Original Research

Behavioural effects of vitamin C with haloperidol in mice

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Abstract

Vitamin C causes a significant change in pharmacological effects of some drugs which may lead to unpredictable responses. Vitamin C-haloperidol interaction has not been confirmed *in vivo*. This study was aimed to investigate the influence of vitamin C on some pharmacological effects of haloperidol (extrapyramidal side effects caused by the antipsychotic haloperidol). Albino male mice (n = 24, body weight of 20 - 40 gm) were divided into four groups and each of consists of six mice. Group I (control): given 1% Tween 80 solution, group II: given vitamin C in a dose of 100 mg/kg, group III: given haloperidol (2 mg/kg), while group IV: given a combination of vitamin C and haloperidol. All the treatments were given by intraperitoneal route of administration. Three sub-acute doses of different treatments were given at 24:0, 5:0, 1:0 hour before scoring. Parameters scored were catalepsy, ptosis, rigidity and akinesia. The experiment was repeated using vitamin C in a dose of 500 mg/kg. Both doses of 100 and 500 mg/kg of vitamin C significantly antagonized the effect of haloperidol by decreasing all the tested parameters. The results indicate that vitamin C decreases extrapyramidal side effects caused by the antipsychotic haloperidol and show that it successfully decreases catalepsy, ptosis, rigidity and akinesia in mice.

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Keywords: Behaviour, haloperidol, interaction, mouse, vitamin C

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Introduction

Drug-drug interaction is the pharmacologic or clinical response to the administration of drug combination different from that anticipated from the known effect of the two agents when given alone [1]. Drug interaction may involve a change in the pharmacokinetic or pharmacodynamic aspects of the drugs [2]. Haloperidol is a high potency first-generation (typical) antipsychotic and one of the most frequently used antipsychotic medications worldwide [3]. Haloperidol has demonstrated pharmacologic activity at several receptors in the brain [4] which exerts its antipsychotic effect through strong antagonistic action on the dopamine receptor (D_2), particularly within the mesolimbic and mesocortical systems of the brain [5]. Haloperidol improves the psychotic symptoms by halting the over-production of dopamine [6]. However, haloperidol treatment includes several extrapyramidal side effects produced as a result of drug-induced damage to the basal ganglia. In addition, it causes irregular movements and locomotor patterns in experimental animals [7].

Vitamin C is a water-soluble vitamin and an essential nutrient. It is found in variable quantities in fruits and vegetables and organ meats [8]. It is an essential and vital for growth and development in many multicellular organisms in humans. It also plays an important role in many physiological processes in humans. It is needed for the repair of tissues in all body parts. One of the important functions of vitamin C includes the formation of protein used to make skin, tendons, ligaments, and blood vessels for healing wounds and forming scar tissue, for repairing and maintaining cartilage, bones and teeth and also has a role in the absorption of iron. It can also act as a reducing and capping agent for metal nanoparticles [9]. Reports have suggested an increase by vitamin C of haloperidol treatment of patients with schizophrenia. A combination

of vitamin C significantly more effective than used alone as antipsychotic role in treatment of psychosis due to catalepsy induced by haloperidol was attenuated by vitamin C [10] and it may potentiate catalepsy induced by haloperidol in rats and squirrel monkeys [11]. However, the addition of vitamin C was not found with any change in psychopathology nor has pharmacokinetic interaction with haloperidol. Accordingly, the purpose of this study is to investigate the influences of two doses of vitamin C on some pharmacological effects of haloperidol.

Materials and methods

Haloperidol was obtained from Janssen Company, Belgium. Vitamin C was obtained from Rotexmedica Company, Trittau, Germany. Healthy male Albino Swiss Webster mice of body weight ranging of 20 to 40 gm bred locally in the animal house of Faculty of Pharmacy, University of Tripoli, Libya. Mice were kept under a standard fixed condition area of temperature and humidity as well as light-dark cycle until used. Ethic approval for the experimental methodology has been obtained from Research Ethics Committee for animal use, Faculty of Pharmacy, University of Tripoli (3/2020) according to the International Ethical Guidelines for the Use of Animals in Research.

Experimental design: Mice (n = 24) were divided into four groups, each group consists of six mice. Group I: is control group and was given 1% Tween 80 solution, group II: is given vitamin C in a dose of 100 mg/kg, group III: is given haloperidol (2 mg/kg) and group IV: is given a combination of vitamin C and haloperidol. All the treatments were given by intraperitoneal route of administration. Sub-acute administration was applied in three doses 24, 5:00 and 1:00 hour before scoring. Mice adapted in the experimental area for three days before starting the experiment (accommodation area). Parameters scored were catalepsy, ptosis, rigidity and akinesia [12]. The experiment was repeated by using vitamin C in a dose of 500 mg/kg.

