious public health problem throughout the world [1,2] and according to World Health Organisation (WHO) it is one of the top 10 cause of morbidity and mortality. Even after vast research in this field, the therapeutic remedy remains unsatisfactory [3]. This is because the only 65% of the depressive patient responds to the current anti-depressant therapeutics [4] and to achieve clinical benefit it takes several weeks which could be a reason to worry as depression tends to increase the risk of suicide in advanced stage [5]. Hence, to undertake the research in the area of depression with new targets having quick onset of action is an immense need in the field of neuro-psychopharmacology.

The currently available anti-depressant agents produce their effect by alterations in the monoamine system [6,7]. Serotonin plays an important role in the pathogenesis of the affective disorders [8]. Serotonin as a neurotransmitter acts through different receptors (5-HT$_{1-7}$) which are metabotropic class of receptors except 5-HT$_3$ which belongs to an inotropic receptor family [9]. 5-HT$_3$ antagonists are presently used in the management of chemotherapy associated nausea and vomiting [10]. In the last decade, 5-HT$_3$ antagonist have been extensively evaluated for the neuro-psychopharmacological potentials in various pre-clinical and some clinical studies [11]. Literature survey reveals some pre-clinical studies showing the links between 5-HT$_3$ receptor and depression. Earlier it is reported that 5-HT$_3$ receptor antagonists reverse the escape deficits in rat learned helplessness, one of the screening methods for anti-depressants [12]. 5-HT$_3$ receptor antagonists have been reported for anti-depressant like effect and it also potentiates the anti-depressant activity of selective serotonin reuptake inhibitors (SSRIs) [13,14]. SSRIs a class of antidepressant agents acts as a functional antagonist of 5-HT$_3$ receptors for their anti-depressant effect [15-17]. Ondansetron have been reported for reducing the fatigue clinically which is one of the possible sign of depression [18].

Based on the structures of available anti-depressants, carboxamide derivatives are found to be most potent anti-depressants. One of the carboxamide derivative 7-chloro-5, 11-dihydrodibenz [b,e][1,4]oxazepine-5-carboxamide has been found to be a potential tricyclic anti-depressant agents [19]. Also, N-substituted imidazole-5-carboxamides [20] and benzo [b] thiophene carboxamides connected to 4-aryl piperazines through a benzylic spacer [21] have been reported for potential anti-depressant activity.

Hence, on the basis of evidence provided by the above literature and from a series of structurally novel compounds 3-methoxy-*N*-*p*-tolylquinoxalin-2-carboxamide (QCM-4) was selected for its anti-depressant potential on the basis of its 5-HT$_3$ antagonist potential performed on guinea pig ileum against 2-methyl 5-hydroxytryptamine (5-HT agonist). QCM-4 showed a high log P value (2.84) and pA$_2$ value (7.3) which is slightly higher than ondansetron (6.9). The present work was designed to investigate the anti-depressant like activity of a novel carboxamide derivative QCM-4 using high predictive and validated models of depression including FST, TST, 5-HTP induced head twitch response in mice, reserpine induced hypothermia and OBX in rats.

MATERIALS AND METHODS

Animals

Male Swiss albino mice (20-25 g) and Wistar rats (170-200 g) were procured from Hisar Agricultural University, Hisar, India (Reg. No. 417/01/a/ CPCSEA). The animals were housed under standard laboratory conditions and maintained on natural light and dark cycle and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. Each group consisted of 6 animals. All the experiments were carried out between 0900 and 1500h. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) of Birla Institute of Technology and Science, Pilani, India (Protocol No. IAEC/RES/16/06).

Drugs and chemicals

QCM-4 was synthesized by medicinal chemistry group of the Institute. Escitalopram (EST), Bupropion (BUP) was procured from Ranbaxy Research Laboratory (India) as a generous gift samples. Vehicle used for formulation was distilled water. The test compound QCM-4 as well as standards EST and BUP were prepared freshly in distilled water on the day of experiment and injected in intra-peritoneal cavity (i.p.) at a dose volume of 10 ml/kg for mice and 2 ml/kg for rats.

Chemistry of QCM-4

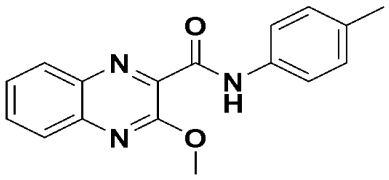

Fig.1: Structure of QCM-4

The compound QCM-4 (fig.1) was synthesized from condensation of the starting material *o*-phenylenediamine (1) with diethyl ketomalonate afforded the ethyl 3, 4-dihydro-3-oxoquinoxalin-2-carboxylate (2), which on treatment with phosphorous oxychloride in presence of catalytic amount of dimethylformamide provided the chloro ester compound (3). The chloro ester compound (3) on hydrolysis using sodium carbonate gave sodium salt of carboxylic acid, which on acidification yielded the 3-chloroquinoxalin-2-carboxylic acid (4).

The key intermediate, 3-methoxyquinoxalin-2-carboxylic acid (5) was synthesized from the intermediate 3-chloroquinoxalin-2-carboxylic acid (4) by nucleophilic displacement with excess amount of sodium methoxide by irradiation with microwaves (fig.2). The target 3-methoxy-*N*-*p*-tolylquinoxalin-2-carboxamide (QCM-4) was synthesized by coupling the 3-methoxyquinoxalin-2-carboxylic acid with *p*-toluidine in the presence of coupling agents EDC·HCl and HOBt under inert atmosphere, (nitrogen gas).

