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RESEARCH

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Long-term scopolamine treatment altered locomotor, exploratory and anxiety-like behaviours of albino rats

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Abstract

Background: Animal models are used to provide an adequate investigation of brain-behaviour, physiological and path physiological relationships to give insight into human behaviour and the underlying processes of drugs affecting the nervous system. Scopolamine; SCO (alkaloid L-(2)-scopolamine [L-(2)-hyoscine]) has a competitive inhibitory effect on muscarinic receptors for acetylcholine. Thus, this study was designated to investigate the effect of long-term SCO treatment on locomotor, exploratory and anxiety-like behaviours of rats using open field test.

Results: The long-term SCO treatment induced a prominent increase in locomotion (hyperactivity) and exploratory behaviour of rats. In addition, anxiety-like behavioural patterns showed a non-significant difference in SCO treated compared to control. Serotonin level was significantly decreased in the scopolamine treated group in comparison with the control group.

Conclusions: Data suggested that long-term SCO treatment resulted in marked neurobehavioural alterations in a rat as an animal model.

Keywords: Scopolamine, Rats, Serotonin, Exploratory behaviour, Locomotion

1 Background

Animal models are often included as an experimental paradigm which depends on using of the non-human species to provide an adequate investigation of brain-behaviour, physiological and path physiological relationships to give insight into human behaviour and the underlying processes [3, 40] of some drugs affecting the nervous system. Among these drugs, scopolamine; SCO (alkaloid L-(2)-scopolamine [L-(2)-hyoscine]) has a competitive inhibitory effect on muscarinic receptors for acetylcholine [36], acts as a non-selective muscarinic antagonist [34], affects the parasympathetic nervous system. Hence, SCO may induce signs of anxiety, avoidance, fear, and it is used to treat nausea and motion sickness in humans [25, 30, 41]. Anxiety is a status that is often accompanied by neurobehavioural signs such as depression and abnormal behaviour [37]. Scopolamine (SCO) is a muscarinic receptor antagonist (non-selective) that suppresses learning and memory through disrupting some indirect pathways and cholinergic transmission [26]. It's been commonly used as a pharmaceutical medication to simulate Alzheimer's disease-related brain damage [5, 44]. Various tests were developed to evaluate different behavioural patterns as indicators for anxiety in rodents [32]. These tests are based on the hypothesis that, anxiety in animals is to some extent equivalent to that in human and animal models can produce a condition of anxiety that may be related to anxiety disorders [3, 9]. Thus, an analogy can be believed, if not a homology, between humans and rodents in anxiety indicators [32]. Open field is a behavioural test based on the observation and measurement of behavioural patterns related

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to anxiety such as locomotion, exploration, and freezing in rodents [11] after their placement in the maze as a novel environment or exposure to a new stimulus [15, 32]. Furthermore, serotonin is included in the behaviour performance and mood regulation, due to its therapeutic effectiveness and extended treatment of anxiety disorders with selective serotonin reuptake inhibitors [31]. Most studies proved that short term use of SCO in treatment caused anxiety in rats and mice [10, 22], however, there is little information about the effect of long term treatment SCO on rats, behaviour and anxiety. Therefore, we aimed to investigate the effect of long term SCO treatment on locomotor, exploratory and anxiety-like behaviours of rats using open field test and measurement of brain serotonin levels.

2 Methods

2.1 Chemicals

Scopolamine (scopolamine hydrobromide, Sigma-Aldrich Co., USA).

2.2 Animals

Thirty male albino rats weighing 130–160 g were purchased and acclimatized for two weeks before the onset of the experiment. The experimental design was approved by Institutional Animal Treatment and Use Committee.

Animals were housed in plastic cages and maintained at appropriate environmental conditions of temperature (21 ± 2 °C), and relative humidity (45 ± 5%) with a reversed 12-h light–dark cycle. Clean fresh water and feed adlibitum were available during the period of the experiment.

Thirty male albino rats were randomly selected and divided into two groups ($n = 15$ per each). Control group; injected with saline intraperitoneally (i.p); and Scopolamine hydrobromide-treated group (SCO) administered SCO at a dose of 2 mg/kg, i.p. dissolved in saline solution [5, 10] daily for seven weeks.

