Zinc Tempers Haloperidol-induced Behavioural Changes in Healthy Mice

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Abstract Objective: Behavioural changes secondary to haloperidol/zinc administration were studied, with a view to understanding the patterns of behavioural responses that may be exhibited after such treatment in healthy mice. **Methodology:** 6 month old Swiss mice (24-30 g each, n=12), were given vehicle (distilled water), haloperidol (2 mg/kg), zinc (as Zinc gluconate, 2.5 and 5 mg/kg) or haloperidol/zinc combination, orally for 21 days. Behaviours were assessed after the first and last dose of haloperidol/zinc. **Results:** Zinc administration led to a significant reduction of haloperidol-induced suppression of horizontal locomotion, rearing and grooming behaviours. Zn also prevented the deterioration in Y-maze and radial-arm maze working-memory that was associated with repeated haloperidol administration. Finally, an enhanced anxiolytic effect was seen with co-administration of haloperidol with the lower dose of zinc. **Conclusion:** Co-administration of haloperidol with zinc can reduce some unwanted effects that are known to be associated with haloperidol therapy; hence zinc may be a beneficial adjunct to haloperidol administration.

Keywords Antipsychotic, Neurobehaviour, Supplement, Trace Element, Memory, Novelty-induced Behaviour, Anxiety

affinity for the D₃ and D₄ dopamine receptors [2]. Its effect on locomotion is mediated by its blockade of central dopaminergic receptors [3]. Haloperidol's blockade of D₂ receptors in the brain is also responsible for its effect on cognition [4] and reinforcement behaviours [5]. Administration of haloperidol results in suppression of voluntary behaviours such as motor activity; this is one of the most prominent observable effects of antipsychotics [6,7]. In experimental animals, it has central-inhibiting effects, causing a decrease in both horizontal and vertical locomotion [8]. Unfortunately, haloperidol causes deficits in learning and memory in both humans and experimental animals [9,10,11], an observation that had been made even in healthy volunteers; supporting the fact that haloperidol alters behaviour even in the non mentally-ill [12,13]. In humans, other adverse reactions that may follow haloperidol administration include drowsiness, lethargy, drooling or hypersalivation and a fixed stare.

The side-effects that may be associated with drugs such as haloperidol constitute major impediments to therapy, and a significant reduction in their occurrence is always desirable; due to the fact that serious side-effects discourage regular and continued use of drugs [14]. Therefore, modulation of some unwanted effects of haloperidol is a desired end-point.

In recent times, there has been a great deal of interest in the use of natural remedies, multivitamins or trace element supplements, either in the direct treatment of mental-health disorders or to help reduce the quantity of medications required for treatment. A number of studies have also reported a strong correlation between nutritional deficiencies [14], mineral excess/deficiencies [15] and mental illnesses [16]. The most common nutritional deficiencies seen in mental disorder patients are of omega-3 fatty [17] and B vitamins [18], while overload or deficiency of essential trace elements like zinc, selenium, copper, manganese [19] and iron have also been implicated.

Zinc (Zn) is an essential mineral, which is found naturally in some foods, and is available as a dietary supplement. It

1. Introduction

Antipsychotic medications are increasingly being used in the management of mental disorders. Their use ranges from treatment of schizophrenia to the management of behavioural symptoms that may occur with certain types of dementia. Haloperidol is a 'typical' antipsychotic drug used in the treatment of adult schizophrenia and mania, and hyperactivity/behavioural disorders in children [1]. It binds with high affinity to dopamine₂ (D₂ receptor), but also has