Yield: 80%; mp: 124-126 °C; FT-IR (KBr, cm^{-1}): 3305 (sharp N-H str.), 3039, 3005, 2916, 2860 (C-H str.), 1666 (C=O str.), 1598, 1514 (C=C, C=N ring str.), 1442 (CH_2 bend), 1242, 1220, 1128, 1089 (C-O str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.63 (s, 1H, amide), 8.07 (d, 1H, quinoxaline), 7.89 (d, 1H, quinoxaline), 7.79–7.75 (m, 1H, quinoxaline), 7.69 (d, 2H, phenyl), 7.65–7.61 (m, 1H, quinoxaline), 7.19 (d, 2H, phenyl), 4.23 (s, 3H, OCH_3), 2.35 (s, 3H, CH_3).

Scheme 1

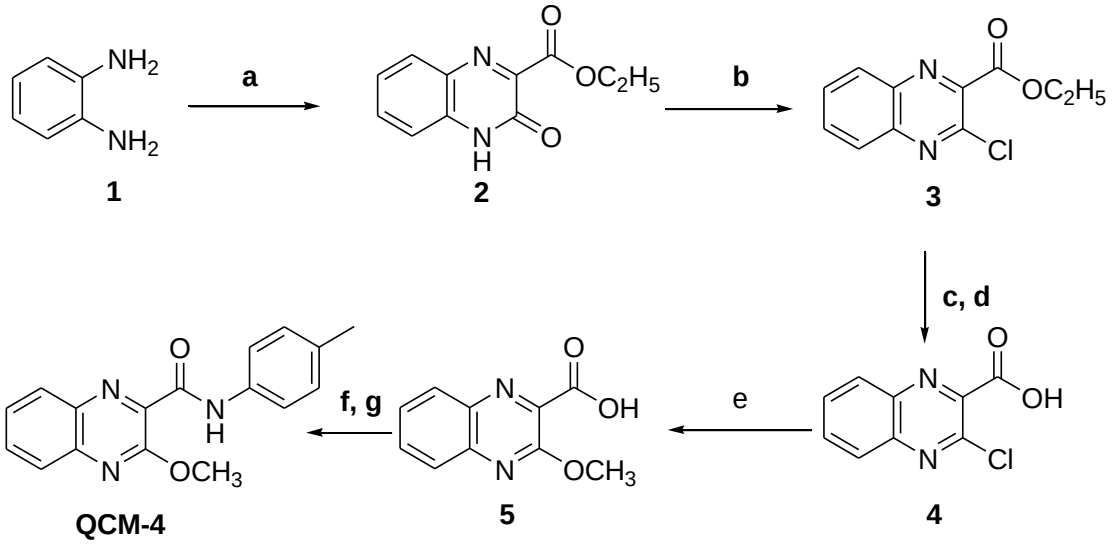

Fig. 2: Synthetic Scheme of QCM-4 [Reagents and conditions: (a) Diethyl ketomalonate, ethanol, reflux, 6 h, 60%; (b) $POCl_3$, DMF, reflux, 30 min 80%; (c) Na_2CO_3, reflux, 6 h (d) dil. HCl, 94%; (e) $NaOCH_3$, Methanol, MWI (420 Watt), 6 min, dil. HCl, 85%; (f) EDC·HCl, HOBt, THF, N_2, 0°C–rt, 1h; (g) toludine, rt, 6h, 80%]

Behavioral assay

Measurement of locomotor activity

The locomotor activity score was measured to rule out the interference of change in locomotor score in the parameters of depression. The locomotor activity was recorded using actophotometer

(INCO, India) consisting a test period of 10 min which includes initial 2 min of acclimatization followed by recording of locomotor score for 8 min. Mice were injected with QCM-4 (0.25, 0.5, 1, 2 and 4 mg/kg i.p.) 30 min before measuring locomotor score [22].

Forced swim test (FST)

The animals were individually forced to swim in a 25×12×25-cm (L × B × H) filled with water (23±2°C) up to a height of 15 cm. Animals were trained for swimming 24 hrs before commencement of test for 15 min. After the initial 2 min of vigorous activity, the animals were observed for immobility for next 4 min, 30 min post treatment of QCM-4 (0.5, 1 and 2 mg/kg i.p.) or EST (10 mg/kg i.p.) or vehicle (10 ml/kg i.p.). An animal is considered to be immobile whenever it remained floating passively in the water in a slightly hunched but upright position, with nose above the water surface [23,24].

Tail suspension test (TST)

Mice were individually suspended by the tail from a horizontal bar by a distance of 50 cm from floor using an adhesive tape (distance from tip of the tail = 2 cm). In a 6 min of test session the duration for which animal remains immobile was recorded. The mice were injected with QCM-4 (0.5, 1 and 2 mg/kg i.p.) or BUP (20 mg/kg i.p.) or vehicle (10 ml/kg i.p.) 30 min prior to reading. An animal is considered immobile when it remained passive, completely motionless and did not exhibit any body movement [25,26].

5-HTP induced head twitch response

The method mentioned elsewhere [27] was adopted with slight modifications [28]. Briefly, the mice were administered with a monoamine oxidase inhibitor, pargyline (75 mg/kg i.p.) 30 min prior

to 5-HTP (5 mg/kg i.p.) injection. QCM-4 (1 and 2 mg/kg i.p.) or EST (10 mg/kg i.p.) or vehicle (10 ml/kg i.p.) was injected 15 minutes prior to 5-HTP injection. Fifteen minutes after 5-HTP administration, the number of head twitches exhibited by the mice during the next 30 min was recorded as head twitch score.