2.3 Open field test (OF)

The open field test (Fig. 1) was firstly developed by Hall [19] to evaluate rodent emotional behaviour. Ohl [32] mentioned that OF test is used to assess unconditioned anxiety, where placing the animal in a novel environment is considered a stimulus that helps in the expression of anxiety symptoms as humans. The test was used to assess locomotor and exploratory behaviour in rodents qualitatively and quantitatively and anxiety behaviour [18, 42].

The open field maze was a wood square arena with 72 cm in length and 36 cm in height, blue lines were drawn on the floor with a marker that divide the floor into sixteen squares; each square is 18 × 18 cm according to Brown et al. [8].

Nine rats were randomly selected from each group. Each rat was individually placed into one of the four corners of the open field and allowed to explore the apparatus for 5 min. The behaviour of rats was recorded using a digital video camera. The maze floor was cleaned after each rat test using 70% ethyl alcohol.

Test parameters were analysed to assess locomotion and exploration (square crossing and rearing) and anxiety behavioural patterns according to Walsh and Cummins [43], Jahkel et al. [23], Choleris et al. [11] and Kalueff and Tuohimaa [24] as showed in Table 1.

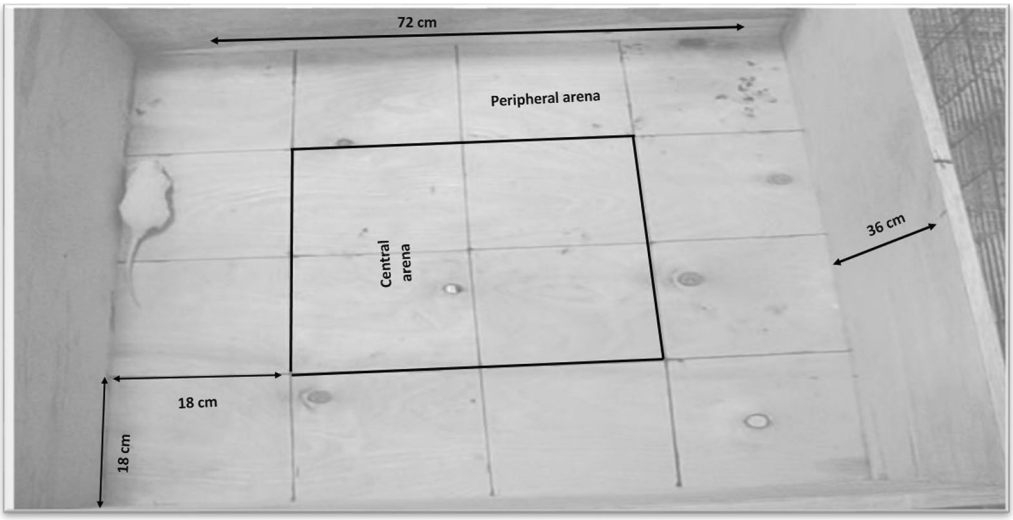

Fig. 1 Open field test apparatus (maze)

Table 1 Open field test scoring parameters

Locomotion and exploration	
Peripheral squares crossing	Number of peripheral squares crossed with all four paws of rat
Rearing	The frequency with which the rat stand against the wall of the maze
Central square crossing	Frequency of central squares crossing with all four paws of rat
Central square duration	Duration of time spent in the central squares by the rat
Anxiety	
Freezing	Duration of time during which the rat was completely stationary
Stretch attend posture	Frequency of the forward elongation of animal head and shoulders followed by retraction to its original position
Urination	The number of streaks of urine
Defecation	Number of fecal boli produced

2.4 Biochemical measurements

After the end of behavioural tests, six rats from each group were humanely euthanized using a low dose of diethyl ether followed by rat decapitation. Then, brain samples were extracted, washed with saline and kept in a deep freezer at − 80 °C. Brain homogenate was prepared for measurement of serotonin level at the National Organization for Drug Control and Research, Giza, Egypt.