plays a key role in protein synthesis, DNA synthesis, cell division and immune function [20]; it is also an important micronutrient which helps in normal growth and development [21]. Zn is vital to the catalytic activity of over a hundred enzymes [22,23], apart from being an integral part of the structure of main antioxidant enzymes like superoxide dismutase [24]. A daily intake of Zn is recommended to maintain a steady state in the body. Zn is also one of the micronutrients that have been linked to behaviour, learning, memory and mental-health disorders such as schizophrenia and depression [25]. Studies have reported alterations in the copper/zinc ratio in patients with schizophrenia [26]. A number of researchers also believe that Zn is probably a neurotransmitter; an opinion buttressed by the observations that Zn is found in high concentrations in certain regions of the brain, such as the hippocampus, and that Zn deficiency can produce behaviours that are similar to those found in schizophrenics [27]. Zn supplementation in this study was in the form of zinc gluconate caplets which contains approximately 13% zinc by weight. We administered 2.5 and 5.0 mg/kg of zinc gluconate which is approximately 0.325 and 0.65 mg/kg of elemental zinc daily. The doses used in this study correspond to about 21 and 42 mg in a young adult human weighing about 60 kg. These doses, though higher than the recommended daily allowance of 8-11 mg/kg in adults are within and slightly above the upper daily limit of 40 mg in adults 19 years and above [23]. There have been reports of increased risk of adverse health effects with long-term Zn intake exceeding the tolerable upper limit [23]. These 'upper limits' however do not usually apply to individuals receiving Zn for medical treatment under the care of a physician, i.e. when Zn is being used as a therapeutic agent [28].

The rationale for this study is that if Zn is likely to become an adjunct agent in the management of certain mental illnesses (where there is Zn deficit), it will be of benefit for us to be familiar with the baseline behavioural effects of Zn/standard antipsychotic in health. We therefore tested the hypothesis that co-administration of Zn with haloperidol can lead to a significant alteration of the behavioural effects of haloperidol in healthy mice.

2. Materials and Methods

2.1. Equipment and Apparatus

Open-field, Y-maze, Radial-arm maze, Elevated plus-maze.

2.2. Dietary Supplements

Zinc gluconate Caplets (each caplet contained 6.5 mg of elemental Zn), Haloperidol tablets.

2.3. Animals

6 month old Swiss mice from our colony (Empire Breeders, Osogbo, Osun State, Nigeria), weighing 24-30 g each were used for this study. Mice were housed in groups of six in plastic cages located in a temperature-controlled quarters (22-25 degree Celsius) with 12 hour light/dark cycle. All animals were fed commercial standard chow (Calories: 29% Protein, 13% Fat and 58% Carbohydrate) from weaning. Mice had free access to food and drinking water except during the behavioural tests. All procedures were conducted in accordance with the approved institutional protocols and within the provisions for animal care and use prescribed in the scientific procedures on living animals, European Council Directive (EU2010/63).

2.4. Experimental Method

Two hundred and forty healthy mice of either sex were randomly assigned into three main groups, based on the behavioural tests carried out on them: Open field (72), working-memory (84) and anxiety tests (84). They were further divided into 6 groups of 12 animals each, for the open field test and 7 groups of 12 animals each, for the cognition and anxiety tests respectively. Animals in each group received vehicle (distilled water) at 10 ml/kg, haloperidol (2 mg/kg) [29], Zn (as 2.5 or 5.0 mg/kg/day of zinc gluconate, administered alone or co administered with haloperidol), or a standard drug (diazepam at 0.5 mg/kg for the anxiety test and scopolamine at 1 mg/kg for cognition test) daily for 21 days, via an oral cannula. All animals were gavaged daily starting at 9.00 a.m; gavage was used as method of drug delivery to simulate administration in humans. Route of administration of drug or vehicle was oral. Doses of Zn and haloperidol were calculated by crushing the tablets and dissolving measured quantities in distilled water. Behavioural tests were carried out after the first (acute) and last (repeated) dose of vehicle or drug.

2.4.1. Behavioural Testing

Behavioural tests were carried out in a quiet room between the hours of 9 a.m. and 2 p.m. On each of the test days, animals were transported to the testing room in their home cages, and allowed to acclimatize for 30 minutes before behavioural tests. At the onset of the tests, each animal was placed in the apparatus and its behaviour videotaped for subsequent analysis. After testing, each mouse was removed from the maze, and returned to its home cage. The maze is cleaned thoroughly with 70 % ethanol and then wiped dry to remove any trace of odour.