Catalepsy: it is a reduced ability to initiate movement and failure to achieve a correct posture, it was measured by the bar test. Mice are positioned so that their hindquarters are on the bench and their forelimbs rest on a one cm diameter horizontal bar elevated by four cm above the bench. Mice are judged to be cataleptic if, they maintain this position for the 30 sec or more. The length of time for which the mouse maintains this position is recorded with a stopwatch with a maximum duration of 180 sec. This procedure is performed 30 min after the administration of haloperidol (2 mg/kg). If the mouse holds the position for the 30 sec or more then catalepsy is said to be induced.

Akinesia test: the mouse is held by the tail so that it is standing on forelimbs only and moving on its own. The number of steps taken with both forelimbs was recorded during the 30 sec trial.

The tremor: it is scored visually in mice using the rating scale: 0: no tremor, 1: occasional isolated twitches, 2: moderate or intermittent tremor associated with short periods of calm and 3: pronounced continuous tremor.

Ptosis: it is scored: 4: eyes completely closed, 2: half-open eyes and 0: wide-open eyes, with 1 and 3 indicating intermediate values.

Righting reflex: it is evaluated by turning the mouse onto its back five times. Normal mice immediately turn themselves over, to right themselves onto all four feet. Righting reflex was scored as follows: 0: no impairment, 1: on side one to two times, 2: on side three to four times, 3: on side five times, 4: on back one to two times, 5: on back three to four times, 6: on back five times, 7: sluggish when placed on back and 8: righting response absent when on back and tail pinched.

Data analysis: Data are presented as the mean ± SEM and analyzed using SPSS version 16. One-Sample Kolmogorov-Smirnov test was applied to find out if the data is parametric or not; if the data is parametric, one-way analysis of variance (ANOVA) followed by LSD (Least Significant Difference). If the data is non-parametric, two-tailed Mann Whitney-U test is used, p value < 0.05 considered statistically significant.

Results

Effect of vitamin C on catalepsy induced by haloperidol: Vitamin C in a dose of 500 mg/kg did not show any catalepsy sign compared to that of the control-treated group while haloperidol and the combined treatment of haloperidol with vitamin C produced catalepsy significantly compared to the control-treated group. The combined treatment of haloperidol and vitamin C produced a profound significant decrease in the catalepsy compared to haloperidol-treated group. While the combined treatment produced a highly increased in the catalepsy compared to vitamin C treated group (**Figure 1**).

In **Figure 2**, vitamin C in a dose of 100 mg/kg did not show any catalepsy sign compared to the control-treated group while haloperidol and the combined treatment of haloperidol with vitamin C produced catalepsy significantly compared to the control-treated group. The combined treatment of haloperidol and vitamin C produced a significant decreased in catalepsy compared to haloperidol treated group. While the combined treatment produced a highly increased in the catalepsy compared to vitamin C treated group.

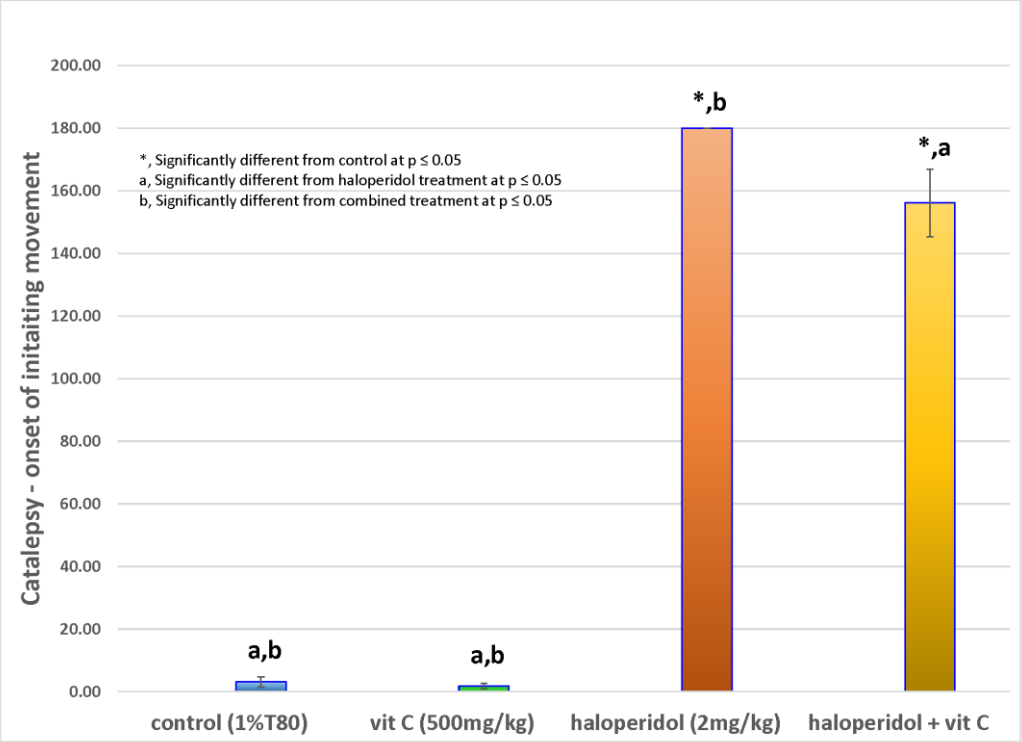

Figure 1: Effect of vitamin C (500 mg/kg) on catalepsy induced by haloperidol in mice