Reserpine induced hypothermia (RIH)

RIH was performed in rats with slight modifications [28] from the originally described method by Costa E *et al*., 1960 [29]. After 30 min of treatment with QCM-4 (1 and 2 mg/kg i.p.) or EST (10 mg/kg i.p.) or vehicle (10 ml/kg i.p.) the rats were injected with reserpine (1 mg/kg i.p.). The animals were gently hand-restrained, while inserting the probe rectally. The effect of QCM-4 on reserpine induced hypothermia was recorded using digital thermometer by measuring rectal temperature at 0, 30, 60, 90 and 120 min following reserpine administration. Hypothermia was measured by calculating temperature difference between 0 and 120th min.

Olfactory bulbectomy surgery (OBX)

The rats were bulbectomized at the age of 7-8 weeks. Bilateral olfactory bulbectomy was performed according to the previously described method [30-32]. Briefly, rats were anaesthetized with the cocktail of xylazine and ketamine (5 and 75 mg/kg i.p. respectively). The animals were fixed in a stereotactic frame (INCO, India), and the skull was exposed by a midline incision. The burr holes (2 mm in diameter) were drilled 8 mm anterior to bregma and 2 mm on either side of the midline at a point corresponding to the posterior margin of the orbit of an eye. The olfactory bulbs were removed by suction, the holes were then filled with haemostatic sponge to control excessive bleeding and the scalp was sutured. Sham-operated rats were subjected to the same surgical procedure, including piercing of the dura mater, but their bulbs were left intact. Following a rehabilitation period of 14 days, olfactory bulbectomized /sham operated rats were treated orally with QCM-4 (1 and 2 mg/kg) or EST (10 mg/kg) or the vehicle (2 ml/kg) once daily between 09:00-11.00 a.m. for 14 days from 15th onwards.

Sucrose preference test

Sucrose preference test was carried for OBX /sham operated rats after 14 days of post surgery treatment with QCM-4 or EST or vehicle. The test was performed as described previously [33,34] with minor modifications. Briefly, before the test, mice were trained to adapt to sucrose solution (1%, w/v): two bottles of sucrose solution were placed in each cage for 24 h and then one bottle of sucrose solution was replaced with water for 24 h. After the adaptation, mice were deprived of water and food for 24 h. Sucrose preference test was conducted at 9:30 a.m. in which mice were housed in individual cages and were free to access to two bottles containing 100 ml of sucrose solution (1% w/v) and 100 ml of water, respectively. After 24 h, the volumes of consumed sucrose solution and water were recorded and the sucrose preference was calculated by the following formula as described in following equation.

$$\% \text{ Sucrose preference} = \frac{\text{Sucrose consumption (ml)}}{\text{Water + Sucrose consumption (ml)}} \times 100 \qquad (1)$$

Modified open field test (OFT)

The OBX / sham operated rats were subjected to an open field test after sucrose preference test according to the method reported earlier [13, 35-37]. The apparatus consisted of a circular (diameter: 90 cm) arena with 75 cm high aluminum walls and floor equally divided into 10 cm squares. A 60 W light bulb was positioned 90 cm above the base of the arena, which was the only source of illumination in the testing room. On the day of experiment, each animal was individually placed in the center of the open field apparatus and the ambulation scores (number of squares crossed), rearing and fecal pellets were counted for 5 min.

Statistical analysis

One specific group of animal was assigned to one specific drug treatment condition and each group comprised of six (n=6) animals. All the values are expressed as mean ± S.E.M. The data were analyzed by one-way ANOVA followed by Dunnett's test using GraphPad InStat 3. P<0.05 was considered as statistically significant.

RESULTS

Effect of QCM-4 on locomotor activity

The locomotor activity was assessed by using the actophotometer. Except at 4 mg/kg there was no significant change observed in the locomotor activity of mice compared to vehicle control group (Fig.3).

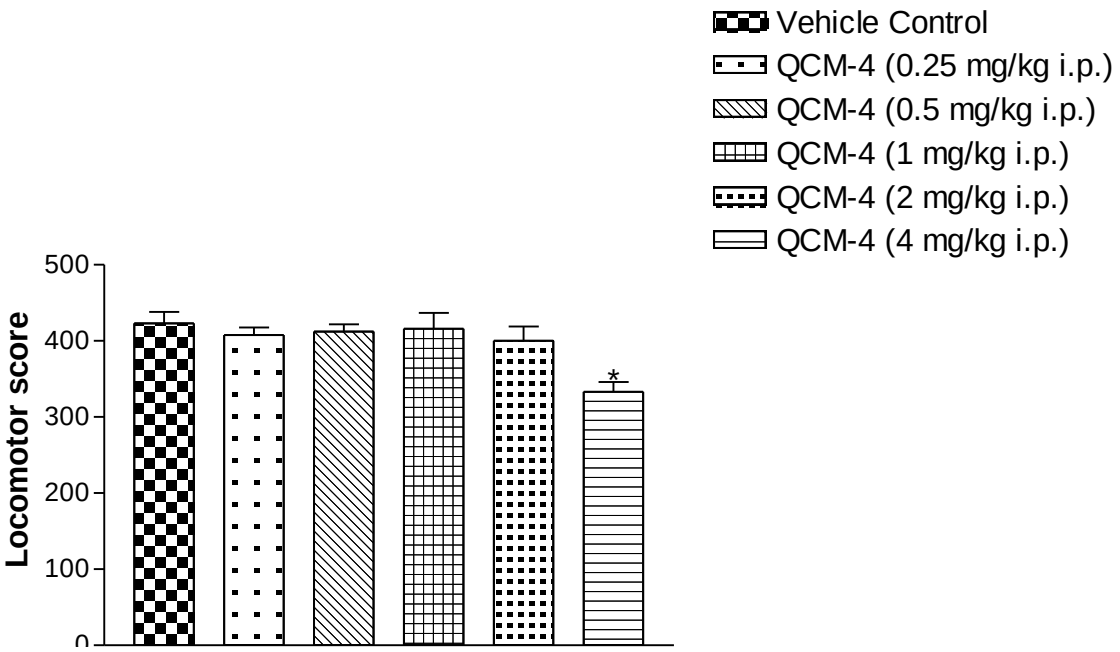

Fig. 3: Effect of QCM-4 on the locomotor activity score in mice. All the values are expressed as mean ± S.E.M., *P<0.05 as compared with vehicle control group to QCM-4 treated group. n = 6/group.