2.4.2. Open Field

Open field novelty-induced behaviours such as horizontal locomotion, rearing and grooming were recorded over a twenty minute period [30]. The open field box is a rectangular arena made up of a hard floor measuring 36 x 36 x 26 cm, its floor was divided into 16 equal squares. The mice were placed in the centre of the field and covered by a small dome which was removed at the beginning of video

recording of their activity. After administration of vehicle or drug, mice were introduced into the field and the total horizontal locomotion (number of floor units entered with all paws), rearing frequency (number of times the animal stood on its hind legs either with its fore arms against the walls of the observation cage or free in the air) and frequency of grooming (number of body cleaning with paws, picking of the body and pubis with the mouth and face washing actions, indicative of a stereotypic behaviour) within the 20 minute interval was recorded.

2.4.3. Memory (Y-maze, Radial arm-maze)

Y-maze was used to measure spontaneous alternation, a measure of working-memory. Spontaneous alternation was assessed using a Y-shaped maze which was composed of three equally spaced arms (120°, 41cm long and 15cm high, 5 cm wide). Each mouse was placed in one of the arm compartments and allowed to move freely until its tail completely entered another arm. The sequence of arm entries was recorded. An alternation was defined as entry into all three arms consecutively [31]. The number of actual alternations is number of sequential arm entries into three arms, designated A, B and C. The percentage alternation is calculated as $\{(Actual\ alternations/Total\ arm\ entry\ minus\ two) \times 100\}$ in a 5 minute interval.

Working-memory in the radial-arm maze was measured as alternation index, which is the ratio of sequential arm entries before error and total arm entry. The apparatus is made up of eight equidistantly spaced arms, each about 33 cm long, all radiating from a small circular central platform. Working-memory was assessed when the rat enters each arm a single time. Re-entry into the arms would result in a working-memory error [31].

2.4.2. Anxiety Model: Elevated plus-maze (EPM)

The EPM validated for use in mice, relies upon the rodents' proclivity toward dark, enclosed spaces (approach) and an unconditioned fear of heights/open spaces (avoidance). It is plus-shaped, with two open arms measuring 25 x 5 x 5 cm lying across from each other and perpendicular to two closed arms measuring 25 x 5 x 16 cm with a centre platform (5 x 5 x 0.5 cm). The closed arms are enclosed by 2 high walls (16 cm) while the open arms have no side wall. Animals are placed in the central platform facing the closed arm and behaviours recorded for 5 minutes. The criterion for arm visit is considered only when the animal decisively moved all its four limbs into an arm [32].

2.5. Statistical Analysis

Data was analysed using Chris Rorden's ezANOVA for windows. Hypothesis was tested using analysis of variance (ANOVA). We tested the hypothesis that acute or repeated oral co-administration of Zn/haloperidol could significantly alter haloperidol-induced behaviours in healthy mice. Two-factor ANOVA was used to test effects of Zn and haloperidol co-administration and duration of administration (acute versus repeated) on behaviour, while one-way ANOVA was used to assess the effects of Zn and haloperidol administration on body weight. Tukey (HSD) test was used for within and between group comparisons. Results were expressed as mean $\pm$ S.E.M, and $p < 0.05$ considered significant.

3. Results

3.1. Effect of Zn/haloperidol Administration on Body Weight

Figure 1. Effect of Zn/haloperidol administration on body weight. Each bar represents mean $\pm$ S.E.M, number of mice per treatment group = 12; VEH: Vehicle, HAL: Haloperidol, ZN: Zinc, HAL/ZN: Haloperidol Zinc co-administration.