2.4.1 Estimation of the serotonin levels

The estimation of serotonin (5-HT) levels in the rat brain was carried out according to the fluorometric method described by Ciarlone [13], where standard solution tubes and brain homogenates were centrifuged at 1000R for 5 min. Then 2.5 ml of supernatant fluid was transferred to tubes containing 1.6 millilitres of 0.2 N acetic acid and 5 ml of heptane. All tubes were centrifuged at 1000 g for 5 min after 30 min on a vortex mixer. The supernatant phase of organic matter was discarded. The aqueous phase was transferred to tubes for the 5-HT analysis. All tubes were boiled for 10 min, then cooled with tap water before being read on a spectrophotofluorometer. The excitation wavelength wavelengths were 355 and 470 nm.

2.5 Statistical analysis

All data were analysed using the independent *T*-test using SPSS version 22 statistical software. The data are presented as mean ± SE. The significance of the results was judged at a 0.05 *P* svalue. The correlation between behavioural parameters was analysed by the Pearson correlation test, followed by the principal component analysis and Varimax rotation. As well as, the correlation between behavioural parameters and biochemical data was analysed by the Pearson correlation test.

3 Results

Statistical analysis using the independent *T*-test revealed significant alterations in behaviours and level of brain serotonin of rats injected by SCO for seven weeks.

Regarding behaviour, Fig. 2 shows that SCO increased locomotor activity (number of peripheral square crossing and rearing) of rats (Fig. 2A). The number of peripheral squares crossed by SCO treated rats (91.25) was significantly ($P=0.01$) more than that crossed by the control group (66.75). Additionally, SCO treated rats expressed a significant ($P=0.02$) increase in rearing frequency (18.75) than that of the control group (9.25). Similar data were observed in exploratory behaviour, where SCO treated rats showed a significant ($P=0.01$) increase in central square crossing (9.25) more than that crossed by those in the control group (2.00) (Fig. 2A). As well as, SCO injected rats were observed to spend a significant ($P=0.02$) longer time in central squares (9.75 s) than in the control group (2.25 s) (Fig. 2B).

Moreover, anxiety-like behaviours were not significantly altered by long term SCO treatment (Fig. 3). Rats in SCO treated group spent a non-significant shorter freezing time (0.25 s) in the open field maze than the control group (0.75 s) (Fig. 3A). Defecation showed a non-significant decrease in SCO treated rats (0.00) compared to the control group (1.75). Meanwhile, SCO injected rats exhibited a non-significant increase in stretch attend posture (SAP) (2.00) with control rats (1.00) (Fig. 3B).

Furthermore, Fig. 4 declares that long-term SCO treatment induced a significant ($P=0.05$) decrease (1.00 µg/gm tissue) in serotonin level in comparison to the control group (1.72 µg/gm tissue).

Pearson correlation between behavioural patterns in Table 2 showed that freezing was negatively correlated with all variables at a moderate degree except with defecation (moderate positive correlation). The number of square crossing (peripheral and central), rearing and central square duration showed a good positive correlation