Effect of QCM-4 on immobility time in forced swim test (FST)

Acute administration of QCM-4 (0.5, 1 and 2 mg/kg i.p.) significantly reduced the immobility time as compared to vehicle control mice. However, no significant effect was observed at 0.25 mg/kg dose of QCM-4 (Fig.4).

Effect of QCM-4 on immobility time in tail suspension test (TST)

Acute administration of QCM-4 (0.5, 1 and 2 mg/kg i.p.) significantly reduced the immobility time as compared to vehicle control mice. However, no significant effect was observed at 0.25 mg/kg dose of QCM-4 (Fig.5).

Effect of QCM-4 on 5-HTP induced head twitches in mice

The administration of pargyline (75 mg/kg i.p.) and 5-HTP (5 mg/kg i.p.) induced the characteristic of head twitch response. QCM-4 (1 and 2 mg/kg i.p.) significantly potentiated the head twitch response as compared to vehicle control group (pargyline + 5-HTP) (Fig. 6).

Effect of QCM-4 on reserpine induced hypothermia in rats (RIH)

Administration of reserpine (1 mg/kg i.p.) elicited a pronounced decrease in core body temperature of rats of vehicle control group. This effect was significantly attenuated by QCM-4 (1 and 2 mg/kg i.p.) (Fig.7).

Effect of QCM-4 on sucrose preference test

14 days post surgery, significantly reduced the percentage of sucrose consumption in the OBX control as compared to Sham control animals. While treatment with QCM-4 (1 and 2 mg/kg p.o.) significantly increased the percentage of sucrose preference in OBX animals as compared to the OBX control animals. Sham treated animals with QCM-4 (1 and 2 mg/kg p.o.) did not exhibit significant change in sucrose preference as compared to sham control animals (Fig.8).

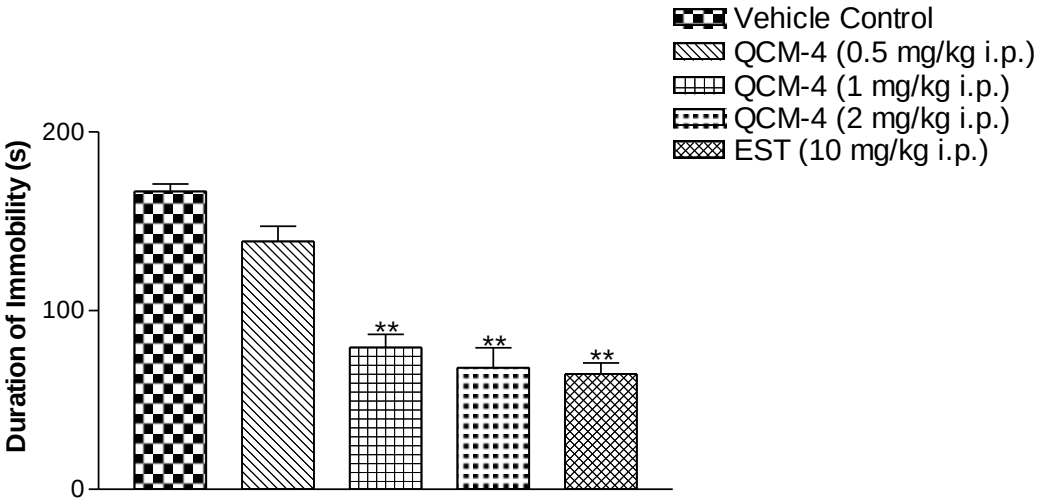

Fig. 4: Effect of QCM-4 on the immobility time in FST in mice. All the values are expressed as mean ± S.E.M., **P<0.01 as compared with vehicle control group to QCM-4 treated group. n = 6/group.