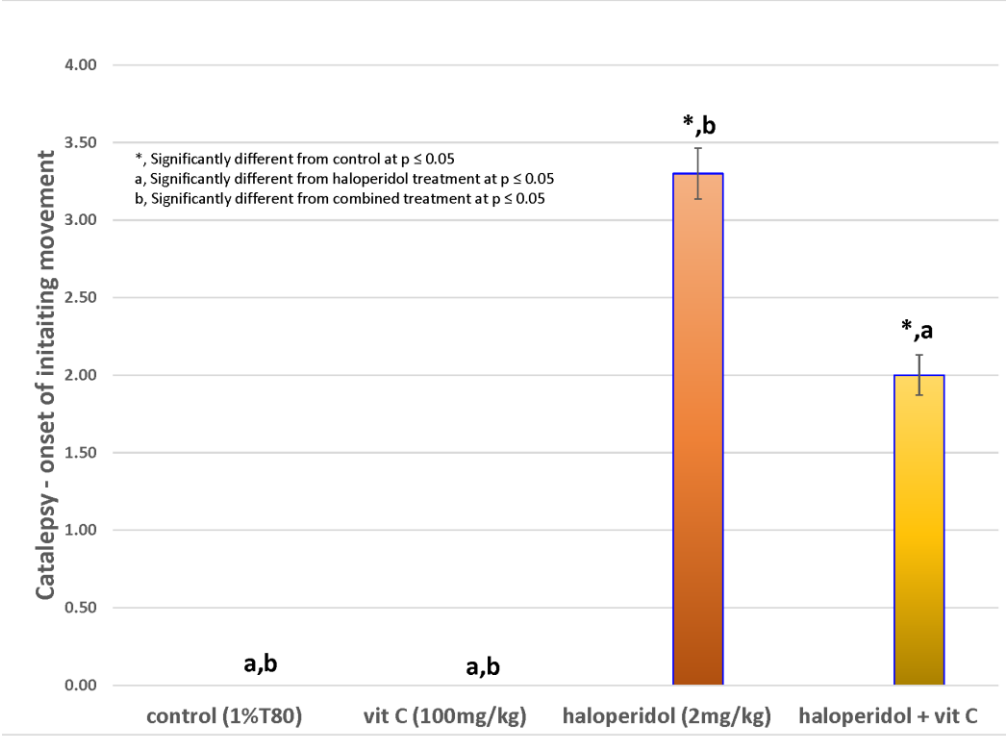

Figure 2: Effect of vitamin C (100 mg/kg) on catalepsy induced by haloperidol in mice

Effect of vitamin C on ptosis induced by haloperidol:

Vitamin C (500 mg/kg) did not show any ptosis sign compared to the control-treated group while haloperidol and the combined treatment of haloperidol with vitamin C produced ptosis significantly compared to the control-treated group. The combined treatment of haloperidol and vitamin C produced a significant decreased in ptosis compared to haloperidol-treated group. While the combined treatment produces a highly increased in the ptosis compared to vitamin C treated group (**Figure 3**). Eye closure was visually scored using the following scale: 0: normal opening, 1: eyes 1/4 closed, 2: eyes 1/2 closed, 3: eyes 3/4 closed and 4: completely closed.

In Figure 4, Vitamin C in a low dose (100 mg/kg) did not show any ptosis sign compared to the control-treated group while haloperidol and the combined treatment of haloperidol with vitamin C produced ptosis significantly compared to the control-treated group. The combined treatment of haloperidol and vitamin C produced a significant decreased in ptosis compared to haloperidol treated group, while the combined treatment produces a profound increase in the ptosis compared to vitamin C treated group.