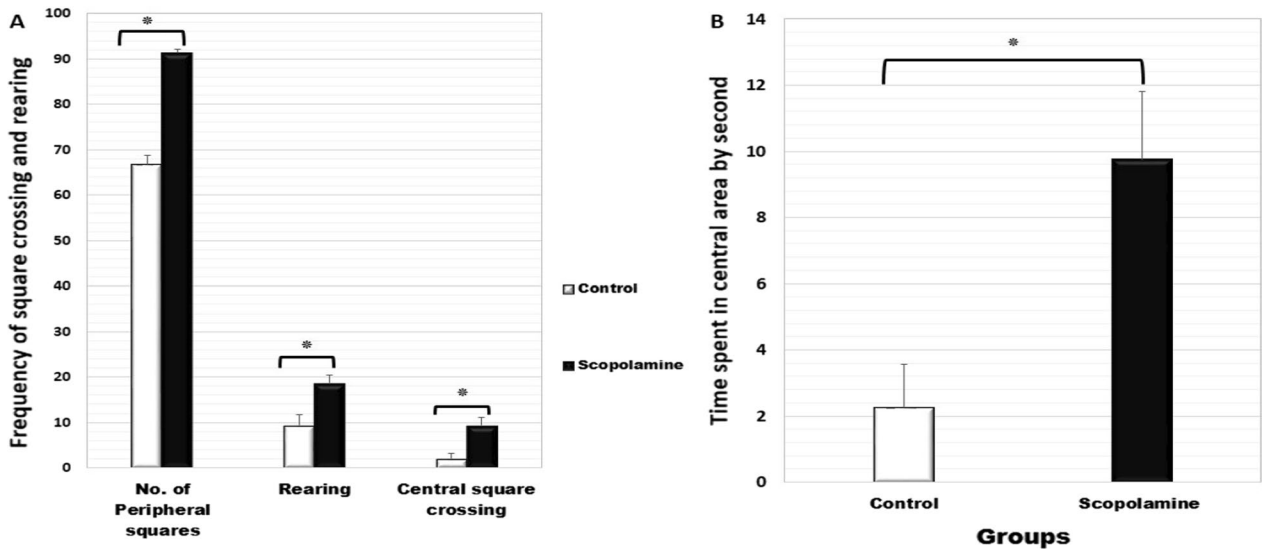

Fig. 2 Effect of long-term scopolamine treatment on locomotor activity and exploratory behaviour of rats. All values are the mean ± SE ($n = 6$). The strike (*) indicates a significant difference between the control and scopolamine treated groups according to the independent *T*-test at $P < 0.05$

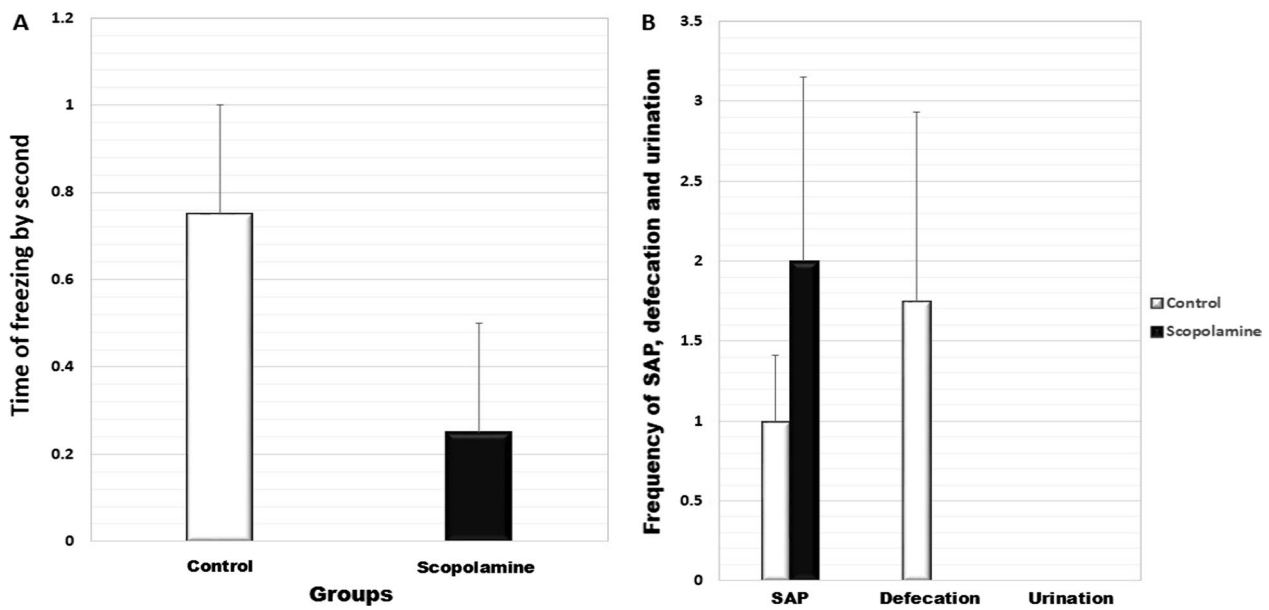

Fig. 3 Effect of long-term scopolamine treatment on anxiety-like behaviour of rats. All values are the mean ± SE ($n = 6$). A significant difference between the control and treated according to the independent *T*-test at $P < 0.05$

with each other. As well as, week to moderate positive correlation with SAP. On the other side, they showed a moderate negative correlation with freezing and defecation.