Figure 1 represents the percentage weight change, defined as the percentage difference between the final and initial body weights divided by initial weight. There was a significant ($F = 29.41$, $p < 0.001$) increase in weight in groups of animals administered haloperidol alone and haloperidol with Zn at 2.5 and 5.0 mg/kg compared to animals administered vehicle. Administration of Zn at 2.5 mg/kg was associated with lesser weight gain when compared to vehicle. Compared to haloperidol however, there was lesser weight gain in groups administered Zn alone at 2.5 and 5.0 mg/kg and haloperidol and Zn at 2.5 and 5.0 mg/kg.

3.2. Effects of Zn/haloperidol administration on horizontal locomotion

Figure 2 shows the effect of zinc/haloperidol on locomotion in the open-field. Two-factor ANOVA of horizontal locomotion scores revealed a significant main effect of Zn or haloperidol (drug) ($F = 28.6$, $p < 0.001$), duration of administration (acute vs. repeated) ($F = 144$, $p < 0.001$), but no interactions between drug x duration of administration ($F = 0.988$, $p < 0.341$). Pairwise comparisons of the effect of vehicle versus (vs.) drug dose following acute administration revealed a significant increase in horizontal locomotion following administration of Zn alone at 2.5

mg/kg, and a significant decrease with haloperidol and haloperidol co-administered with Zn at 2.5 and 5.0 mg/kg. Repeated administration resulted in significant decrease in locomotor activity with haloperidol, both doses of Zn alone and when Zn (2.5 and 5.0 mg/kg) was co-administered with haloperidol. Compared to haloperidol however, there was a significant increase in locomotor activity at both doses of Zn when administered alone or when co-administered with haloperidol.

3.3. Effects of Zn/haloperidol Administration on Rearing

Figure 3 shows the effect of Zn/haloperidol on rearing in the open field. Two-factor ANOVA of rearing scores revealed significant main effect of Zn and haloperidol (drug) dose ($F = 19.00$, $p < 0.001$), duration of administration (acute vs. repeated) ($F = 314$, $p < 0.001$) and strong interactions between Zn and haloperidol dose x duration of administration ($F = 19.32$, $p < 0.005$). Pairwise comparisons of the effect of vehicle vs. Zn and haloperidol dose following both acute and repeated administration revealed a significant increase in rearing activity when Zn was administered at 2.5 mg/kg, and a decrease with administration of haloperidol, Zn at 5.0 mg/kg and when Zn (2.5 and 5.0 mg/kg) was co-administered with haloperidol compared to vehicle.

Figure 2. Effects of Zn/haloperidol administration on horizontal locomotion. Each bar represents mean $\pm$ S.E.M, * $p < 0.05$ vs. VEH, # $p < 0.05$ vs. HAL, number of mice per treatment group = 12, VEH: Vehicle, HAL: Haloperidol, ZN: Zinc, HAL/ZN: Haloperidol Zn co-administration.

Figure 3. Effects of Zn/haloperidol administration on rearing activity. Each bar represents mean $\pm$ S.E.M, * $p < 0.05$ vs. VEH, # $p < 0.05$ vs. HAL, number of mice per treatment group = 12, VEH: Vehicle, HAL: Haloperidol, ZN: Zinc, HAL/ZN: Haloperidol Zn co-administration

Figure 4. Effects of Zn/haloperidol administration on grooming behaviour. Each bar represents mean $\pm$ S.E.M, * $p < 0.05$ vs. VEH, # $p < 0.05$ vs. HAL, number of mice per treatment group = 12, VEH: Vehicle, HAL: Haloperidol, ZN: Zinc, HAL/ZN: Haloperidol/Zn co-administration.

