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Anti-depressant-like activity of a novel serotonin type-3 (5-HT_3) receptor antagonist in rodent models of depression

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N-Cyclohexyl-3-methoxyquinoxalin-2-carboxamide (QCM-13), a novel 5-HT_3 antagonist identified from a series of compounds with higher pA_2 (7.6) and good log P (2.91) value was screened in rodent models of depression such as forced swim test (FST), tail suspension test (TST), interaction studies with standard anti-depressants and confirmatory studies such as reversal of parthenolide induced depression and reserpine induced hypothermia. In FST (2 and 4 mg/kg) and TST (2 and 4 mg/kg), QCM-13 significantly reduced the duration of immobility in mice without affecting the base line locomotion. QCM-13 (2 and 4 mg/kg) was also found to have significant interaction with standard anti-depressants (fluoxetine and bupropion in FST and TST respectively). Further, reversal of parthenolide induced depression in mice and reserpine induced hypothermia in rat models indicate the serotonergic influence of QCM-13 for anti-depressant potential.

Keywords: Anti-depressant-like effect, Depression, Forced swim test, 5-HT_3 antagonist, Quinoxalin-2-carboxamide, Serotonin

Depression is a major affective disorder seen in most of the patients, associated with diminished health status and increased health care utilization. It is a highly prevalent and disabling condition associated with significant morbidity and mortality[1]. Depression is seen as a transient mood state virtually in almost all individuals at different parts of the life, however it becomes a severe medical condition when symptoms like abnormalities in mood, neurovegetative functions (such as appetite and sleep disturbances), cognition (such as inappropriate guilt and feelings of worthlessness), and psychomotor activity (such as agitation and retardation) appears[2]. Anti-depressants like tricyclic anti-depressants (TCAs), monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs), serotonin nor-epinephrine reuptake inhibitors (SNRIs), various atypical anti-depressants and norepinephrine dopamine reuptake inhibitors (NDRIs) are available for commercial use. Many new classes of drugs have been found to have antidepressive effects such as tianeptine (acting via pituitary-adrenal axis inhibition)[3], leptin (a hormone secreted by adipose tissue)[4] and rolipram (a phosphodiesterase inhibitor)[5]. Despite the availability of these drugs and advent of new molecules in the pharmacotherapy of depression, it is unfortunate that this disorder goes ineffectively diagnosed and treated[6]. The anti-depressant action of the aforementioned agents produces their effect by acting on serotonergic/noradrenergic/dopaminergic system (selective and non-specific uptake inhibitors, receptor agonists and antagonists, enzyme inhibitors, etc.)[7,8]. The serotonergic system has pivotal role in affective disorders[9-12]. Serotonin a neurotransmitter present in both peripheral and central nervous system, involved in various patho-physiological conditions by acting on their receptors (5-HT_{1-7})[8]. All the serotonin receptors are belonging to the superfamily of G-protein coupled receptor, whereas the 5-HT_3 receptor is ligand-gated ion channel receptor[7]. The 5-HT_3 receptors are widely distributed in median raphe, hypothalamus, hippocampus and amygdala[7,13].

Several pre-clinical investigations have been made that show a possible relationship between 5-HT_3 receptors and depression as well as other cognitive disorders[14]. Patrick *et al.*[15] have shown that 5-HT_3 antagonists such as zacopride, ondansetron and ICS 205-930 reverse the learned helplessness behaviour in rats, which is one of the most widely used method for screening of anti-depressants. *Ptychodiscus brevis* toxin induced depression of spinal reflexes was found to be modulated through 5-HT_3 receptors[16].

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Commercially available anti-depressants such as fluoxetine, imipramine, phenelzine and iproniazid also showed anti-depressive activity by blocking 5-HT_3 receptors[17]. Among the various synthetic chemical moieties available, derivatives of carboxamides have been found as potent anti-depressants. A carboxamide derivative 7-chloro-5, 11-dihydrodibenz [*b, e*][1, 4]oxazepine-5-carboxamide, has been found to be a potential tricyclic anti-depressant[18]. N-substituted imidazole-5-carboxamides[19] and benzo[b]thiophene carboxamides connected to 4-aryl piperazines through a benzylic spacer[20] have been found to have potential anti-depressant activity.

