

Central Cholinergic Markers and Spatial Memory Performance in Rats Following Administration of *Rauwolfia* *Vomitoria* and Chlorpromazine

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Abstract

The study was designed to compare the effects of *Rauwolfia vomitoria* and chlorpromazine on metabolic activities, spatial memory performance, central cholinergic markers, oxidative stress markers and neurohistoarchitecture in the brain of adult wistar rats. Twenty five adult male wister rats weighing between 200 - 230 g were divided into five groups (A-E) of five rats each. Group A (control) received 2mls of normal saline daily, Group B received 5mg/kg of chlorpromazine, Group C received 10 mg/kg of chlorpromazine, Group D received 150 mg/kg of *R. vomitoria* and Group E received 300 mg/kg of *R. vomitoria* orally. All the medications were given daily for 21 days. Body weights were taken weekly and recorded. A y-maze apparatus was used to assess the spatial memory performance in the rats at days 14 and 21 of the experiment. All the animals were euthanized using 20mg/kgbw of intramuscular ketamine, cardially perfused with 4% paraformaldehyde and the brains were removed, some were homogenised for analysis of central cholinergic markers and others for histological analysis. Low doses of chlorpromazine increase body weight and *Rauwolfia vomitoria* increase central cholinergic markers. The findings of the study show that *R.vomitoria* and chlorpromazine exert differential and dosage dependent effects on body weight, central cholinergic neurons and learning performance. The conclusion from the study indicates that *R. vomitoria* could be a better alternative drug in the treatment of psychosis with lesser metabolic and central nervous system side effects.

Keywords: *Rauwolfia vomitoria*, Chlorpromazine, Psychosis, Spatial memory, Central Cholinergic markers.

Introduction

Psychosis is one of the common mental disorders known to man and it is characterized by thought disorders, cognitive dysfunction, hallucination, and in some cases, memory impairment (Tollefson, 1996). The long-term functional outcome in patients with psychosis is determined by their learning potentials and memory capacities during rehabilitation. However, the effects of antipsychotic drugs on these components of cognitive functions are poorly understood, particularly when they are administered for a long period of time (Alvin *et al.*, 2008). Deficit in spatial memory is one of the cognitive impairments that have been consistently associated with psychosis (Park and Holzman, 1992 and Keefe *et al.* 1995). Functional neuroimaging studies have confirmed deficits in prefrontal cortical activation during performance of spatial working memory tasks in psychotic, compared to non-psychotic, individuals (Callicott *et al.*, 1998 and Manoach *et al.* 2000).

Metabolic disorders are often associated with the use of many psychotropic agents, including antidepressants, mood stabilizers and antipsychotic drugs (Kunz *et al.*, 2008). Antipsychotic agents help restore the chemistry of the brain, so that normal mood and behavior can be restored (Trujillo, 1996). There are several classes of antipsychotic drugs used in the treatment of major mental and behavioral disorders with each class having varying degrees of mechanism of action (Kane *et al.*, 1989). However, many of the antipsychotic agents have several adverse effects which occur in varying degrees depending on dosage regimes and duration of the treatment (Marken, 2000 and Cantrell *et al.*, 1999).

Acetylcholine (Ach), a neurotransmitter is synthesised in neuronal terminals in a reaction catalysed by choline acetyltransferase and is degraded into choline and acetate by acetyl cholinesterase (Ellman *et al.*, 1991). However, the functions of the acetylcholine in the brain are less well understood, but they are thought to be related to motor activities, learning, memory and the control of sleep stages. Studies have shown that agonists or stimulators of all these transmitter systems affect learning, memory recall and cognitive process (Everitt and Robinson, 1997). Chlorpromazine (CPZ), one of the first generation (typical) antipsychotics and one of the most common drugs

used in the treatment of psychotic disorders worldwide, has been associated with a lot of side effects (Adams *et al*, 2005). The major challenges facing the first generation antipsychotic agents such as chlorpromazine and others are in their limitation in the exacerbation of adverse motor functions. However, the newer agents used, which have fewer side effects are relatively expensive and unavailable, especially in the developing countries (Gardner *et al.*, 2005 and Miyamoto *et al.*, 2005).