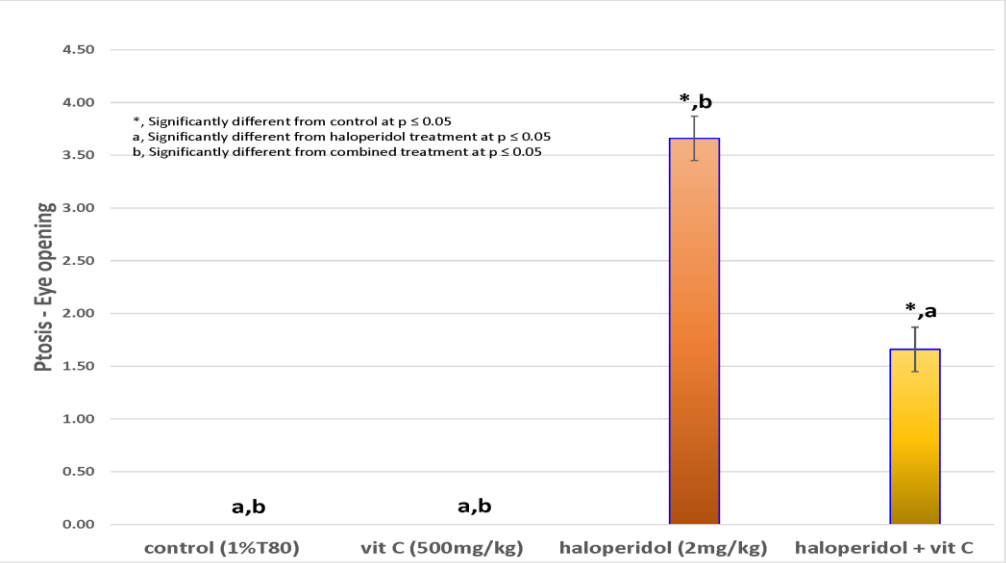

Figure 3: Effect of vitamin C (500 mg/kg) on ptosis induced by haloperidol in mice.

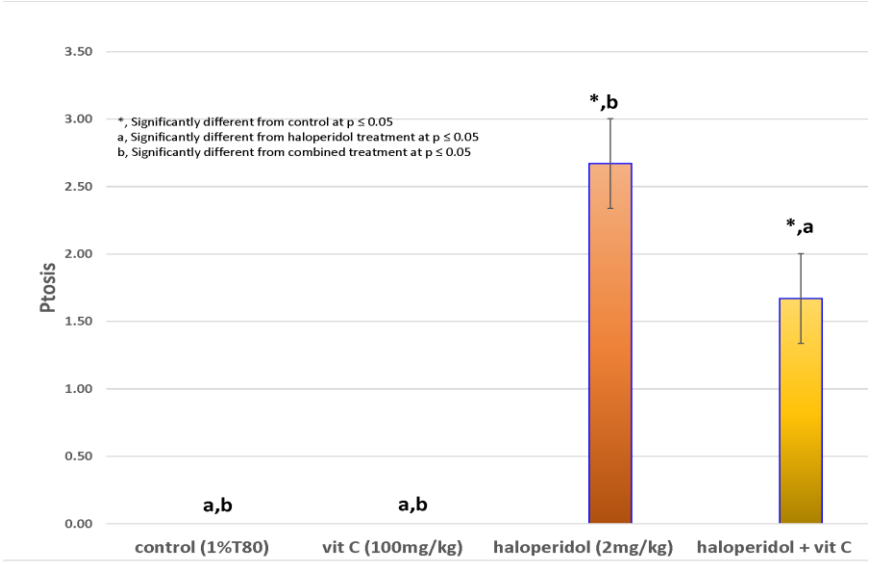

Figure 4: Effect of vitamin C (100 mg/kg) on ptosis induced by haloperidol in mice

Effect of vitamin C on rigidity induced by haloperidol

Vitamin C in a high dose (500 mg/kg) did not show any rigidity sign compared to the control-treated group while haloperidol and the combined treatment of haloperidol with vitamin C produced rigidity significantly compared to the control-treated group. The combined treatment of haloperidol and vitamin C produced a significant decreased in mouse rigidity compared to haloperidol treated group while the combined treatment produced a highly increased in rigidity compared to vitamin C treated group (**Figure 5**).

In **Figure 6**, vitamin C in 100 mg/kg did not show any rigidity sign compared to the control-treated group while haloperidol and the combined treatment of haloperidol with vitamin C produced rigidity significantly compared to the control-treated group ($p < 0.01$). The combined treatment of haloperidol and vitamin C produced a significant decreased in rigidity compared to the haloperidol treated group ($p < 0.05$) while the combined treatment produces a highly increased in the rigidity compared to vitamin C treated mice ($p < 0.01$), (**Figure 6**).

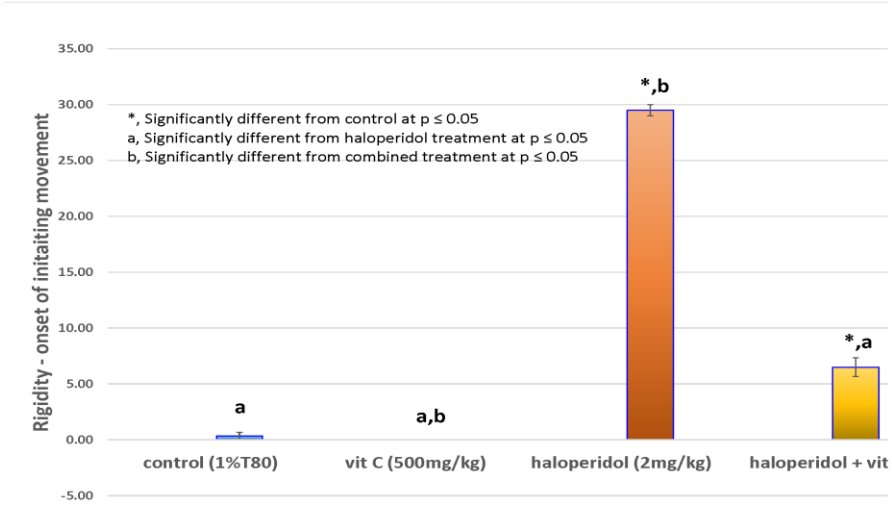

Figure 5: Effect of vitamin C (500 mg/kg) on rigidity induced by haloperidol in mice

