Ketamine induces schizophrenia-like condition in rats via amendment of neurotransmitters and behavior: antipsychotic effect of silkworm pupae

Gehan S. Georgy[a], Amany M. Gad[a,d], Hend Mohamed Anwar[b], Rehab H. Taha[c], Aliaa A. Hassan[b]

Departments of [a]Pharmacology, [b]Biochemistry, National Organization for Drug Control and Research, Egyptian Drug Authority (EDA), [c]Department of Sericulture Research, Agriculture Research Center, Giza, [d]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Sinai University, El Ismailia, Egypt

Correspondence to Gehan S. Georgy, PhD, Associate professor of pharmacology, Department of Pharmacology, National Organization for Drug Control and Research, Egyptian Drug Authority (EDA), 12511, Giza, Egypt. Tel: 01227622981; e-mail: gehan_gorgy11@hotmail.com

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Background
The pupae of mulberry silkworms, family Bombycidae, possess a great number of proteins that cover all of the necessary amino acids obligatory for well-being.
Objective
In this study, we aimed to evaluate the probable antipsychotic effect of pupae of mulberry silkworms in a rat model of schizophrenia prompted by ketamine on the cerebral cortex, hippocampus, and striatum, the brain areas involved in neuropsychiatric complaints.
Materials and methods
To this end, male albino rats were classified as follows: group 1 was the control group; group 2 animals were administered 135 mg/kg, p.o. silkworm pupae for 3 weeks; group 3 animals received vehicle for 3 weeks, and ketamine (30 mg/kg, i.p.) for the last 5 consecutive days of the experiment; and group 4 was the silkworm pupae and ketamine-treated group.
Results
The results revealed that treatment with silkworm pupae improved the exploration of schizophrenic rats in the novel object test and almost normalized their locomotor activity in the open field test. Additionally, silkworm pupae modulated the content of catecholamines and oxidative state in the cerebral cortex, hippocampus, and striatum of schizophrenic rats; however, the acetylcholine esterase activity was restored in the hippocampus only. Histopathological damages caused by ketamine are partially reduced by silkworm pupae.
Conclusion
Our data suggest that silkworm pupae, via neurobehavioral modulatory pathway, exhibit beneficial effects against psychomimetic influence of ketamine.

Keywords:
ketamine, neurotransmitters, novel object, open field, oxidative stress, schizophrenia, silkworm pupae

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Introduction

Silkworm pupae are used as biological nourishment, food material for animals, and traditional drug in countries of Asia. The pupae of mulberry and nonmulberry silkworms are a new existing source of protein with high-value essential amino acids vital for humanoid health [1]. Lately, proteins of silkworm pupae, with many essential amino acids, have been observed to have several biomedical uses [2]. Methionine, valine, and phenylalanine in addition to omega-3 fatty acids, minerals, and vitamins are constituents of silkworm pupae with intense antioxidant influence [3,4]. Wattanathorn *et al.* [5] referred to its ability to protect against Alzheimer's disease. Finally, it increases the vitality of the immune cells, prevents arteriosclerosis and thrombosis, as well as has hypoglycemic effects [2,6]. Consequently, silkworm pupae are recommended as food complement as its huge protein contents enrich its utilization in biomedical field.

Schizophrenia is a grave intellectual disorder that distresses about 1% of the population universally. Delusions, hallucinations, and agitation as well as cognitive impairments are among its clinical signs. Absence of motivation, blunted affect, and social loneliness are also elaborated in this complaint, generating an undesirable influence on the standard of patient health [7,8]. Previous studies have demonstrated neurotransmitters and oxidative imbalance in the pathophysiology of schizophrenia

[9–11]. Amalgamation of genetic and environmental issues exacerbates the condition [12]. Ketamine, in subanesthetic doses, yields hallucinations, obsession, as well as cognitive impairments reminiscent of schizophrenia [13–15]. The acute and frequent ketamine use is endorsed in the preclinical models to reconstruct the behavioral and neurochemical features of the syndrome [16,17]. Its metabolites are responsible for CNS activities allied with general anesthesia, and they mutually undergo hepatic biotransformation via cytochrome P450 [18]. Its bioavailability mainly depends on the route of administration with a relatively low protein binding and large volume of distribution [19]. Consequently, it rapidly crosses the blood–brain barrier to induce anesthesia [20]. Ketamine produces glucuronidated hydroxylated derivatives, which are more water-soluble compounds and simply expelled in urine [21].