Factor analysis was performed using the Varimax rotation method (Table 3). It was noticed that the first component was highly correlated with No of peripheral squares (0.819), No of central squares (0.950), central square duration (0.883), rearing (0.926), and the second component was highly correlated with SAP (0.914) and Freezing (− 0.851). Additionally, the scree plot for factor analysis demonstrated that the data were reduced into two components as observed in Fig. 5.

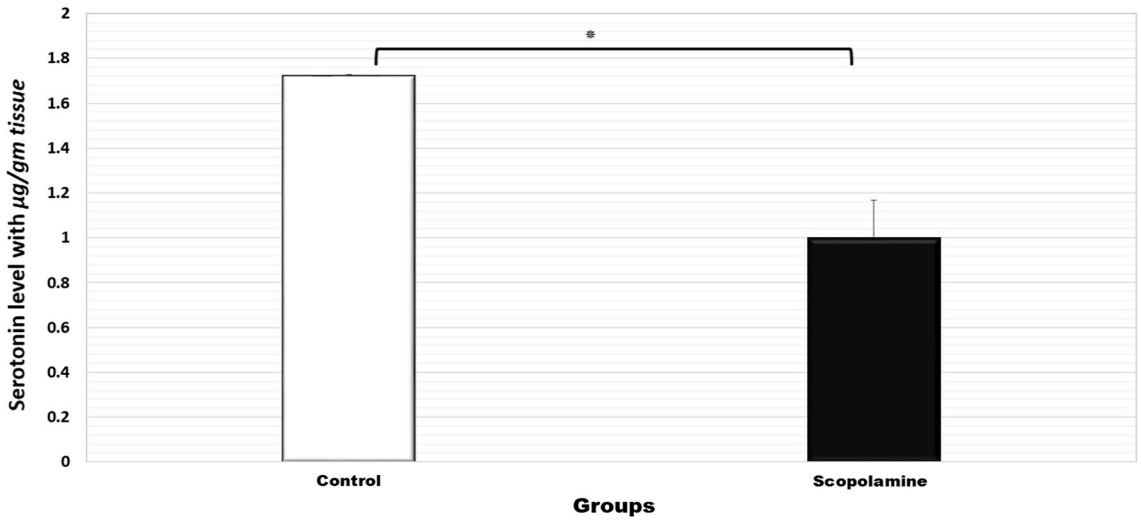

Fig. 4 Effect of long-term scopolamine treatment on brain serotonin level of rats. Data expressed as means ± SE ($n = 5$). *A strike indicates a significant difference at $P \leq 0.05$

Table 2 Pearson correlation between different behavioural parameters in open field test

Pearson correlation	Freezing	No of peripheral Sq crossing	No of central Sq crossing	Central square duration	Rearing	SAP	Defecation
Freezing	1	−.419	−.306	−.417	−.417	−.632	.517
No of peripheral Sq crossing	−.419	1	.705	.724*	.718*	.278	−.395
No of central Sq crossing	−.306	.705	1	.892**	.871**	.237	−.401
Central square duration	−.417	.724*	.892**	1	.821*	.478	−.262
Rearing	−.417	.718*	.871**	.821*	1	.211	−.259
Stretch attend posture (SAP)	−.632	.278	.237	.478	.211	1	−.164
Defecation	.517	−.395	−.401	−.262	−.259	−.164	1

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

No number, *Sq* square

Table 3 Rotated component matrix of factor analysis (Varimax rotation)

	Rotated component matrix[a]	
	Component	
	1	2
Freezing	−.254	−.851
No of peripheral Sq crossing	.819	.231
No of central Sq crossing	.950	
Central square duration	.883	.332
Rearing	.926	.146
Stretch attend posture (SAP)	.123	.914

Extraction method: principal component analysis

Rotation method: Varimax with Kaiser normalization

[a] Rotation converged in 3 iterations

In Table 4, Pearson correlation between behavioural patterns and serotonin level showed a good negative correlation between serotonin level and peripheral and central squares crossing, and rearing. In addition, a moderate negative correlation was found between serotonin level and central squares duration. Moreover, serotonin level showed a moderate positive correlation with defecation and freezing, while no correlation was present between serotonin level and stretch attend posture.