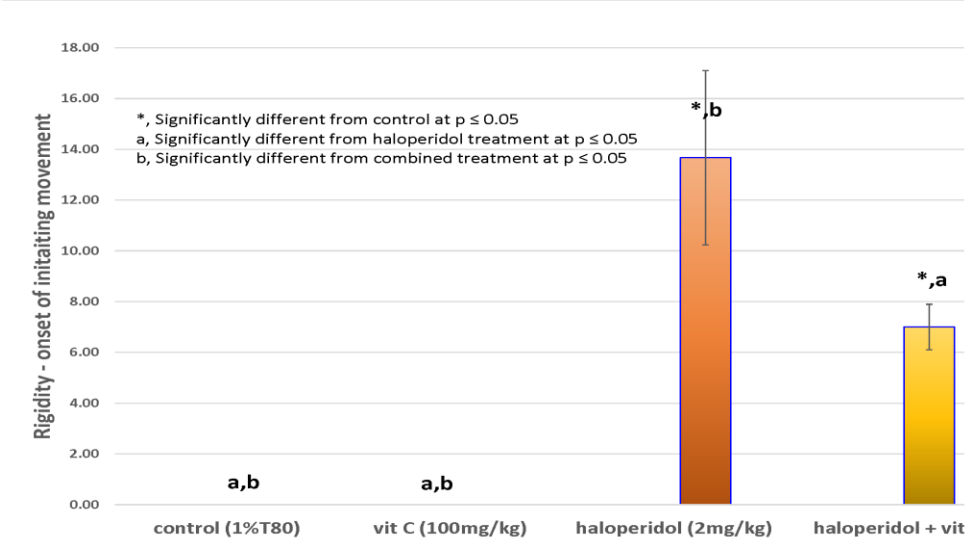

Figure 6: Effect of vitamin C (100 mg/kg) on rigidity induced by haloperidol in mice

Effect of vitamin C on akinesia induced by haloperidol

Vitamin C (500 mg/kg) did not show any akinesia sign compared to the control-treated group while haloperidol and the combined treatment of haloperidol with vitamin C produced akinesia significantly compared to the control-treated group. The combined treatment of haloperidol and vitamin C produced a very highly significant decreased in akinesia compared to haloperidol treated group ($p < 0.001$) while the combined treatment produced a highly increased in the akinesia compared to vitamin C treated mice (**Figure 7**).

Vitamin C in a low dose (100 mg/kg) did not show any akinesia sign compared to the control-treated group while haloperidol ($p = 0.000$) and the combined treatment of haloperidol with vitamin C ($p = 0.000$) produced akinesia significantly compared to the control-treated group. The combined treatment of haloperidol and vitamin C produced a significant decreased in akinesia compared to haloperidol treated group ($p < 0.05$) while the combined treatment produced a very highly increase in the akinesia compared to vitamin C treated group ($p < 0.001$), (**Figure 8**).

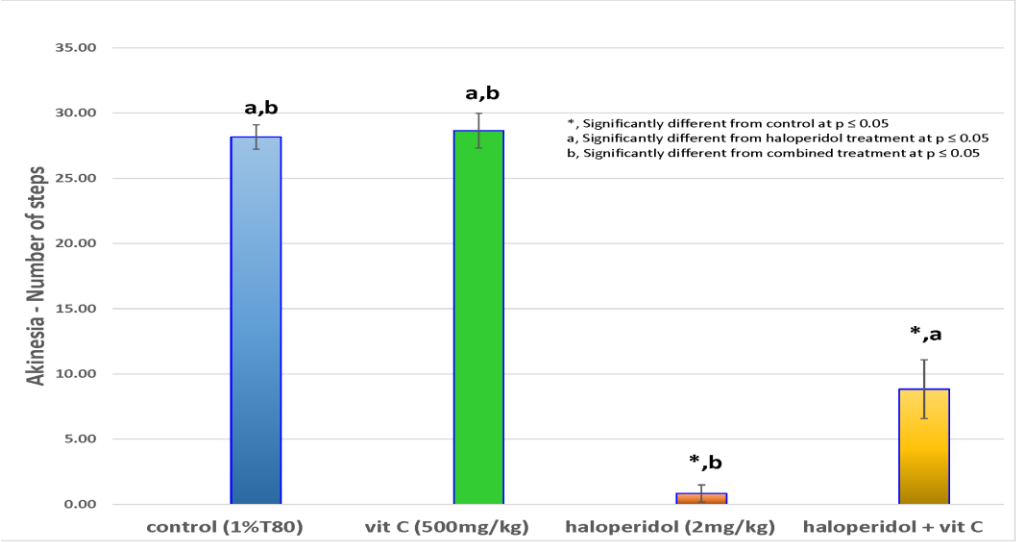

Figure 7: Effect of vitamin C (500 mg/kg) on akinesia induced by haloperidol in mice for 30 min.