As silkworm pupae have important antioxidant effects and neuromodulatory properties, we aimed to evaluate its possible antipsychotic effect in an animal model of schizophrenia on the cerebral cortex, hippocampus, and striatum, the brain areas involved in neuropsychiatric complaints.

Materials and methods

Animals

Adult male Sprague-Dawley albino rats (250–300 g) were obtained from National Organization for Drug Control and Research (NODCAR), Cairo, Egypt. They were retained in a well-ventilated room under appropriate conditions of temperature, humidity, and light (25±2°C, 60–70% humidity and 12 h dark and light cycles). They were nourished on a commercially ordinary diet; tap water was given *ad libitum*. Care and use of the animals were done under the direction of the Research Ethics Committee of National Organization for Drug Control and Research. Unnecessary disturbance of animals was avoided. Animals were handled smoothly and with embracing. The animal protocol was approved by the Ethical Committee of NODCAR (NODCAR/1/53/19).

Preparation of *Bombyx mori* pupae powder

The silk *Bombyx mori* pupae were obtained from Sericulture Research Department of Plant Protection Research Institute, Agriculture Center, Giza, Egypt. The *B. mori* pupae were separated from their sheath and dried at 60°C for a period of 4 days. Then, the dried pupae were grounded to powder, packed, and kept in the cool dry place for use.

Experimental design

After 2 weeks of acclimatization, rats were indiscriminately allocated into the following groups:

Group 1: it was the control group which received the vehicle (distilled water) orally for 3 weeks and then 0.9% saline i.p. for 5 days.

Group 2: animals treated with silkworm pupae orally at a dose 135 mg/kg for 3 weeks [5].

Group 3: animals received vehicle (distilled water) for 3 weeks, then symptoms of schizophrenia was induced by ketamine (30 mg/kg, i.p.) administered once a day for 5 consecutive days [22]. Ketamine hydrochloride (50 mg/ml; Troikaa Pharmaceutical, Ahmedabad, Gujarat, India) was used, diluted in 0.9% saline.

Group 4: animals received pupae for 3 weeks and then ketamine for 5 consecutive days.

Behaviors related to positive (locomotor activity) and cognitive (novel object recognition test) symptoms of schizophrenia were assessed. Then, rats were euthanized by decapitation, and brain areas (cerebral cortex, striatum, and hippocampus) were dissected and rapidly frozen at −80°C until the later assessments. Three animals from each group were set for histological studies by hematoxylin and eosin staining. The used animal debris was frozen till being incinerated.

Behavioral tests

Novel object recognition test

The apparatus is prepared from a polyvinyl arena. In adaptation phase, each rat was located and left for 10 min to freely discover the unfilled arena. On the succeeding day, two identical, familiar objects were put in opposite symmetrical corners, and each animal was placed with for 4 min. On the third day, one object was exchanged with a new shape-different one, and the animal was allowed to sightsee for 4 min. The time spent by the animal reconnoitering both objects was recorded. Discrimination ratio was calculated by dividing the time of empirical attempts toward the new object to both objects. Discrimination ratio designates the recognition memory [23,24].

Open field test

Open field test was carried out according to the former method argued by Sethi *et al.* [25]. During 3 min, the ambulation (horizontal locomotor activity) and the rearing (upright movements) frequencies were assessed.

Estimation of neurotransmitters content

Monoamines, serotonin (5-HT), as well as norepinephrine and dopamine (NE and DA) were assessed in the cerebral cortex, hippocampus, as well as striatum according to Ciarlone [26]. The process is established on a fluorometric test as a fluorescent product outcome from reaction with ortho-phthalaldehyde on assessment of 5-HT and alkaline sulfite-iodine solution on evaluating NE and DA.