3.4. Effects of Zn/haloperidol Administration on Grooming

Figure 4 shows the effect of Zn/haloperidol on grooming in the open field. Two-factor ANOVA of grooming scores revealed a significant main effect of Zn and haloperidol dose ($F = 38.2$, $p < 0.001$), significant effect of duration of administration (acute vs. repeated) ($F = 83.4$, $p < 0.004$) and strong interactions between Zn and haloperidol dose $\times$ duration of administration ($F = 16.20$, $p < 0.002$). Pairwise comparisons of the effect of vehicle vs. zinc and haloperidol dose following acute administration showed significant

increase in grooming when Zn was administered at 2.5 mg/kg and when Zn (2.5mg/kg) was co-administered with haloperidol, while a decrease was seen with haloperidol compared to vehicle. Repeated administration revealed a significant increase in grooming when Zn was administered at 2.5 mg/kg and a decrease with haloperidol, Zn at 5.0 mg/kg and when Zn at 5.0 mg/kg was co-administered with haloperidol. Compared to haloperidol however, there was a significant increasing in grooming at both doses of Zn (2.5 and 5.0 mg/kg) when either administered alone or co-administered with haloperidol.

Figure 5. Effects of Zn/haloperidol administration on Y-maze memory tasks. Each bar represents mean $\pm$ S.E.M., * $p < 0.05$ vs. VEH, $^{\mu}p < 0.05$ vs. SCOP, $^{\#}p < 0.05$ vs. HAL, number of mice per treatment group = 12; VEH: Vehicle, SCOP: scopolamine HAL: Haloperidol, ZN: Zinc, HAL/ZN: Haloperidol/Zn co-administration.

3.5. Effect of Zn/haloperidol Administration on Y-maze Spatial Working-memory Tasks

Figure 5 shows the effect of Zn/haloperidol administration on % alternation in the Y-maze. Two-factor ANOVA revealed significant main effect of Zn and haloperidol dose ($F = 44.1$, $p < 0.001$), significant effect of duration of administration (acute vs. repeated) ($F = 8.16$, $p < 0.013$) and no interactions between Zn and haloperidol dose $\times$ duration of administration ($F = 1.90$, $p < 0.541$). Pairwise comparisons of the effect of vehicle vs. scopolamine (SCOP), Zn or haloperidol dose following acute administration revealed a significant decrease in memory scores (measured as % alternation) with SCOP and a significant increase with haloperidol, Zn at both doses (2.5 and 5.0 mg/kg), when administered alone or when co-administered with haloperidol. Repeated administration of vehicle, scopolamine, Zn or haloperidol resulted in a significant decrease in memory scores with SCOP and haloperidol and a significant increase with Zn (2.5 and 5.0 mg/kg) when administered alone or when Zn (5.0 mg/kg) was co-administered with haloperidol, compared to vehicle.

In comparison to SCOP, there was a significant increase in memory scores with haloperidol and Zn (at 2.5 and 5.0 mg/kg) when acutely administered either alone, or co-administered with haloperidol. Repeated administration revealed a significant decrease in memory scores with haloperidol and an increase with both doses of Zn administered alone or co-administered with haloperidol. In comparison to haloperidol, there was a significant increase in memory task scores with Zn at 5.0 mg/kg alone and when Zn at 2.5 mg/kg was co-administered with haloperidol, following acute administration. Repeated administration

revealed a significant increase at both doses (2.5 and 5.0 mg/kg) of Zn, either administered alone or co-administered with haloperidol.

3.6. Effect of Zn/haloperidol Administration on Radial Arm-maze Spatial Working-memory Tasks

Figure 6 shows the effect of Zn/haloperidol administration on alternation index in the radial-arm maze, measured as alternation index/5 minute, which is a fraction of number of correct alternation before first error and total number of alternations made in a five minute period. Two-factor ANOVA revealed significant main effect of Zn and haloperidol dose ($F = 50.4$, $p < 0.001$), significant effect of duration of administration (acute vs. repeated) ($F = 7.98$, $p < 0.005$) and no interactions between Zn and haloperidol dose $\times$ duration of administration ($F = 1.91$, $p < 0.08$). Pairwise comparisons of the effect of vehicle vs. scopolamine (SCOP), Zn or haloperidol dose following acute administration revealed a significant decrease in memory scores (measured as % alternation) with SCOP and a significant increase with Zn at both doses (2.5 and 5.0 mg/kg) when administered alone, and when Zn at 2.5 mg/kg was co-administered with haloperidol. Repeated administration of vehicle, scopolamine Zn or haloperidol resulted in a significant decrease in memory scores with SCOP and haloperidol and a significant increase with Zn at 2.5 mg/kg when administered alone or co-administered with haloperidol, compared to vehicle.