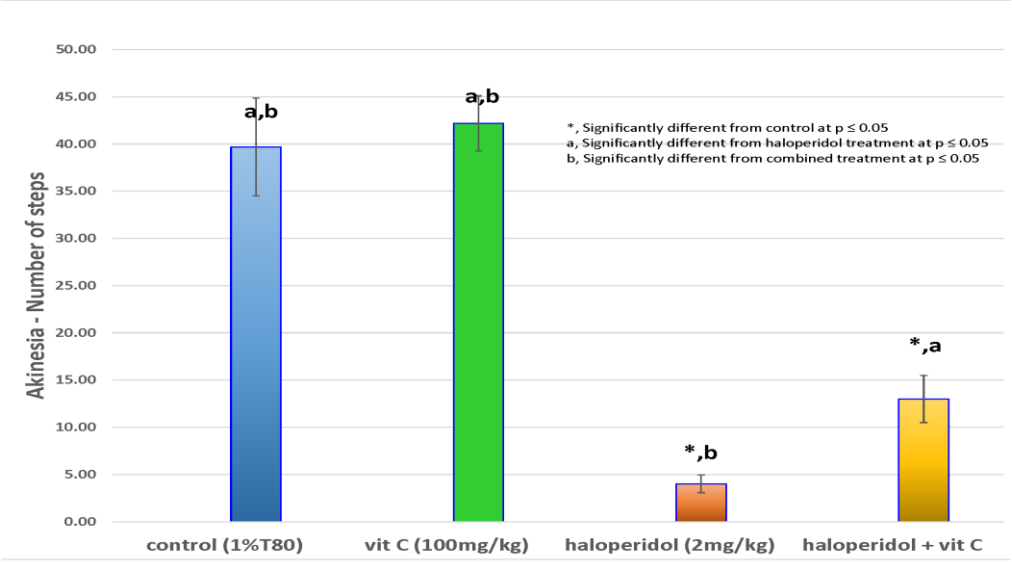

Figure 8: Effect of vitamin C (100 mg/kg) on akinesia induced by haloperidol in mice for 30 min.

Effect of vitamin C on akinesia induced by haloperidol for 180 min

Effect of high dose of vitamin C (500 mg/kg) on akinesia induced by haloperidol for 180 min is shown in **Figure 9**. Scoring akinesia for 180 min, there is no significant difference between control-treated groups after 90 min and after 180 min compared to akinesia after 30 min of drug injection. The statistical analysis showed no significant difference between vitamin C treated groups after 90 min and after 180 min compared to akinesia after 30 min of drug injection. Haloperidol treated group did not show any difference of akinesia after 90 min and after 180 min compared to akinesia after 30 min of drug injection. The combined treatment of haloperidol and vitamin C did not show any difference in inducing akinesia after 90 min and after 180 min compared to akinesia after 30 min of drug injection (**Figure 9**).

Effect of the low dose of vitamin C (100 mg/kg) on akinesia induced by haloperidol for 180 min is shown in **Figure 10**. Scoring akinesia for 180 min, there is no significant difference between control-treated groups after 90 min and after 180 min compared to akinesia after 30 min of drug injection. The statistical analysis showed is no significant difference between vitamin C treated groups after 90 min and after 180 min compared to akinesia after 30 min of drug injection. Haloperidol treated group did not show any difference of akinesia after 90 min and after 180 min compared to akinesia after 30 min of drug injection. The combined treatment of haloperidol and vitamin C treated group did not show any difference of akinesia after 90 min and after 180 min compared to akinesia after 30 min of drug injection (**Figure 10**).