Estimation of choline esterase activity

Choline esterase (ChE) content in the cerebral cortex, hippocampus, as well as striatum was evaluated using the kit reagents provided by Biodiagnostic (Giza, Egypt). Owing to the reaction between thiocholine and DTNB, a yellow compound (2-nitro-5-mercaptobenzoate) was obtained and measured at 405 nm.

Estimation of oxidative stress biomarkers

The cerebral, hippocampal, and striatal thiobarbituric acid reactive substance as well as reduced glutathione (GSH) were evaluated using the kit reagents delivered by Biodiagnostic.

Histopathological study

Autopsy samples from the cerebral cortex, hippocampus, and striatum of rats representing different groups were preserved in 10% neutral formalin solution for 24 h. Samples were cleared in xylene and implanted in paraffin wax at 56°C in hot air oven for 24 h. The blocks were organized for segmenting at 4-µm thicknesses by a sledge microtome. The tissue stained with hematoxylin and eosin was inspected using a light electron microscope [27].

Statistical analysis

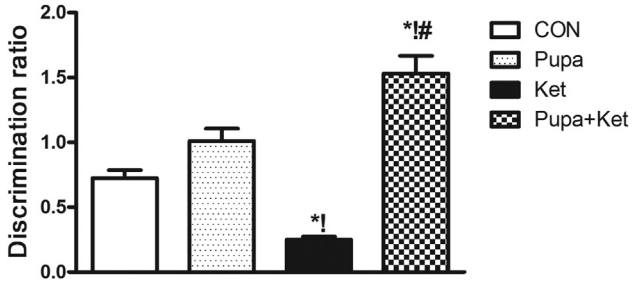
Prism computer program (Graph Pad software Inc. V5, San Diego, California, USA) was used to perform statistical analysis. *P* value less than or equal to 0.05 was deemed to be significant statistically. All data were stated as means±SE. A statistical comparison between groups was accomplished using one-way analysis of the variance followed by Tukey–Kramer multiple comparison test.

Results

Effect of silkworm pupae on behavioral alterations in the novel object recognition test in schizophrenic rats

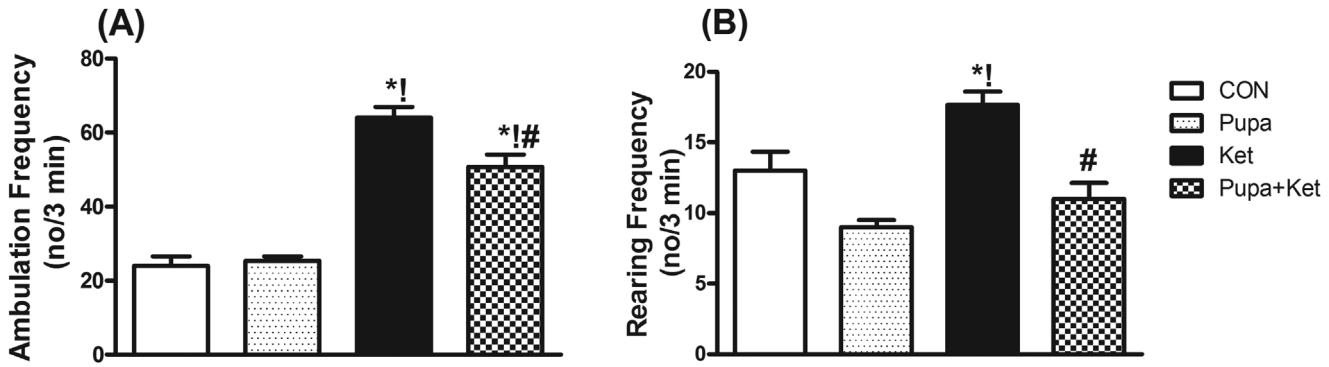
As shown in Fig. 1, rats treated with ketamine were less able to reconnoiter the new object, as displayed through debility in the discrimination ratio compared with the normal control group. Pretreatment with silkworm pupae in ketamine-received rats improved the exploration of the novel object and elevated the

Figure 1



Effect of oral treatment with silkworm pupae (135 mg/kg) daily for 3 weeks on discrimination ratio – novel object recognition test in schizophrenic rats. Data represents mean±SEM (*n*=6). *Significant from control, !significant from silkworm pupae (Pupae), #significant from ketamine (Ket) at *P* value less than or equal to 0.05.