4 Discussion

Scopolamine is a potential anxiolytic drug that reduces aggressive behaviour in non-human primates under certain environmental conditions [20, 33]. The open field test was a traditional test frequently used to analyse anxiety, in addition, to assessing exploratory behaviour and

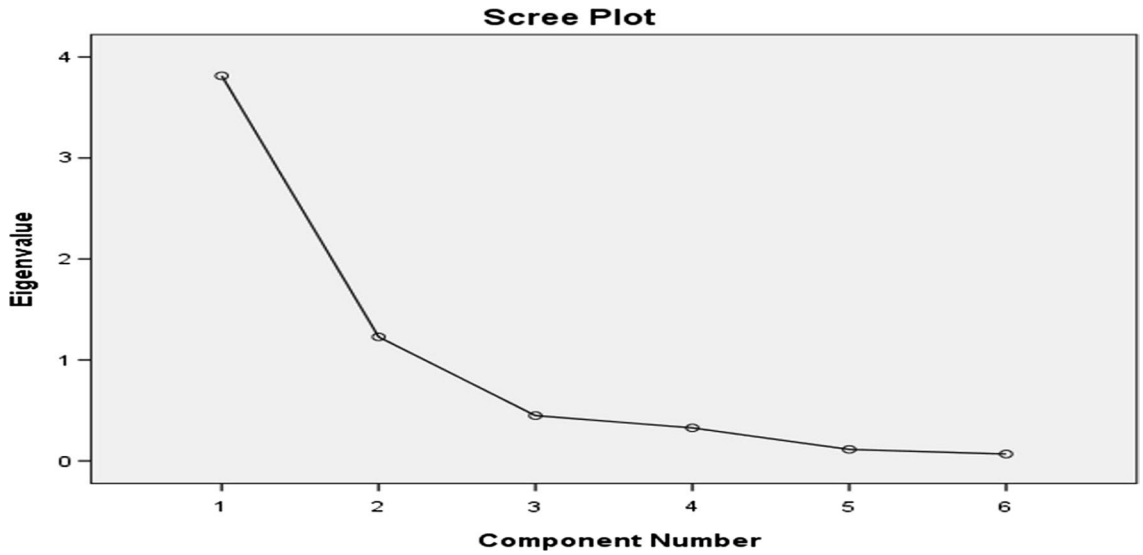

Fig. 5 Scree plot of factor analysis

Table 4 Pearson correlation between different behavioural parameters and biochemical data

Pearson correlation	Freezing	No of peripheral Sq crossing	No of central Sq crossing	Central square duration	Rearing	SAP	Defecation	Serotonin
Freezing	1	− .419	− .306	− .417	− .417	− .632	.517	.370
No of peripheral Sq crossing	− .419	1	.705	.724*	.718*	.278	− .395	− .922**
No of central Sq crossing	− .306	.705	1	.892**	.871**	.237	− .401	− .803*
Central square duration	− .417	.724*	.892**	1	.821*	.478	− .262	− .685
Rearing	− .417	.718*	.871**	.821*	1	.211	− .259	− .813*
Stretch attend posture (SAP)	− .632	.278	.237	.478	.211	1	− .164	− .021
Defecation	.517	− .395	− .401	− .262	− .259	− .164	1	.502
Serotonin	.370	− .922**	− .803*	− .685	− .813*	− .021	.502	1

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

No number; *Sq* square

activity of rodents [21]. In the current study, long-term SCO administration increased activity and exploration of rats in open field maze. Likewise, Christmas and Maxwell [12] revealed that benzodiazepines (anxiolytic drugs) at low doses stimulated the animal locomotion in a novel environment. As well, Gentsch et al. [17] stated that, injecting chlordiazepoxide as an anxiolytic drug in spontaneously hypertensive rats induced an increase in both locomotion and exploratory behaviour. However, diazepam (anxiolytic drug) decreased locomotion and exploration of rats in the open field test [16].