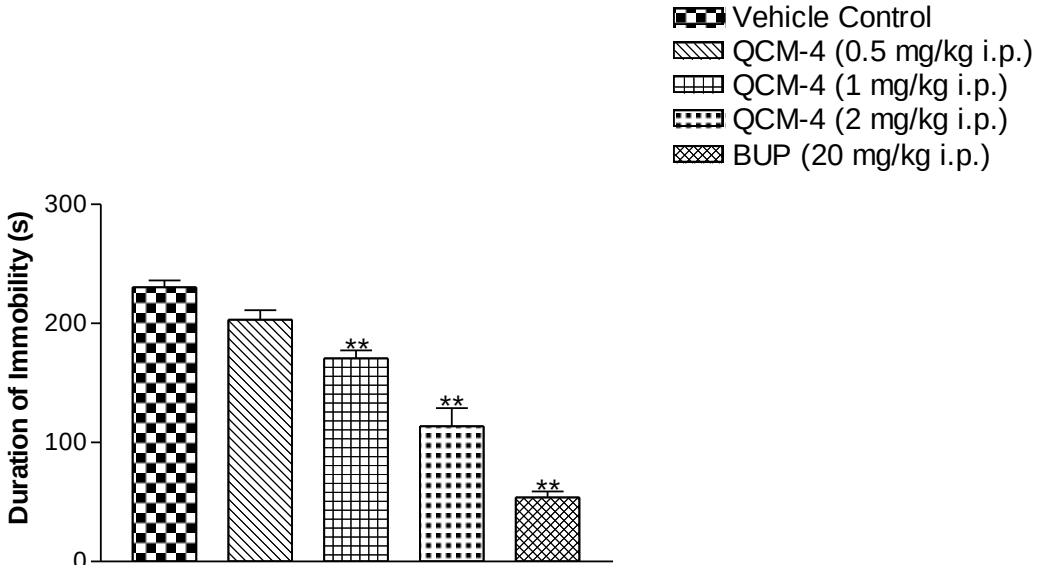

Fig. 5: Effect of QCM-4 on the immobility time in TST in mice. All the values are expressed as mean ± S.E.M., **P<0.01 as compared with vehicle control group to QCM-4 treated group. n = 6/group.

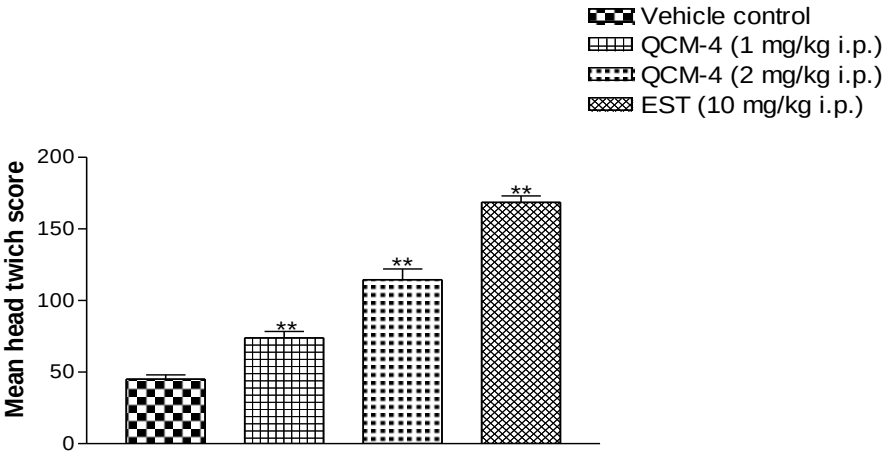

Fig. 6: Effect of QCM-4 treatment on the 5-HTP induced head twitches in mice. All the values are expressed as mean ± S.E.M., **P<0.01 as compared with vehicle control group to QCM-4 treated group. n = 6/group.

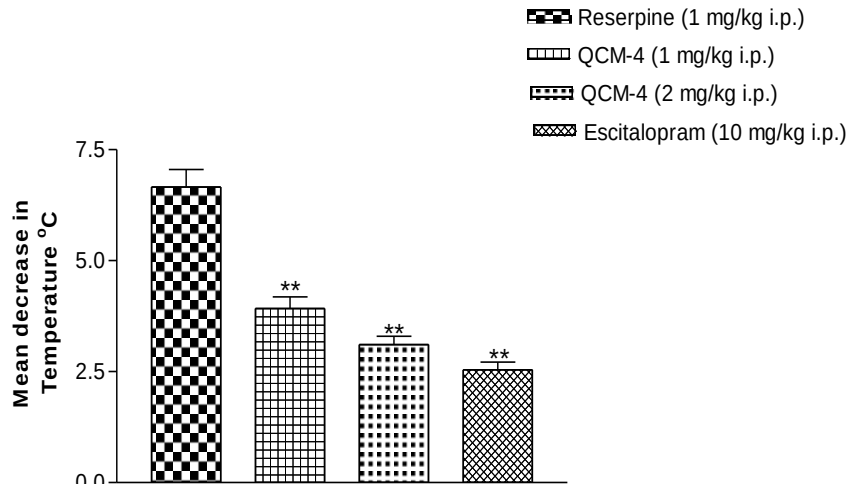

Fig. 7: Effect of QCM-4 on reserpine induced hypothermia in rats. All the values are expressed as mean ± S.E.M., **P<0.01 as compared with vehicle control group to QCM-4 treated group. n = 6/group.