Keeping above in view and activities of various substituted and non-substituted carboxamide moieties reported the present study has been undertaken to investigate the anti-depressant effects of N-cyclohexyl-3-methoxyquinoxalin-2-carboxamide in validated animal models of depression.

Materials and Methods

Animals—Swiss Albino mice (22–27 g) and Wistar rats (150-200 g) of either sex were purchased from Hissar Agricultural University, Hissar, India. Animal experimentation was conducted in adherence to the Institutional Animal Ethics Committee of Birla Institute of Technology & Science, Pilani, India (Protocol No. IAEC/RES/04/02). Male and female animals were housed separately in groups of 6 per cage and maintained in standard laboratory conditions with alternating light and dark cycle of 12 h each at 23° ± 2° C and 65 ± 2% RH for at least one week before the commencement of the experiments. The animals had free access to food (standard pellet chow feed) and filtered water *ad libitum*. New animals were used in each experiment.

Drugs and chemicals—QCM-13 (N-cyclohexyl-3-methoxyquinoxalin-2-carboxamide) was synthesized by the medicinal chemistry group of the institute. Fluoxetine (FLX) and bupropion (BUP) were procured from Glenmark Pharmaceuticals and Ranbaxy Research Laboratories (India) respectively, as generous gift samples. The test drugs QCM-13, FLX, BUP were freshly prepared in distilled water. Volume administered by oral and ip route was constant volume of 10 ml/kg for each mouse and 1 ml/kg for each rat respectively.

Chemistry

The compound QCM-13 (*N*-cyclohexyl-3-methoxy-quinoxalin-2-carboxamide) (Fig. 1) was synthesized from the starting material, *o*-phenylenediamine **(1)** in a sequence of reactions (Fig. 2). Initial condensation with diethyl ketomalonate followed by chlorination using phosphorous oxychloride, afforded the chloro ester compound **(3)** which on saponification followed by nucleophilic displacement with sodium methoxide gave the key intermediate, 3-methoxyquinoxalin-2-carboxylic acid **(5)**. This key intermediate was coupled with cyclohexylamine to obtain the desired product in good yield in presence of EDC·HCl and

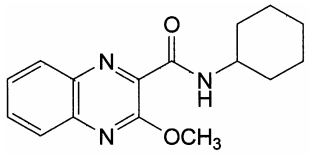

Fig. 1—Structure of QCM-13

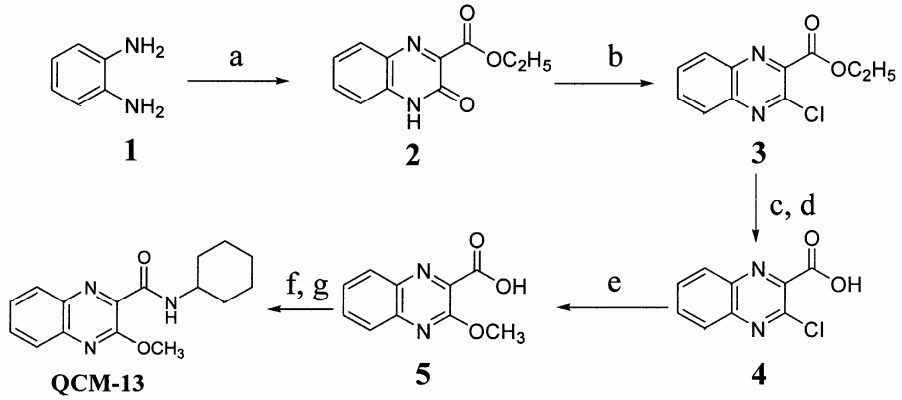

Fig. 2—Synthetic route of QCM-13 [Reagents and conditions: (a) Diethylketomalonate, ethanol, reflux , 6 h., 60%; (b) $POCl_3$, DMF, reflux, 30 min. 80%; (c) (c) Na_2CO_3, reflux, 6 h. (d) dil. HCl, 94%; (e) $NaOCH_3$, Methanol, MW, 6 min., dil. HCl, 85%; (f) EDC.HCl, HOBt, THF, N_2, 0°C–RT, 1h .; (g) Cyclohexylamine, 6 h]

HOBt as coupling agents. The structure of the QCM-13 was confirmed by spectral data.