The observed hyperactivity and increased exploration suggested that rats were less anxious in the maze. This is supported by Walsh and Cummins [43], Jafarian et al. [22] and Cheon et al. [10] who demonstrated that a high frequency of the number of peripheral square crossing and rearing in open field test indicating increased locomotor activity and/or a lower level of anxiety. In addition, Walsh and Cummins [43] and Stanford [39] reported that rats spent most of their time in the central area and explore more in open field test reflect a low level of anxiety. On the other hand, Archer [1] found that hyperactivity in a novel maze may indicate a high-stress state of animals. The observed increase in activity was correlated with the recorded non-significant decrease in freezing, where rats spent more time performing rearing and exploration and thus little time remained for freezing. The reduction in freezing time and defecation frequency confirmed our

suggestion that long-term SCO treatment decreased anxiety in rats [11, 38]. Furthermore, the recorded non-significant increase in stretch attend posture meant that rats had the motivation to explore open field maze and thus, became less afraid in the maze [11]. On the contrary, Bindra and Thmpson [6] proposed frequent defecation of rats in a novel environment as a sign of emotionality and it is not an index of anxiety. Unlike our hypothesis, stretch attend posture is considered anxiety-like behaviour and indicated that the animal was hesitant to move due to a high level of anxiety [7]. Serotonin has a marked role in animal anxiety [27, 28]. Thus, the observed anxiolytic behaviour caused by long-term SCO treatment may be explained by the reported decrease of serotonin in the current study. This is proved by Bert et al. [2] demonstrated that serotonin (5-HT) increased with anxiety and decreased with anxiolytic drugs in different rat stocks and strains. Moreover, anxiety was increased following a potentiated 5-HT release during the stay on the X-maze [4]. Furthermore, central administration of 5-HT led to an anxiogenic-like behaviour [45]. Additionally, Cook and Sepinwall [14] found that non-selective 5-HT antagonists application induced an anxiolytic-like behaviour. On the other side, Murphy et al. [29] demonstrated that reduced serotonin tissue levels as a result of serotonin transporter deletion increased anxiety-like behaviours which is clearer in female mice than males. The obtained decrease in brain serotonin level caused by SCO treatment is agreeable with Ramakrishnan et al. [35] who reported that intraperitoneal injection of SCO to mice caused a significant decrease in serotonin level in comparison with the control group. Our data were disagreeable with Zaki et al. [46] who observed that administration of SCO at a dose of 20 mg\kg via intraperitoneal injection for seven days increased serotonin level in SCO treated rats compared to control rats. This difference in data is attributed to different doses, duration and tested animal strain, age and sex [2]. Further studies are highly required to evaluate the effect of different doses of SCO on the anxiolytic behaviour of rats as an animal model at different durations and doses and in various behavioural tests.

5 Conclusions

Our study suggested that long-term SCO treatment induced neurobehavioural alterations included an increase in locomotor and exploratory behaviours and a decrease in anxiety which was accompanied by low brain serotonin levels in rats as an animal model. However, further studies are required to investigate the effect of long-term SCO treatment on anxiety-related behaviour of rats using other anxiety assessment behavioural tests.

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Authors' contributions
All authors (AKA, AEL, HE, FK) equally contributed to this study. The conception OR design of the work: HE and AKA shared the work conception and design. Analysis: AKA performed the analysis of behaviour, AEL carried out the biochemical analysis, and AKA and FK performed the work statistical analysis. Interpretation, work drafting and revision of data: FK and AKA shared data writing and revision. Manuscript reviewing: HE reviewed the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials
The datasets generated and/or analysed during the current study are not publicly available but are available from the corresponding author if needed.

Declarations

Ethics approval and consent to participate
The experimental design was approved by Beni-Suef University Institutional Animal Treatment and Use Committee (BSU-IACUC), approval number (018.50). http://www.bsu.edu.eg/Content.aspx?section_id=3291&cat_id=43&lang=enln.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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