Figure 2



Effect of oral treatment with silkworm pupae (135 mg/kg) daily for 3 weeks on behavioral alterations, ambulation frequency (a), and rearing frequency (b) in open field test in schizophrenic rats. Data represents mean±SEM (*n*=6). *Significant from control, !significant from silkworm pupae (Pupae), #significant from ketamine (Ket) at *P* value less than or equal to 0.05.

discrimination ratio compared with ketamine-induced group.

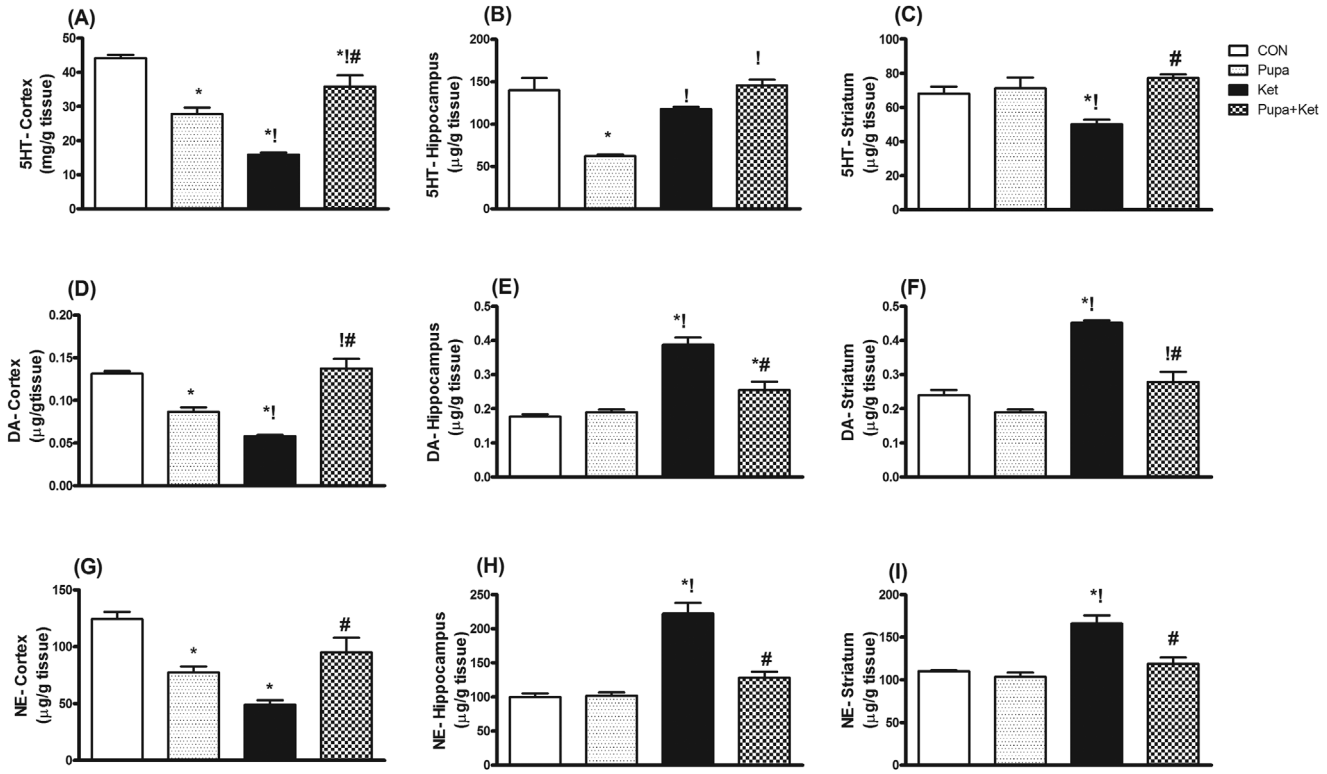
Effect of silkworm pupae on behavioral alterations in the open field test in schizophrenic rats