Behavioural assays

Dose response studies: Dose-response studies were carried out using the mouse spontaneous locomotor activity (SLA) test, forced swimming test (FST) and tail suspension test (TST), to determine the appropriate doses of QCM-13 that significantly influenced the depressive state without affecting the baseline locomotor status. The QCM-13 (0.25-8.0 mg/kg, ip) was administered to mice 30 min before subjecting them to assessment of SLA or in FST. Selected doses (2.0 and 4.0 mg/kg/ ip) were used for the interaction studies.

Tail suspension test (TST): Tail suspension test is the most commonly used behavioural model for screening of anti-depressants in mice. The method is based on the principle that a mouse suspended by tail above a fixed height of 50 cm from the ground shows alternate periods of agitation and immobility[21,22]. Animals were allowed to acclimatize to the experimental room for 1-2 hr before the behavioural procedure. After 30 min of injection of control (distilled water) or drug, mice were individually suspended by the tail from a horizontal bar at a distance of 50 cm from floor using adhesive tape (distance from tip of tail = 2 cm). A 6 min test session was employed. Each animal under test was visually isolated from the other animals during the test. The behavioural parameter recorded was the number of seconds spent in a completely immobile posture, expressed as immobility. The animal was considered immobile when it was passive, completely motionless and didn't show any body movement[22,23].

Forced swim test (FST): The method described by the Porsolt *et al.*[24] was followed. In brief the method is based on the principle that a mouse forced to swim in a cylindrical vessel will cease struggling and remained afloat passively, which is termed immobility[25]. Animals were allowed to acclimatize to the experimental room for 1-2 hr before the behavioural procedure. After 30 min of injection of control (distilled water) or drug, mice were individually placed in an open glass cylinder (22.5 cm diam. and 30 cm height) filled with water at a height of 15 cm maintained at 26° ± 1° C. At this height the animals were not able to support themselves by bottom and side walls of the chamber. The water was changed for each animal subjected to FST as used water has been shown to alter the behaviour[26]. Each mice was forced to swim for 15 min on the pre-test day i.e. 24 h before commencing the test[27,28]. After the initial 2 min of the vigorous movement, the mice acquire an immobile posture which was characterized by motionless floating in water making only those movements necessary to keep its head above the water[28]. Following the test, the mice were dried using a towel and returned to their cages. Each animal was used only once in the test.

Spontaneous locomotor activity (SLA): To rule out the effect of drug on immobility period, spontaneous horizontal locomotor activity of control and the test animals were recorded as per the procedure[28,29] using an actophotometer. A count was recorded when a beam of light falling on a photocell is cut off by the animal passing across the beam[23]. The animals were individually placed in a square arena (30 × 30 × 25 cm), with walls painted black and a photocell assembly. After an initial 2 min acclimatization period, the digital locomotor scores were recorded for the next 10 min in a dimly lit room. The arena was cleaned with dilute alcohol and dried between trials.

Interaction studies: The interaction study with standard anti-depressants and serotonergic modulators were carried out in mice using the FST/TST to deduce the exact mechanism of the QCM-13. Mice were treated individually with a single dose (ip) of control; FLX (10 and 20 mg/kg)/BUP (10 and 20 mg/kg; for TST and FST respectively) post 15 min of QCM-13 administration. The doses of standard anti-depressants were obtained from pilot studies or from previous studies[28,30]. After 30 min of after the anti-depressant injection, mice were subjected to FST/TST. The equipment used to assess the rodent's behaviour was sprayed with alcohol and wiped thoroughly between trials to eliminate the residual odour.

Parthenolide (PTL) induced depression test: Parthenolide (PTL) is a germacranolide sesquiterpine lactone obtained from the plants of Astracae family[28] (eg. *Tanacetum parthenium*). PTL plays an important role as anti-migraine agent[31] and causes the inhibition of 5-HT release[27,32] the mechanism responsible for its depressant like activity[27]. QCM-13 was tested for antagonizing the depressant effects of PTL. Mice were trained 24 h before the test for 15 min and given PTL (1 mg/kg). After 30 min of the PTL dosing, QCM-13 was given in significant doses (2 and 4 mg/kg/ip). Then individual mice were subjected (after 45 min of PTL dosing) to FST. The test was performed as described by Redrobe and Bourin[33].