In comparison to SCOP, there was a significant increase in memory task scores with haloperidol, Zn (at 2.5 and 5.0 mg/kg) when administered alone, and when Zn at 2.5 mg/kg was co-administered with haloperidol acutely. Repeated

administration revealed a significant decrease with haloperidol and an increase with Zn at 2.5 mg/kg when administered alone or co-administered with haloperidol. Compared to haloperidol, there was a significant increase in

memory scores with Zn at 2.5 and 5.0 mg/kg when administered either alone or co-administered with haloperidol following both acute and repeated administration.

Figure 6. Effects of Zn/haloperidol administration on radial arm maze memory tasks. Each bar represents mean $\pm$ S.E.M, * $p < 0.05$ vs. VEH, # $p < 0.05$ vs. SCOP, $\mu p < 0.05$ vs. HAL, number of mice per treatment group = 12; VEH: Vehicle, SCOP: scopolamine, Haloperidol, ZN: Zinc, HAL/ZN: Haloperidol/Zn co-administration.

Figure 7. Effects of Zn/haloperidol administration on % time spent in the open arm of the elevated plus maze. Each bar represents mean $\pm$ S.E.M, * $p < 0.05$ vs. VEH, # $p < 0.05$ vs. DIZ, $\mu p < 0.05$ vs. HAL, number of mice per treatment group = 12; VEH: Vehicle, DIZ: diazepam, Haloperidol, ZN: Zinc, HAL/ZN: Haloperidol/Zn co-administration.

3.7. Effect of Zn/haloperidol Administration on Anxiety Behaviour

Figure 7 shows the effect of Zn/haloperidol administration on % time spent in the open arm of the EPM. Two-factor ANOVA revealed a significant main effect of Zn and haloperidol dose ($F = 49.00$, $p < 0.001$), duration of administration (acute vs. repeated) ($F = 39.2$, $p < 0.001$) and strong interactions between Zn and haloperidol dose $\times$ duration of administration ($F = 33.25$, $p < 0.002$). Pairwise comparisons of the effect of vehicle against diazepam (DIZ), Zn or haloperidol dose, following acute administration revealed a significant increase in time spent in the open arms with DIZ, haloperidol, and Zn at both doses (2.5 and 5/0 mg/kg) when administered either alone or co-administered with haloperidol, with both acute and repeated administration. In comparison to DIZ, there was a significant decrease in time spent in the open arms with haloperidol and following Zn at 5.0 mg/kg co-administered with haloperidol following acute and repeated administration. There was a significant increase in open arm time with Zn at 2.5 mg/kg administered alone or co-administered with haloperidol following acute administration, and with Zn at 2.5 mg/kg co-administered with haloperidol, following repeated administration. In comparison to haloperidol, there was a significant increase in time spent in the open arms with Zn at 2.5 and 5.0 mg/kg administered alone and with Zn at 2.5 mg/kg co-administered with haloperidol, following acute and repeated administration. A significant decrease was seen compared to haloperidol when Zn at 5.0 mg/kg was co-administered with haloperidol following repeated administration.