As shown in Fig. 2, rats that received ketamine showed significant elevation in both the horizontal activity (ambulation frequency) to 2.7 folds and the vertical activity (rearing frequency) by 31% compared with control rats. On the contrary, ambulation and rearing frequencies were significantly reduced in rats treated with silkworm pupae compared with ketamine-treated rats.

Effect of silkworm pupae on neurotransmitters alterations in schizophrenic rats

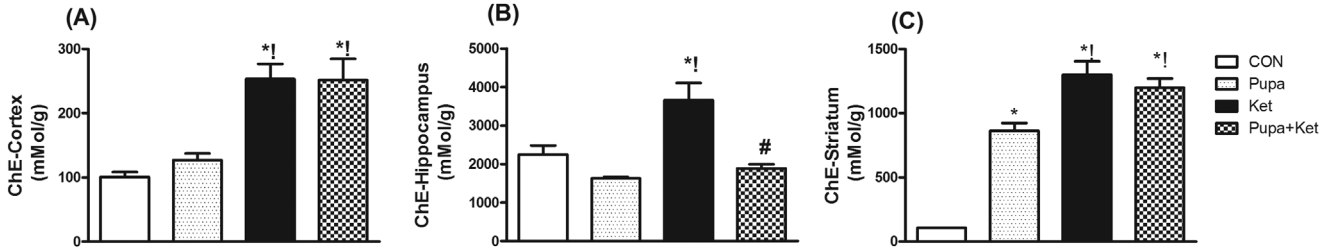
As shown in Fig. 3a–c, 5-HT was significantly reduced in the cerebral cortex and striatum brain area by 64 and 26%, respectively, in rats under the effect of ketamine compared with control rats. Additionally, 5-HT was reduced in both cortical and hippocampal regions owing to silkworm pupae treatment. On the

Figure 3



Effect of oral treatment with silkworm pupae (135 mg/kg) daily for 3 weeks on neurotransmitters contents, serotonin (a, b, c), dopamine (d, e, f), and norepinephrine (g, h, i) (µg/g) in the cerebral cortex, hippocampus, and striatum of schizophrenic rats. Data represents mean ±SEM (n=5–7). *Significant from control, !significant from silkworm pupae (Pupae), #significant from ketamine (Ket) at P value less than or equal to 0.05.

Figure 4



Effect of oral treatment with silkworm pupae (135 mg/kg) daily for 3 weeks on cholinesterase (ChE) activity (U/g) in the cerebral cortex, hippocampus, and striatum of schizophrenic rats. Data represents mean±SEM (n=6). *Significant from control, !significant from silkworm pupae (Pupae), #significant from ketamine (Ket) at P value less than or equal to 0.05.

Table 1 Effect of oral treatment with silkworm pupae (135 mg/kg) daily for 3 weeks on oxidative stress parameters, MDA (nmol/g) and glutathione (mg/g) in the cerebral cortex, hippocampus, and striatum of schizophrenic rats

	Cerebral cortex		Hippocampus		Striatum	
	MDA (nmol/g)	GSH (mg/g)	MDA (nmol/g)	GSH (mg/g)	MDA (nmol/g)	GSH (mg/g)
CON	192.3±14.75	2.9±0.15	825.8±1.6	4.6±0.08	572±7.7	5.1±0.3
Pupa	180.2±11.64	2.3±0.1*	834.7±8.0	5.0±0.07	571.3±7.9	5.1±0.2
Ket	158.5±5.1	1.7±0.04*!	1152±60.9*!	3.6±0.04*!	1217±45.9*!	2.6±0.1*!
Pupa+Ket	112.4±1.6*!#	2.4±0.07*#	845.7±9.4 #	6.1±0.24*!#	346.5±19.7*!#	4.9±0.2 #

contrary, its treatment nearly normalized 5-HT content in cerebral cortex as well as striatum in rats under the effect of ketamine.