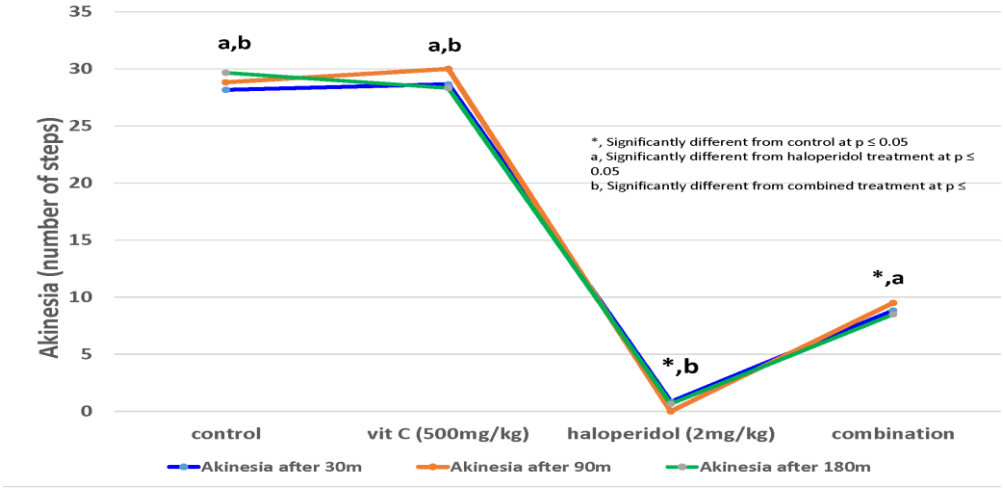

Figure 9: Effect of vitamin C (500 mg/kg) on akinesia induced by haloperidol in mice for 180 min.

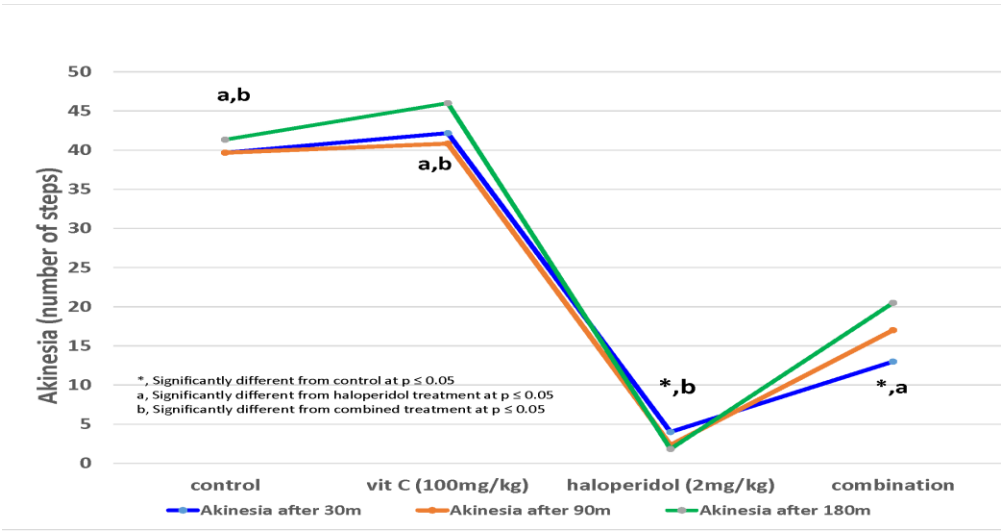

Figure 10: Effect of vitamin C (100 mg/kg) on akinesia induced by haloperidol in mice for 180 min.

Discussion

In this study, antipsychotic haloperidol produced akinesia, ptosis, rigidity and catalepsy as side effects. These extrapyramidal side effects were significantly antagonized by vitamin C intake. Vitamin C reduced these effects but not abolished them and higher doses may need to completely abolish. Tremor and righting reflex was not observed in haloperidol treated group, a higher dose of haloperidol may show such effects. In the brain, dopamine affects movement, emotions and the reward system [13]. It affects various functions from hypertension and hormonal regulation to voluntary movement and reward [14]. Vitamin C may act to increase dopamine neurons number, following transplantation by stimulating dopaminergic differentiation of neural precursors from the fetal ventral mesencephalon [15]. An increase in oxidative stress levels after classical antipsychotics treatment reported. Haloperidol induced a six-fold increase in reactive oxygen species (ROS) and by antioxidants treatment, it lowered ROS levels and protection of the cells was observed [16]. Haloperidol treatment causes dopaminergic receptor blockade resulting in increased dopamine turnover rate. This can lead to generation of ROS by-products of its metabolism [17]. Treatment with antioxidant significantly reversed the oxidative effects of haloperidol [18]. The decrease in enzymatic activities of superoxide dismutase and glutathione peroxidase is due to haloperidol induce a decrease in the genetic expression of these enzymes [19]. The antioxidant vitamin E reduces haloperidol-induced impairment of locomotor activity in rats [20]. Currently, vitamin C antagonizes haloperidol behavior, which may be through its antioxidant properties. Oxidative stress caused by free radicals may cause death of substantia nigra dopamine neurons as in Parkinson's disease [15]. Vitamin C in a certain dose, increased the expression of dopamine neurons; it also significantly increased the number of dopamine neurons compared with non-treated controls with an almost two-fold increase in dopamine neurons being observed [21]. This could be one of the mechanisms vitamin C antagonize haloperidol's side effects. Catalepsy induced by haloperidol is through repeated antagonism of D_2, but not D_1-like receptors [22]. Catalepsy, induced by haloperidol, was attenuated by vitamin C [23]. Vitamin C can increase the amount of dopamine, by decreasing dopamine auto-oxidation and this leads to stimulation of D_1 and D_2 receptors [15]. Vitamin C antagonize catalepsy induced by haloperidol; this may be through its antioxidant and increasing the survival of dopamine neurons and by activating differentiation pathways and promoting the dopaminergic differentiation of neurons [24].