4. Discussion

Unwanted behavioural side-effects constitute one of the limitations to the use of antipsychotic medications; therefore, research has continued on the path of discovering newer and better drugs. However, the newer drugs tend to have their own side-effects, therefore, they may not necessarily be the 'magic bullet' that is desired. Recent breakthroughs in the understanding of the pathogenesis of several mental illnesses point to imbalances or deficiencies of trace elements in the body. Of these, Zn has received a certain degree of attention; and some researchers are of the opinion that correction of bodily Zn deficiency may be crucial to management of schizophrenia [15]. Zn supplementation may be a beneficial adjunct to standard medications; however, there is a need to first understand the behavioural phenotype that is expressed by Zn administration alone, and in co-administration with a standard medication in health. Using healthy mice, we chose to investigate the potentials of Zn as a modulator of the behavioural effects of a standard antipsychotic. The aim was to document the ability of Zn to reduce unwanted behavioural effects of haloperidol in health; thereby allowing a clearer perspective when both are co-administered as

therapy.

In our study, we utilized mixed-gender mice in order to be able to extrapolate our results to humans generally and also because psychiatric disorders affect both genders of humans. Behavioural indices that were measured in this study were open field novelty-induced behaviours, working-memory and anxiety. Our results show that (1) significant weight gain was observed with haloperidol, and a lesser weight gain seen with Zn/haloperidol co-administration (2) open-field exploration and grooming were suppressed by haloperidol; these effects were reversed to varying extents with Zn (3) haloperidol-induced memory-impairment in the Y-maze and radial-arm maze was significantly attenuated by Zn (4) anxiolytic potential of haloperidol is significantly boosted by Zn.

In this study, administration of haloperidol caused a significant increase in weight compared to vehicle. Zn supplementation in this study was associated with lesser weight gain compared to weight changes seen with haloperidol. Co-administration of zinc with haloperidol however resulted in increased weight gain compared to Zn alone groups, although this was a significant reduction from haloperidol-associated weight gain. Weight gain is one of the documented side-effects of haloperidol [33], although compared to other antipsychotics; haloperidol has been less consistently associated with weight gain [34]. There have been reports of increase weight with haloperidol [35,36], and there is also documentation of no weight gain [37]. In the last decade or more, reports of a link between beneficial therapeutic response and treatment-emergent weight gain have been seen [36]. The underlying mechanisms involved in antipsychotic-induced weight gain however are still a subject of extensive debates. Although increase in food or calorie intake, fat deposition hormones and peptides have been considered as possible causes, the main biological mechanisms are largely unknown. Management of antipsychotic-induced weight gain has become important largely due to the complications of uncontrolled weight gain [38]. More recently, behavioural and non-pharmacologic interventions have been considered [39]. The ability of Zn to curb excess weight gain if used as a therapeutic adjunct will be of immense benefit, clinically.

Administration of haloperidol in this study resulted in central inhibition (with acute and repeated administration). Horizontal locomotion and rearing were depressed compared to vehicle; this correlates with results of previous studies that concluded that haloperidol exerted a depressant effect on general explorative ability [40,41]. Zn supplementation in this study caused a mixed response, with an initial increase in locomotor activity and rearing at 2.5 mg/kg following acute administration, leading to a decrease with repeated administration; although, no significant difference was seen in locomotor activity and rearing at 5 mg/kg (acute) and 2.5 mg/kg (repeated administration) respectively. The results seen with Zn administration in this study confirms that Zn supplementation can significantly alter behaviour in animals; and is consistent with other studies that have reported an

acute increase in locomotor activity which later transforms to no significant effect or a decrease in response, usually with repeated administration of Zn [42-45]. Co-administration of zinc with haloperidol however resulted in an improvement in locomotor activity and rearing compared to that seen with haloperidol alone, although at no time did the values return to baseline (vehicle).