Concerning DA and NE, Fig. 3d–i reveals that ketamine administration reduced catecholamine level in the cerebral cortex by 54% in case of DA and 66% in case of NE. However, their level was enhanced in hippocampus and striatum following ketamine treatment. Regarding the group pretreated with silkworm pupae, DA and NE contents were almost restored in all regions.

Effect of silkworm pupae on choline esterase activity in schizophrenic rats

As shown in Fig. 4a–c, ChE activity was highly elevated in the cerebral cortex, hippocampus, and striatum in ketamine-treated group compared with the control group. Pre-administration of silkworm pupae followed by ketamine normalized ChE activity in the hippocampus brain area. This effect was not shown in either cerebral cortex or striatum.

Effect of silkworm pupae on MDA and glutathione contents in schizophrenic rats

As shown in Table 1, hippocampal and striatal malondialdehyde (MDA) contents were highly increased in the ketamine group compared with the control group. These results were upturned significantly in silkworm-administered rats. Concerning GSH, its content was reduced in ketamine-administered rats in each brain region relative to normal rats. However, silkworm pupae administration augmented GSH level in cerebral cortex, hippocampus, and striatum compared with ketamine-treated rats.

Effect of silkworm pupae on histopathological examination in schizophrenic rats

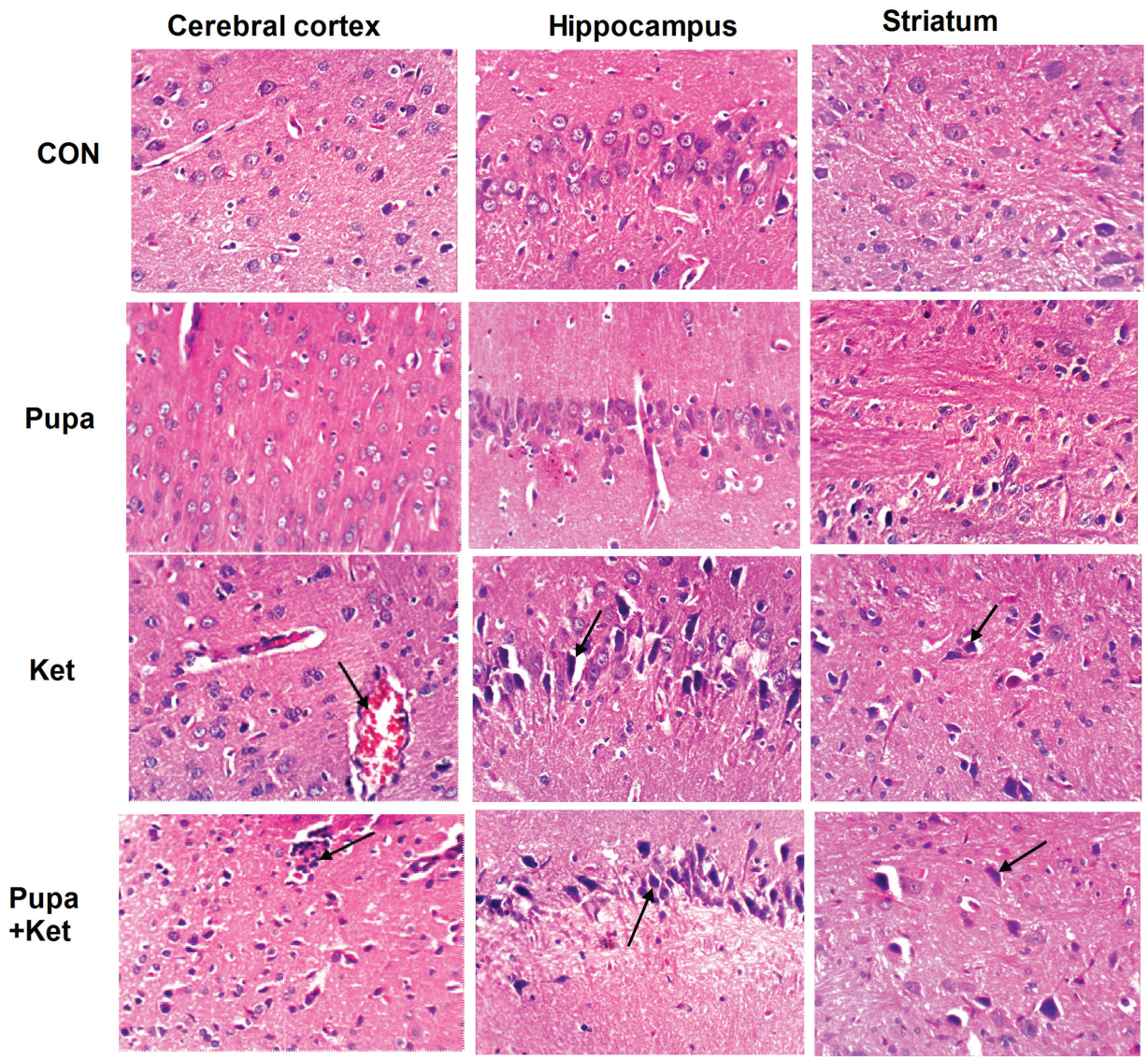
Figure 5 displays that control as well as rats treated with silkworm pupae revealed normal appearance of neurons of the cerebral cortex, hippocampus, and striatum. On the contrary, rats treated with ketamine exhibited necrosis of neurons in all studied brain areas, in addition to congestion of the cerebral cortex with focal gliosis and focal hemorrhage in hippocampus. These observations were mildly improved in schizophrenic rats treated with silkworm pupae.