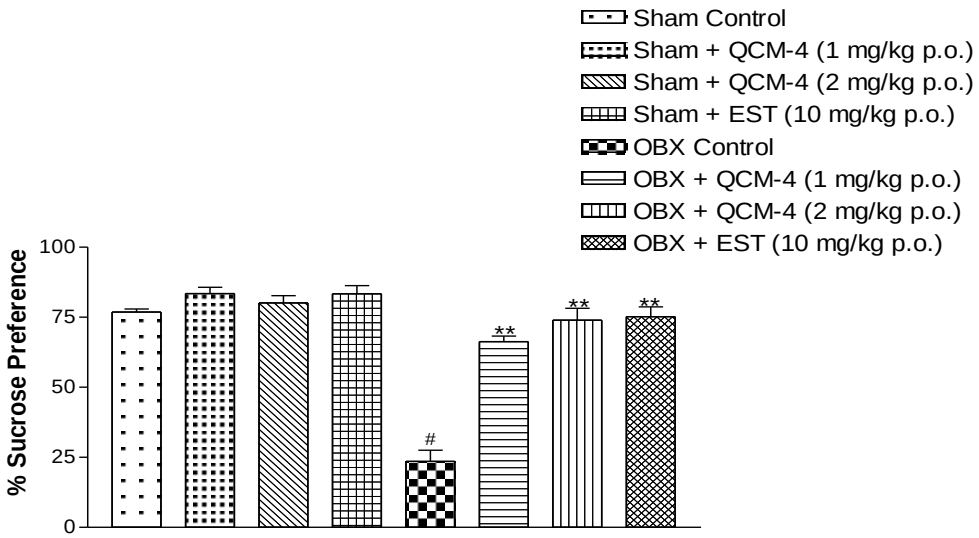

Fig. 8: Effect of QCM-4 on sucrose preference test in olfactory balbectomy induced depression in rats. All the values are expressed as mean ± S.E.M., #P<0.01 as compared with sham control group to OBX control group, **P<0.01 as compared with OBX control group to QCM-4 treated OBX animals, n = 6/group.

Effect of QCM-4 on modified Open field test (OFT)

The effects of QCM-4 treatment for 14 days of post surgery on the behavior of OBX /Sham operated rats were analyzed in the modified open field paradigm. Removal of the olfactory bulbs produced a characteristic hyperactivity in the OBX rats when compared to Sham rats in the modified open field test. Treatment with QCM-4 (1 and 2 mg/kg p.o.) significantly reduced the ambulation, rearing and fecal pellets (Table 1) in OBX rats as compared to the vehicle control OBX rats. Sham treated animals with QCM-4 (1 and 2 mg/kg p.o.) did not exhibit any significant changes in behavioral parameters as compared to Sham control animals.

Table 1: Effect of QCM-4 on open field test (OFT) in olfactory balbectomy induced depression in rats.

Groups	No. of ambulation	No. of fecal pellets	No. of rearings
Sham Control	98.33 ± 2.89	2.50 ± 0.43	12.67 ± 0.76
Sham + QCM 4 (1 mg/kg p.o.)	100.83 ± 2.61	2.33 ± 0.49	9.83 ± 0.48
Sham + QCM 4 (2 mg/kg p.o.)	107.00 ± 4.72	2.00 ± 0.26	8.67 ± 0.76
Sham + EST (10 mg/kg p.o.)	109.83 ± 4.56	1.83 ± 0.31	8.33 ± 0.67
OBX Control	227.83 ± 3.86#	6.50 ± 0.43#	35.50 ± 2.51#
OBX + QCM-4 (1 mg/kg p.o.)	156.33 ± 4.08 **	3.83 ± 0.48 **	26.67 ± 1.82 **
OBX + QCM-4 (2 mg/kg p.o.)	125.00 ± 3.06 **	3.17 ± 0.48 **	17.17 ± 1.58 **
OBX + EST (10 mg/kg p.o.)	113.50 ± 3.39 **	2.83 ± 0.48 **	15.50 ± 1.73 **

All the values are expressed as mean ± S.E.M., #P<0.01 as compared with sham control group to OBX control group, **P<0.01 as compared with OBX control group to QCM-4 treated OBX animals, n = 6/group.

DISSCUSSION

Depression is a significant contributor to the global burden of disease and affects people in all communities across the world. Presently, depression is estimated to affect 350 million people worldwide. Hence, to addresses such serious concerned of public health and burden of society it is time to come up with new therapeutics with quick onset of action and fewer side effects than existing class of treatment. Hence, moving our research in the field of depression, the present attempt deals with a novel 5-HT_3 antagonist QCM-4 as a potential approach against depression.

In order to eliminate the effect of QCM-4 on basal locomotor activity of mice, a dose dependent response was measured by using actophotometer as immobility is the crucial parameter of depression in the pre-clinical models [38]. We found no significant change in locomotor score indicating no interference of QCM-4 with basal locomotor score. QCM-4 dose dependently reduced the immobility time in FST and TST models of depression having high predictive validity [39,40]. FST and TST both models suggests a link between the action of monoamines and depression [41,42].

Furthermore, serotonergic system is considered to be one of the most important monoamine involved in depression. To revel this we dealed with a mechanistic model of depression which includes 5-HTP induced head twitches and reserpine induced hypothermia. 5-HTP induced head twitches was potentiated significantly by QCM-4 showing the mechanism pathway through increasing the synaptic levels of serotonin against depression [24]. Reserpine blocks the transport of monoamines into the synaptic vesicles by depleting monoamines. Depletion of serotonergic transmission in brain is characterized by reduced temperature and ptosis [43]. Antidepressants antagonize the property of reserpine to reduce the temperature against depression [43]. QCM-4 significantly blocked the hypothermic effect of reserpine and showed antidepressant like action.

Sucrose preference is regarded as one of the key symptom of depression leading to anhedonia indicating loss of interest or pleasure [44,45]. Though the exact mechanism for this is not clear, it is thought to be because of damage to serotonergic and dopaminergic systems leading to depression like symptom of inability to experience the pleasure or happiness [44,46]. The reduction in sucrose consumption in OBX animals was reversed by QCM-4 showing anti-depressant activity.

OBX model is one of most reliable model for assaying antidepressant property of a new chemical entity as it is mostly based on lowering the levels of serotonin [47,48]. OBX animals develop a specific abnormal features characterized by increase in ambulation, rearing and fecal contents in OFT [32,36] and potential antidepressant have ability to reverse this hyperactive behavior in OBX model [37]. QCM-4 reversed the hyperactive behavior in OBX animals by reducing the ambulation, rearing and fecal content in OFT model.