Reserpine induced hypothermia: Reserpine produces a depressant effect on CNS of test animals which leads to a decrease in the locomotor activity, induces hypothermia and (blepharo) ptosis[34]. In the present study, reserpine induced hypothermia was taken as a measure to test the drug under study. The rats were gently hand-restrained, and the lubricated digital thermometer probe was inserted into the rectum. The rectal temperature of the rats treated with reserpine (1 mg/kg, i.p)/ QCM-13 (2.0 and 4.0 mg/kg, ip)/ escitalopram (ESC) (10 mg/kg, ip) was recorded at 30, 60 and 120 min after the drug administration. Reserpine was injected 15 min post drug administration. The differences in the rectal temperature between the baseline and 60th min values were tabulated as reserpine showed recovery at 120th min. On the day preceding the experiments, the rectal temperature of the rats were assessed in a similar manner in order to habituate the animals to the experimental procedure.

Statistical analysis—All the results are expressed as mean ± SE. The data from the dose response studies were subjected to one-way ANOVA followed by post hoc Bonferroni test. The behavioural scores from interaction studies were analyzed using two-way ANOVA followed by Dunnet test. All the comparisons were made against the control (vehicle treatment), or as specified otherwise. The level of statistical significance was fixed at $P<0.05$ (Prism-3 software was used.)

Results

Spontaneous locomotor activity—There was no significant influence of the tested doses of QCM-13 (2 and 4 mg/kg body weight) on the spontaneous locomotor activity of mice when compared to control group. However the at doses of QCM-13 at 0.5, 1.0, 8.0 mg/kg significantly influenced the locomotor activity (Fig. 3).

Forced swim test—The acute treatment with QCM-13 significantly decreased the duration of immobility in mice compared with vehicle treatment (Fig. 4) when tested for the doses of 2.0 and 4.0 mg/kg. This effect was dose dependent and was not observed at lower doses (0.5 mg/kg).

Tail suspension test—The acute treatment with QCM-13 significantly decreased the duration of immobility in mice compared with vehicle treatment (Fig. 5) when tested for the doses of 2 and 4 mg/kg. This effect was dose dependent and was not observed at lower dose (0.5 mg/kg) tested.

Interaction studies—The dose of QCM-13 (2 mg/kg) that induced a significant ($P<0.05$) decrease in the duration of immobility was selected for the interaction studies. It was found to be an overall significant difference in the duration of immobility among the different groups tested for FST and TST interaction studies. In FST, pre-treatment with QCM-13 (2.0 mg/kg) potentiated the anti-depressant effect of FLX (10 and 20 mg/kg) as seen by decrease in duration of immobility(s) in the mice (Fig. 6). Similarly, for TST, pre-treatment with QCM-13 (2 mg/kg) potentiated the anti-depressant effect of BUP (10 and 20 mg/kg) (Fig. 7).

Parthenolide (PTL) induced depression—In the confirmatory study with parthenolide test, there was a significant increase in the duration of immobility for

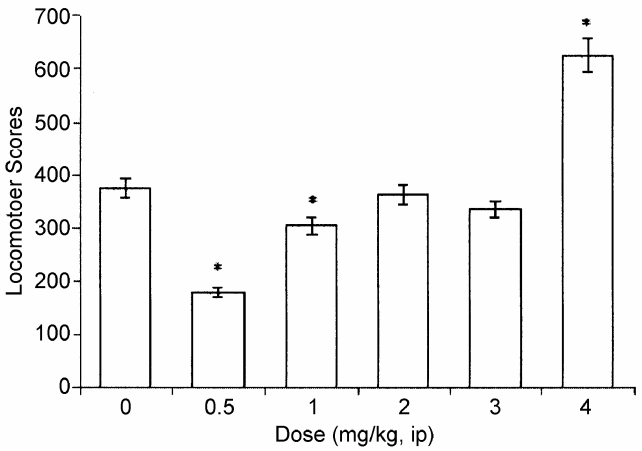

Fig. 3—Effect of QCM-13 on spontaneous locomotor activity in mice [Values are mean± SE from 6 in each group. *P < 0.05 compared with vehicle-treated group]