Vitamin C regulates N-Methyl-D-aspartate (NMDA) receptor through the redox phenomena. It has an inhibitory role on NMDA receptor functioning because vitamin C secretion into the extracellular space accompanies glutamate release. Vitamin C may prevent the over activity of NMDA receptors [25]. ROS are produced by NMDA receptor activation [26]. Hyper-glutamatergic function observed after haloperidol treatment [27]. Haloperidol induced significantly enhanced formation of ROS in the whole blood of rats. Vitamin C reduced ROS production [28]. It may antagonize the haloperidol effect by its inhibitory role on NMDA receptor functioning and through scavenging the ROS that induced by haloperidol-induced hyper-glutamatergic function. In conscious dogs, haloperidol administration induced a continuous rise of plasma cortisol levels. It stimulates cortisol release suggests a complex role, or dopamine transmission involving different dopaminergic pathways and different dopamine receptors [29]. The effect of haloperidol on stress hormone (cortisol) in the brain is controversial, haloperidol may increase or decrease cortisol in different brain regions. Antipsychotics treatment in schizophrenia patients showed reduction in baseline cortisol [30] and increased cortisol is a predictor of antipsychotic treatment effects in schizophrenia [31]. In schizophrenia patients, antipsychotic medications reduce hypothalamic-pituitary-adrenal (HPA) axis activation, and that augment stress hormone (cortisol) release exacerbate psychotic symptoms [30]. Studying the central dopaminergic regulation of adrenocorticotropin (ACTH) release in humans, haloperidol administered intravenously to twelve healthy male subjects and measured cortisol for 120 min following injection. Haloperidol produced significant increases in plasma cortisol. Thus, central dopaminergic pathways in the tuberohypophyseal system may regulate the secretion of ACTH from the human pituitary [32]. Therefore, haloperidol can directly regulate the hypothalamic-pituitary-adrenal (HPA) axis through pharmacological action via D_2 receptor antagonism, a reduction in cortisol levels, follows the administration of haloperidol is reported [33].

Vitamin C modulates gamma aminobutyric acid (GABA) systems via activation of $GABA_A$ receptors and possible inhibition of $GABA_B$ receptors [34]. In schizophrenia patients, chronic exposure to haloperidol has distinct effects on the availability of $GABA_A$ receptor with specific subunit compositions [34]. Responses mediated by $GABA_A$ receptors have strongly been potentiated by vitamin C [35]. It can stimulate GABA production in the brain. Vitamin C may antagonize haloperidol induces increase in cortisol through GABA which is responsible for reducing cortisol production

[36]. The cataleptic effect of neuroleptics depends on the balance between the dopaminergic and serotonergic systems and that the serotonergic system exerts an inhibitory influence on the dopaminergic system [37]. Haloperidol-induced increases of dopamine metabolites, which were attenuated by prior administration of 8-hydroxy-2-di-*n*-propylaminotetralin (8-OH-DPAT, 5-$HT1_A$ receptor full agonists). Stimulation of somatodendritic and postsynaptic 5-HT-1_A receptors could attenuate haloperidol-induced catalepsy. Cataleptic effects of a submaximal dose of haloperidol were attenuated by prior administration of 8-OH-DPAT (5-$HT1_A$ receptor full agonists) [38]. Vitamin C is cofactor for tryptophan-5-hydroxylase required for the conversion of tryptophan to 5-hydroxytryptophan in serotonin production [16]. An increase in serotonin production by vitamin C may antagonize the catalepsy induced by haloperidol. Following the akinesia parameter for 180 min, vitamin C behaves as the control without any changes up to 180 min while haloperidol produces akinesia starts from 30 min and was not changed up to 180 min. Vitamin C partially antagonizes akinesia induced by haloperidol at 30 min and up to 180 min without changes. Chronic administration of haloperidol has half-lives of up to 21 days [39]. In this study, the treatment was carried out by sub-acute administration, therefore, the effects were observed only for up to 180 min.

Conclusion

This study concludes that the effects of sub-acute treatment with vitamin C (100 and 500 mg/kg) on extrapyramidal side effects caused by the antipsychotic haloperidol successfully decreases catalepsy, ptosis, rigidity and akinesia in mice.

Author's contribution

S.M. Aburawi contributed to the development of the idea and design of the study. E.N. Burawi and H.E. Alalem collected the data. All authors contributed data tools, and performed analysis and/or interpretation. S.M. Aburawi and S.E.A. Saad drafted the manuscript and revised it for important intellectual context. All authors approved the final version of the manuscript.

Conflict of Interest

The authors declare that they have no competing interests

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