The present neuro-behavioral investigation suggests an anti-depressant like activity of a novel 5-HT_3 receptor antagonist QCM-4 in rodent models of depression. The mechanism for anti-depressant like effect is not exactly clear. Insights from the mechanistic models targeting serotonergic system particularly, including antagonism of 5-HTP induced head twitches in mice and reserpine induced hypothermia in rats predicts that QCM-4 acts by elevating the serotonergic neurotransmission for anti-depressant like effect [49]. An increased availability of serotonin to act through other serotonergic receptors mainly 5-HT_1 and 5-HT_2 through blockage of the post synaptic 5-HT_3 receptor could possibly explain the anti-depressant like activity of QCM-4 [49,50].

CONCLUSION

From the results obtained in the present study we say that the novel 5-HT_3 receptor antagonist QCM-4 has potential anti-depressant like effect which reversed the behavioral alterations in the acute and chronic pre-clinical model of depression. Hence, QCM-4 could be a potential therapeutic candidate for depression. Our further studies will be focused on the role of QCM-4 in chronic stress induced behavioral and biochemical alterations in rodent and on the molecular mechanisms of QCM-4 for the anti-depressant like effect.

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REFERENCES

1. Nestler EJ, Barrot M, Dileone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. Neuron 2002; 34: 13-25.
2. Farida A, Singh PP, Manoj, Anil K, Gaur RK. Drug prescribing for major depressive psychosis patients in geriatric clinic of a teaching hospital in northern India. Indian J Pharm Sci 2013; 5: 269-272.
3. Berton O, Nestler EJ. New approaches to antidepressant drug discovery: beyond monoamines. Nature Reviews Neuroscience 2006; 7: 137-151.
4. Duman RS. Depression: A case of neuronal life and death? Biol Psychiatry 2004; 56: 140-145.
5. Blier P. The pharmacology of putative early-onset antidepressant strategies. Eur Neuropsychopharmacol 2003; 13: 57-66.
6. Rajkumar R and Mahesh R. Assessing the neuronal serotonergic target-based anti-depressant stratagem. Impact of invivo interaction studies and knockout models. Curr Neuropharmacol 2008; 6: 215-234.