Discussion

Management of schizophrenia is a great challenge. To our knowledge, this study is the first to inspect the effect of pupae silkworm using an animal model of psychosis. The antipsychotic activity of pupae silkworm was examined in three brain areas convoluted in neuropsychiatric syndrome (cerebral cortex, hippocampus, and striatum). The study shows that pre-administration of pupae reverses the behavioral, neurotransmitters alterations, histopathological, as well as oxidative stress parameters induced by administration of ketamine. The effect of ketamine could be related to synergistic effects on several pathways by acting on monoaminergic, muscarinic receptors, all being convoluted in neuropsychiatric diseases [28].

In the current study, behavioral tests linked to locomotor and cognitive-memory symptoms of schizophrenia were assessed via open field test and novel object recognition test, respectively. Schizophrenic rats exhibited hyperlocomotion presented by increased ambulation and rearing frequencies in the open field test, whereas cognitive dysfunction was approved by reduced discrimination ratio in the novel object recognition test. Increased locomotor activity in rats corresponds to psychomotor agitation in schizophrenia [29]. The detected hyperlocomotion and impairment of cognitive functions following ketamine administration has been moderately endorsed to N-methyl-d-aspartate receptors blockade and indirect DA agonist activity of ketamine, therefore, increase glutamate and DA release [11]. Herein, ketamine-treated rats showed DA elevated contents in hippocampus and striatum versus reduced cerebral cortex content. Thus, in schizophrenia, hippocampal and striatal regions undergo hyperdopaminergia, whereas the cerebral

Figure 5



Representative photomicrographs of the cerebral cortex, hippocampus, striatum of control (CON), silkworm pupae (Pupa), ketamine (Ket) and Ket-pretreated with Pupae (Pupa+Ket) groups, showing normal intact tissues in control groups (CON, Pupa), whereas the Ket group displayed marked necrosis, pyknosis and atrophy of neurons, congestion of cerebral cortex with focal gliosis and hippocampal focal hemorrhage. These damages are partially reduced in Pupa+Ket group.

cortex shows hypodopaminergia [30,31]. Ketamine-NMDA antagonist effect shows the changes occurred in neurotransmitter systems, thus promotes a decrease in DA release in the cortex. This effect explains the present reduction in cortical DA and NE contents, which may be accountable for the observed cognitive deficiency [32]. Another effect of ketamine is its anticholinergic outcome, displaying memory dysfunction, as ketamine is an antagonist to nicotinic acetylcholine receptor alpha-7 [33]. Acetylcholine plays a serious role in the early phase construction of memory [34]; thus, the anticholinergic effect of ketamine redirects the animal performance in the novel object recognition test, showing memory impairment.