The roles of dopamine, dopamine receptors [41] and N-methyl-D-aspartate (NMDA) receptors [46,47] in the regulation of locomotor activity, emotionality, motivation and memory have been studied extensively. Zn is an NMDA receptor inhibitor that accumulates within the synaptic vesicles of some glutamatergic neurons and modulates neuronal excitability and synaptic plasticity by multiple mechanisms that are poorly understood. Haloperidol on the other hand is a dopamine receptor antagonist [48]. The co-administration of both drugs tempered the hypolocomotion seen when haloperidol is given alone; the mechanisms responsible for these may not be unrelated to Zn's ability to modulate both excitatory and inhibitory neurotransmission [49]. While excitatory NMDA receptors are directly inhibited by Zn, non-NMDA receptors appear unaffected by this inhibition. In contrast, Zn potentiates GABA (A) receptor-mediated inhibitory transmission, influencing transmitter release. Finally, studies have shown that both direct and indirect stimulation of dopamine (D₁) receptors can increase locomotor activity in mice [50], whereas Zn can inhibit antagonist binding to D₁ receptors via allosteric modulation of the binding site.

Grooming is an important and 'ancient' behaviour observed in a number of animals. In this study, a decrease in grooming frequency was seen with haloperidol, which is consistent with a general dopamine receptor depolarizing blockade effect seen with haloperidol administration. On the other hand, Zn administration caused an increase in grooming. Co-administration of Zn and haloperidol also increased grooming above what was seen with haloperidol, and at 2.5 mg/kg, effect was higher than baseline (vehicle). Dopamine is important in ensuring sequential grooming patterns [51] and activation of D₁ receptors result in intense grooming [52]. GABA (A or B) receptor activation however reduces grooming behaviour [53]. An inhibitory effect of Zn on GABA (A) receptors or its possible effect on D₁ dopamine receptors may be responsible for the increase in grooming that was seen; haloperidol on the other hand has low affinities for brain D₁ receptors [54].

The results of spatial working-memory tests (Y-maze, radial-arm maze) revealed memory-impairment with repeated administration of haloperidol, although, acute administration of haloperidol in the Y-maze appeared to improve memory; with Zn, both acute and repeated administration showed memory-enhancement. Co-administration of Zn with haloperidol at both doses showed memory-enhancement compared to haloperidol, although lower than baseline (vehicle). Haloperidol-induced memory-deficits had been previously documented, therefore

the effect seen here is in agreement with those of previous studies that have also reported memory-deficits with repeated haloperidol use [2,54]. On the other hand, Zn had been reported to modulate both ion channels and synaptic plasticity, making it important in both learning and memory [55]. Working-memory is regulated by a number of brain regions (such as hippocampus and prefrontal cortex) through effects of cholinergic, glutamatergic or γ amino butyric acid (GABAergic) projections to these sites [56]. Activation of GABAergic impulses impairs spontaneous alternation working-memory, while increased cholinergic and glutamatergic transmission improves working-memory [57]. The effect of Zn on memory may be mediated through its effects on either GABA or glutamate receptors.

Zn administration has been reported to be anxiolytic [45,58], and this is also evident in this study. Haloperidol administration also resulted in an anxiolytic effect, although the effect was much lower than what was seen with diazepam. Zn supplementation had a more profound anxiolytic effect compared to either haloperidol or diazepam, while co-administration of Zn at 2.5mg/kg with haloperidol resulted in anxiolysis that exceeded that seen with both diazepam and haloperidol. A complex, multi-feedback mechanism [59] involving serotonin, dopamine, glutamate, GABA and norepinephrine have been implicated in anxiety-related disorders [60]. The response seen with the administration of Zn may be a consequence of its effects on GABA (A) or at the NMDA receptors, both of which are critical to anxiety states.

5. Conclusions

This study concludes that co-administration of haloperidol with Zn can reduce some unwanted behavioural effects that are known to be associated with haloperidol therapy; hence zinc may be a beneficial adjunct to haloperidol administration. To ensure its suitability as an adjunct antipsychotic, more studies need to be carried out in assessing the effects of Zn/haloperidol, in an animal model of psychosis.

Conflict of Interest

All authors of this paper declare that there is no conflict of interest related to the content of this manuscript.

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