7. Hoyer D, Hennen JP, Martin GR. Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav 2002; 71: 533-554.
8. Vanpraag HM. Can stress cause depression. Prog Neuropsychopharmacol Biol 2004; 28: 897-907.
9. Barnes NM, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology 1999; 38: 1083-1152.
10. Mahesh R, Perumal RV, Pandi PV. Cancer chemotherapy induced nausea and vomiting: Role of mediators, development of drugs and treatment methods. Pharmazie 2005; 60: 83-96.
11. Wolf H. Preclinical and clinical pharmacology of the 5-HT_3 receptor Antagonists. Scand J Rheumatol 2000; 29: 37-45.
12. Martin KF, Hannon S, Phillips I, Heal DJ. Opposing roles for 5-HT_{1B} and 5-HT_3 receptors in the control of 5-HT release in rat hippocampus *in vivo*. Br J Pharmacol 1992; 106: 139-142.
13. Rajkumar R, Mahesh R, Borah M. Antidepressant-like effects of serotonin type-3 antagonist, ondansetron: An investigation in behaviour-based rodent models. Behav Pharmacol 2008; 19: 29-40.
14. Redrobe JP, Bourin M. Partial role of 5-HT_2 and 5-HT_3 receptors in the activity of antidepressants in the mouse forced swimming test. Eur J Pharmacol 1997; 325: 129-135.
15. Bagdy E, Solyom S, Harsing LG Jr. Feedback stimulation of somatodendritic serotonin release: a 5-HT3 receptor-mediated effect in the raphe nuclei of the rat. Brain Res Bull 1998; 45: 203-208.
16. Lucchelli A, Santagostino-Barbone MG, Barbieri A, Candura SM, Tonini M. The interaction of antidepressant drugs with central and peripheral (enteric) 5-HT3 and 5-HT4 receptors. Br J Pharmacol 1995; 114: 1017-1025.
17. Varea E, Blasco-Ibanez JM, Gomez-Climent MA, Castillo-Gomez E, Crespo C, Martinez-Guijarro FJ, Nacher J. Chronic fluoxetine treatment increases the expression of PSANCAM in the medial prefrontal cortex. Neuropsychopharmacology 2007; 32: 803-812.
18. Barnes NM. 5-HT_3 receptor antagonists ameliorate fatigue: so much potential, so little knowledge. Gut 2005; 54: 1056-1057.
19. Gibbs IS, Heald A, Jacobson H, Wadke D, Weliky I. Physical characterization and activity in vivo of polymorphic forms of 7-chloro-5,11-dihydrodibenz [b,e][1,4] oxazepine-5-carboxamide, a potential tricyclic anti-depressant. J Pharm Sci 1976; 65: 1380-1385.
20. Hadizadeh F, Hosseinzadeh H, Motamed-Shariaty Sadat V, Seifi M, Kazemi S. Synthesis and anti-depressant activity of N-substituted imidazole-5-carboxamides in forced swimming test model. Iran J Pharm Res 2008; 7: 29-33.
21. Pessoa-Mahana H, Rodrigo AR, Ramiro AM, Claudio SB, Pessoa-Mahana CD. Synthesis of benzo[b]thiophene carboxamides connected to 4-aryl piperazines through a benzylic spacer; potential ligands with 5-HT1a binding affinity. Synth Commun 2007; 37: 3559-3567.
22. Pathan AR, Viswanad B, Sonkusare SK, Ramarao P. Chronic administration of pioglitazone attenuates intracerebroventricular streptozotocin induced-memory impairment in rats. Life Sci 2006; 79: 2209-2216.
23. Chandrashekar R, Manohar VR, Rao SN. Antidepressant activity of aqueous extract of fruits of terminalia chebula in rats. Indian J Pharm Sci 2012; 4: 449-451.
24. Pandey DK, Rajkumar R, Mahesh R, Radha R. Depressant- like effect of parthenolide in a rodent behavioural anti-depressant test battery. J Pharm Pharmacol 2008; 60: 1643-1650.
25. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: A new method for screening anti-depressants in mice. Psychopharmacology (Berl) 1985; 85: 367-370.
26. O'Leary OF, Bechthold AJ, Crowley JJ, Hill TE, Page ME, Lucki I. Depletion of serotonin and catecolamines block the acute behavioral response to different classes of anti-depressant drugs in the mouse tail suspension test. Psychopharmacology (Berl) 2007; 192: 357-371.
27. Heal DJ, Philpot J, O'Shaughnessy KM, Davies CL. The influence of central noradrenergic function on 5-HT-mediated head-twitch responses in mice: possible implications for the actions of antidepressantdrugs. Psychopharmacology (Berl) 1986; 89: 414420-414425.
28. Devadoss T, Pandey DK, Mahesh R, Yadav SK. Effect of acute and chronic treatment with QCF-3 (4-benzylpiperazin-1-yl) (quinoxalin-2-yl) methanone, a novel 5-HT_3 receptor antagonist, in animal models of depression. Pharmacol Rep 2010; 62: 245-257.
29. Costa E, Garatini S, Valzelli L. Interaction between reserpine, imipramine and chlorpromazine. Experientia 1960; 16: 461-463.
30. Ivan S, Tomislav K, Mirko D. Neurochemical and Behavioural Changes in Rat Models of Depression. Croat Chem Acta 2011; 84: 287-299.
31. Tasset I, Medina FJ, Penaz J, Jimena I, Del M, Saldedo M et al. Olfactory Bulbectomy Induced Oxidative and Cell Damage in Rat: Protective Effect of Melatonin. Physiol Res 2010; 59: 105-112.
32. Song C, Leonard BE. The olfactory bulbectomised rat as a model of depression. Neurosci Biobehav Res 2005; 29: 627-647.
33. Casarotto PC, Andreatini R. Repeated paroxetine treatment reverses anhedonia induced inrats by chronic mild stress or dexamethasone. Eur Neuropsychopharmacol 2007; 17: 735-742.
34. Luo DD, An SC, Zhang X. Involvement of hippocampal serotonin and neuropeptide Y in depression induced by chronic unpredicted mild stress. Brain Res Bull 2008; 77 :8-12.
35. Klein D, Brown TS. Exploratory behavior and spontaneous alteration in blind and anosmic rats. J Comp Physiol Psychol 1969; 68: 107-110.
36. Kelly JP, Wyrnn AS, Leonard BE. Olfactory bulbectomized rat as a model of depression: An update. Pharmacol Ther 1997; 74: 299-316.
37. Van Reizen H, Leonard BE. Effects of psychotropic drugs on the behavior and neurochemistry of olfactory Bulbectomised rats. Pharmacol Ther 1990; 47: 21-34.
38. Boissier JR, Simon P. Action of caffeine on the spontaneous motility of the mouse. Arch Int Pharmacodyn Ther 1965; 158: 212-221.
39. Thiebot MH, Martin P, Puech AJ. Animal behavioural studies in the evaluation of antidepressant drugs. Br J Psychiatry 1992; 160: 44-50.
40. Willner P. Animal models of depression: An overview. Pharmacol Ther 1990; 45: 425-455.
41. Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology (Berl) 1995; 121: 66-72.
42. Lucki I. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. Behav Pharmacol 1997; 8: 523-532.
43. Englert LF, Ho BT, Taylor D. The effects of (-)-Δ^9 – tetra-hydrocannabinol on Reserpine-induced hypothermia in rats. Br J Pharmacol 1973; 49: 243-252.
44. Kalueff AV, Gallagher PS, Murphy DL. Are serotonin transporter knockout mice 'depressed'?: hypoactivity but no anhedonia. Neuroreport 2006; 17: 1347-1351.
45. Strekalova T, Gorenkova N, Schunk E, Dolgov O, Bartsch D. Selective effects of citalopram in a mouse model of stress-induced anhedonia with a control for chronic stress. Behav Pharmacol 2006; 17: 271-287.
46. Bekris S, Antoniou K, Daskas S, Papadopoulou-Daifoti Z. Behavioural and neurochemical effects induced by chronic mild stress applied to two different rat strains. Behav Brain Res 2005; 161: 45-59.
47. Lumia AR, Teicher MH, Salchli F, Ayers E, Possidente B. Olfactory bulbectomy as a model for agitated hyposerotonergic depression. Brain Res 1992; 587: 181-185.
48. Bourin M, Fiocco AJ, Clenet F. How valuable are animal models in defining anti-depressant activity? Human Psychopharmacol 2001; 16: 9-21.
49. Schafer WR. How do antidepressant work? Prospect for genetic analysis of drug mechanism. Cell 1999; 98: 551-554.
50. Greenshaw AJ. Behavioral pharmacology of 5-HT, receptor antagonists: A critical uptake on therapeutic potential. Trends Pharmacol Sci 1992; 14: 265-270.
51. Kilpatrick GJ, Butler A, Ireland SJ, Michel AD, Tyers MB. Affinities of 5-HT uptake inhibitors for 5-HT, receptors in both binding and functional studies. Br J Pharmacol 1989; 98: Suppl 859P.