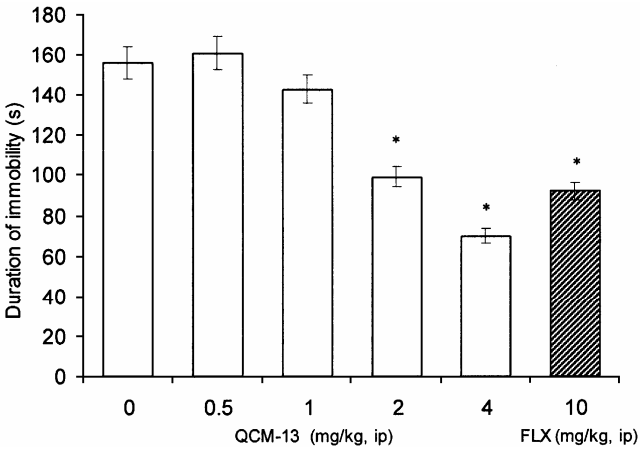

Fig. 4—Effect of QCM-13 and FLX on the duration of immobility in mice FST [Values are mean mean ± SE from 6 animals in each group. *P < 0.05 compared with vehicle-treated group]

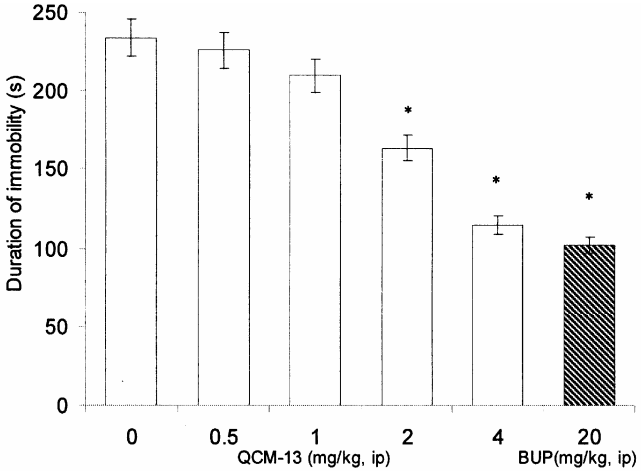

Fig. 5—Effect of QCM-13 and BUP on duration of immobility in mice TST [Values are mean mean ± SE from 6 animals in each group. *$P < 0.05$ compared with vehicle-treated group]

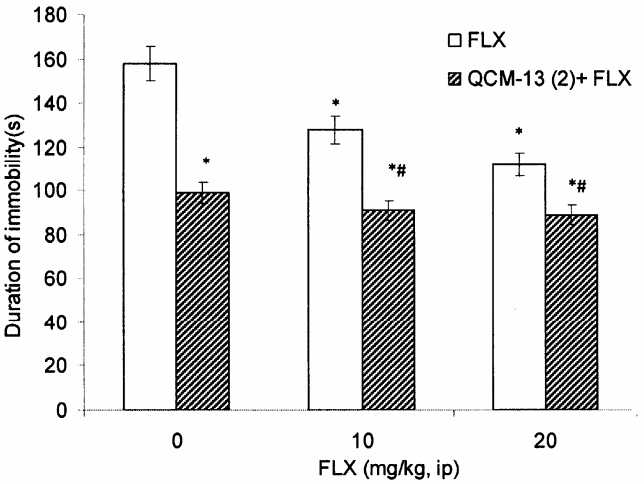

Fig. 6—Effect of QCM-13 (2 mg/kg ip) pre-treatment on anti-depressant activity of FLX (10 and 20 mg/kg ip) in mice FST [Values are mean mean ± SE from 6 animals in each group. *$P < 0.05$ compared with vehicle-treated group and # $P < 0.05$ with FLX treated group]

PTL (1 mg/kg) treated group when compared to control group (vehicle treated) ($P<0.05$) (Fig. 8). Further, QCM-13 at 2 and 4 mg/kg doses significantly reversed the depressant like activity of parthenolide ($P<0.05$) shown by the decrease in the immobility duration of the test compound treated animals.