In harmony with our findings, Irifune *et al.* [35] showed that ketamine induced a marked hyperlocomotion in rodents through DA agonist activity and diminished cognition through disruption of long-term potentiation in schizophrenics compared with control in some studies on animal species [36–39].

Collectively, the elevated levels of DA in our study are explained by dopaminergic hyperactive function hypothesis [40,41]. Chatterjee *et al.* [42] revealed that ketamine administration caused changes in dopaminergic and serotonergic neurotransmission. The present reduction in serotonergic system in studied areas may be explained by increase in the density of 5-HT transporters in different brain

regions [40]. Postmortem studies propose that schizophrenia is related with overactivity of DA and lack of 5-HT roles [43]. Our findings reveal that pupae have the ability to restore and improve monoaminergic levels in the studied areas. The mental memory is enhanced by the metabolites of silkworm, identified as Brain Gold, by stimulating nucleic acid and monoamine neurotransmitters synthesis [2]. Moreover, the elevated striatal DA and NE contents in ketamine group, responsible for over motor activity, was normalized by previous administration of pupae in schizophrenic rats. According to some studies [42,44], antipsychotic drugs oppose the hypermotor activity persuaded by ketamine. This effect was attained in the pupae-treated group.

Oxidative stress is an essential issue described in the pathophysiology of schizophrenia [10,45]. Increased reactive oxygen species levels generate harmful effects on signal transduction, frequently by boosting membrane lipid peroxidation and protein and nucleic acid damage [46]. Likewise, Behrens and Sejnowski [47] and Monte *et al.* [48] stated that ketamine in subanesthetic dose provoked brain oxidative stress in mice. In the current study, administration of pupae altered GSH and MDA levels in the cerebral cortex, hippocampus, and striatum. In agreement, several studies referred to the antioxidant effect of silkworm pupae in various conditions [3,49]. GSH increased redox-sensitive protein activity such as NMDA and DA receptors [50], thus modulating neurotransmitter functions of glutamatergic and dopaminergic pathways [51]. Therefore, the increase in GSH levels and the resultant decrease in MDA levels in treatment group could explain the valuable effects of its antipsychotic properties. The beneficial effect of silkworm pupae is probably due to the presence of essential amino acids, omega-3 fatty acids, minerals, and vitamins [2].

In schizophrenic rats, the observed alterations in behavior as well as neurotransmitters are confirmed by necrosis of pyramidal neurons in brain areas and focal gliosis in the cerebral cortex as manifest by the histopathological findings. Rudin *et al.* [52] showed that subanesthetic dose of ketamine caused apoptosis and neuron damage in neonatal mice, confirmed by DNA fragmentation, in different brain regions. Conversely, in the current study, pupae by preserving monoamines and improving oxidative state triggered neuroprotection, revealed as improvement of the concomitant behavioral deficits.

Conclusion

Finally, our data suggest that silkworm pupae offer beneficial effects against psychomimetic effects of ketamine probably via neuromodulatory pathway and antioxidant effect, indicating that it may be an adjuvant approach for the development of new therapeutic strategies within this psychiatric disorder. Future studies evaluating the potential effects of silkworm pupae proteins for the benefit of human health would be welcomed to enhance our results.

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Authors' contribution: A.A.H. and R.H.T. developed the idea of the research. All authors designed the research. R.H.T. prepared the pupae powder. A.M.G., H.M.A., and A.A.H. carried out the experiment. G.S.G. and A.M.G. carried out the behavioral tests and isolation of brain regions. G.S.G., A.M.G., and H.M.A. carried out the biochemical tests and construed the statistics. G.S.G. was the main author in writing the manuscript. All authors revised the final manuscript.

Conflicts of interest

There are no conflicts of interest.

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