Reserpine induced hypothermia—Treatment with QCM-13 (2 and 4 mg/kg body weight) significantly ($P<0.05$) inhibited the hypothermic response induced by reserpine (1 mg/kg body weight, ip) (Fig. 9). The maximum reversal of reserpine induced hypothermia was observed at 60th min and was dose dependent.

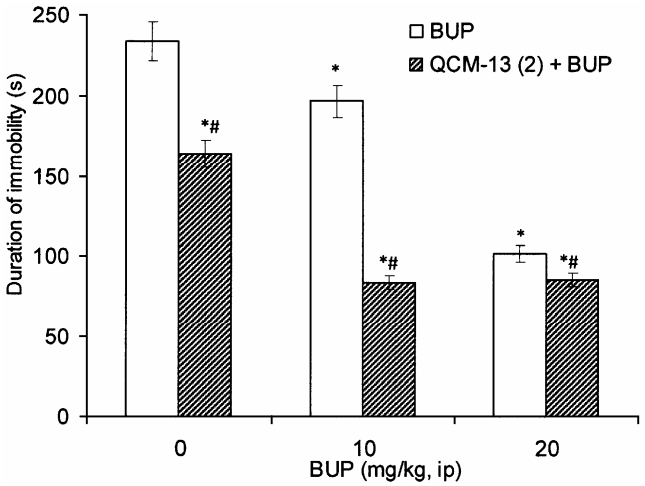

Fig. 7—Effect of QCM-13 (2 mg/kg ip) pre-treatment on anti-depressant effect of BUP (10 and 20 mg/kg) in mice TST [Values are mean mean ± SE from 6 animals in each group. *$P < 0.05$ compared with vehicle-treated group and # $P < 0.05$ with BUP treated group]

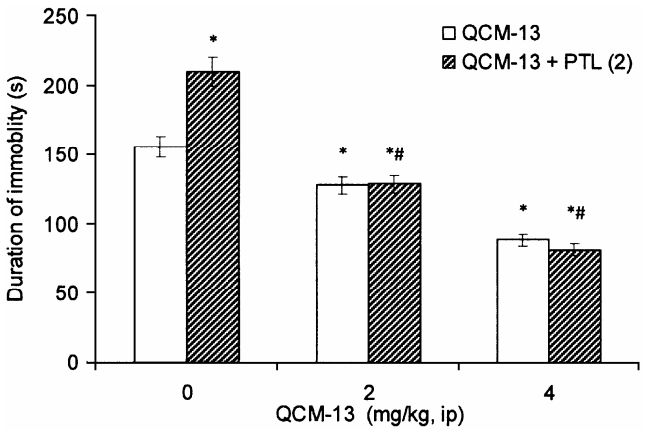

Fig. 8—Effect of QCM-13 (2 and 4 mg/kg/ip) pre-treatment on anti-depressant effects of PTL (1 mg/kg) in mice FST [Values are mean ± SE from 6 animals in each group. *$P < 0.05$ compared with vehicle-treated group and # $P < 0.05$ with PTL (2 mg/kg, ip)]

Discussion

Due to increase awareness of health and increasing thoughts of being good living, people are now becoming more concerned towards the diseases and disorders and their measures of treatment without compromising the comfort. Depression is among the most widely occurring disorders. However, because of improper response and non-compliance of the existing drugs, investigators seek new drugs. Anti-depressant effect of new synthetic compounds has been drawing more attention gradually because of increasing incidence of depression[2,35,36] and predominance of these chemical moieties in therapy.

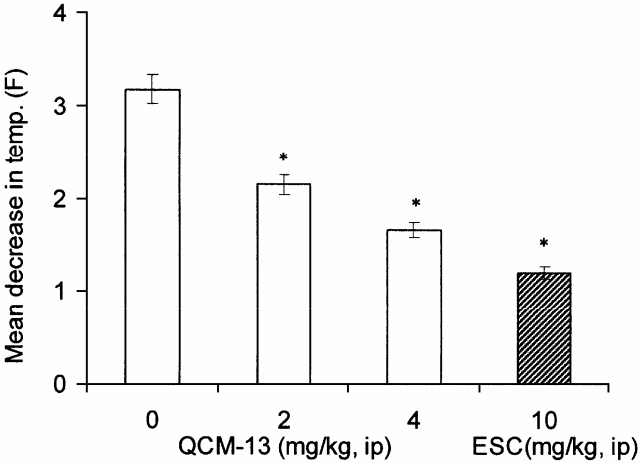

Fig. 9 — Effects of ESC (10 mg/kg po) and QCM-13 (2 and 4 mg/kg po) treatment on reserpine induced hypothermia in rats. Values are mean ± SE from 6 animals in each group. *$P < 0.05$ compared with vehicle-treated group at 60th min]

In the present study, the anti-depressant like effects of QCM-13 was evaluated in validated animal models of depression with the aim to discover a novel agent to encompass all the problems in the existing anti-depressant therapy. The practice of using whole animal assay is considered to be a rapid method for the identification of neuro-psychopharmacological effect of novel compounds. This investigation encompassed acute FST, TST, interaction studies, and various confirmatory studies on animal models of depression. The predictive validity of the aforementioned anti-depressant assays is already reported to be adequate.

While interpreting the anti-depressant-like effect of any test substance based on swimming and exploratory behaviour of rodents, the influence of the test substance in baseline locomotion in animal is of prime concern[37]. QCM-13 (2 and 4 mg/kg, ip) significantly decreased the duration of immobility in mice FST and TST. Reduction in duration of immobility reflects the anti-depressant properties of drugs[24]. The anti-depressant-like effect of the test drug seems not be associated with any motor effects, as it did not show significant change in locomotion of mice. Interactions with selective serotonin reuptake inhibitors (SSRIs) are necessary for conclusive assessment of anti-depressant potential[38]. QCM-13 (2.0 mg/kg) significantly enhanced the anti-depressant action of FLX and BUP. Due to species dependent variation, BUP failed to exhibit anti-depressant effects in FST with Swiss Albino mice[39]. Hence, interaction study in TST was expected to throw some light on the influence on dopaminergic system. In the present study, QCM-13 pre-treatment was found to augment the anti-depressant effects of BUP (10 and 20 mg/kg) in TST indicating the influence on dopaminergic system. Though the exact mechanism is not clear, it could involve the release of dopamine by inhibiting 5-HT_3 receptor[2].

Depressant effect induced by PTL was considered as a model to identify anti-depressants acting through serotonergic mechanism[27,40]. Reversal of depressant effects of PTL showed that QCM-13 acts via serotonergic system. The presence of 5-HT_3 receptors in neuro-anatomical region indicates the regulation of 5-HT transmission indirectly[41].

Depletion of biogenic amines (nor-epinephrine, dopamine, serotonin) in the brain has been observed to induce catalepsy, ptosis and hypothermia. Reserpine is a well known monoamine depleting agent that blocks transmission of monoamines in the synaptic vesicles[42]. In the present study reserpine induced hypothermia was taken as a model for confirmation of the mechanism involved. Both QCM-13 and standard drug ESC were found to decrease the body temperature induced by reserpine indicating that the drugs act by increasing the amount of biogenic amines at the synaptic cleft.

In conclusion, QCM-13 exerts anti-depressant-like effect in all the tested validated animal models and these effects may be mediated by the central monoaminergic neurotransmitter systems predominantly by 5-HT_3 antagonistic action. The convergence of these findings suggests that QCM-13 may be useful as a potential candidate for the management of depression. Regardless of their mechanism of action, a major drawback of all marketed anti-depressants is the 3 to 5 week delay to achieve therapeutic efficacy. A combination with 5-HT_3 antagonist can potentially accelerate the onset of anti-depressant action. Further studies are now required to investigate the effects of QCM-13 to characterize the drug treatment at the cellular levels, by using molecular techniques. The development of more specific ligands may also allow a more directed approach, with their long-term effectiveness.

Acknowledgment

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