Rauwolfia vomitoria, a shrub plant, which is widely distributed in Bangladesh, India, Burma, Thailand, Malaysia and Indonesia, and also found in the southern part of Nigeria where it has an indigenous name of Ira-Igbo (Yoruba), other dense and gallery forests of Africa (Obembe *et al.*, 1994), has remained the first line of antipsychotic supplements (Makanjuola, 1987) among traditional herbal practitioners in many nations because of its minimal side effects (Obembe, *et al.*, 1994). However, the mechanisms of the antipsychotic action are unknown. Due to paucity of available data on the effects of oral administrations of chlorpromazine (CPZ) and *Rauwolfia vomitoria* (RV) on the spatial memory, oxidative stress and the central cholinergic system in rats, the present study was designed to compare the effects of these two drugs on the central cholinergic markers and spatial memory performance with the aim of hiring prospectus on the two drugs.

Materials and Methods

Animals grouping

All procedures used were approved by the University of Ilorin Animal Ethics Committee Screening Committee which parallels the National Institute of Health (NIH) guidelines on the use of animals in experimental research. Twenty-five male albino rats (150-200 g) were randomly divided into 5 groups (Group A, B, C, D and E) with each group comprising of 5 rats. The rats were caged in mesh cages with proper feeding and under a natural light and dark rhythm, at room temperature and proper ventilation for a period of 14 days for acclimatisation before commencement of the experiment. The rats were weighed once every week and the weight recorded throughout the 21 days that the experiment lasted.

Chlorpromazine and *Rauwolfia vomitoria* extraction and Administration

Chlorpromazine hydroxide (Largactil) was purchased from a reputable government pharmacy in Ilorin, Nigeria. A tablet of Chlorpromazine (100mg) was dissolved in 40 mls of normal saline to produce 2.5mg/ml drug solution. The solution was administered at 5 and 10 mg/kg body weight of rats, using orogastric cannula. *R. vomitoria* leaves were obtained and authenticated in the herbarium of the department of Plant Biology, University of Ilorin, Ilorin. The leaves were washed, air dried and blended to fine substances. 100g of the fine substance was dissolved in 1000mls of distilled water, well shaken and left for 48 hours to dissolve properly. The mixture was filtered and the filtrate was evaporated to dryness in a water bath at 40 °C. The resulting paste was weighed and dissolved in normal saline (drive) and stored in the refrigerator until in use. 10g from the paste was dissolved in 20ml of normal saline. The extract solution was administered at 150 mg/kgbw and 300 mg/kgbw using orogastric cannula. The drug and extract solution were orally administered daily for 21 days.

Behavioural study (the Y-maze test)

The behavioural test was conducted in a large quiet room. The spatial memory was assessed using the Y-maze apparatus. Behaviours were scored with a stop watch; all events were observed manually. The Y-Maze experiments were done on days 14 and 21 of the administrations of chlorpromazine and *Rauwolfia vomitoria*.

Y- maze composed of three equally spaced arms (120°, 41cm long and 15cm high) was used to assess the spontaneous alternation in the rats. The floor of the apparatus was 5cm wide and was leveled with saw shaves. Each rat was placed in one of the arm compartments and was allowed to move freely until its tail completely entered another arm. The sequence of arm entries was manually recorded and the arms were labeled as either A, B, or C. An alternation is defined as entry into all three arms consecutively.

The number of maximum spontaneous alternations was calculated as “the total number of arms entered minus two, and the percentage alternation was calculated as {(actual alternations /maximum alternations) x 100}”

(Hiramatsu and Inoue, 2000). For each animal the Y-maze testing was carried out for 10 minutes. Recorded data include correct triplet arm entries (Right decisions), wrong triplet arm entries (Wrong decisions) and total arm entries.

Animal sacrifice and Sample collection

At the end of the experiments, the animals were anesthetized with intramuscular injection of 20 mg/kgbw of ketamine; skin excised and blood sample collected from the left ventricle of the heart. Thereafter, the rats were transcardially perfused with 4% paraformaldehyde. The brains were removed and processed for histological and histochemical analyses.

The other hemisection of the brains was homogenised, centrifuged at 1500 x g for 10min to remove nuclei and cell debris and the resulting supernatant was then used for total Glutathione peroxidase, Acetylcholine and Calcium ion levels in the tissue.

Statistical Analysis

Data from morphometry and biochemical collation were analysed using ANOVA followed by "Bonferroni's Multiple Comparison Test". Data were presented as means $\pm$ SEM (standard error of mean), P value < 0.05 was considered statistically significant.

Results

The result from the present study shows that Chlorpromazine increases body weight in a dosage-and period-dependent manner. The morphometric analysis of the weekly weight changes in the animals showed no significant ($P < 0.05$) increase or decrease on the 7th day and 21st days analyses between the animals with different drug and dosage exposures. However, there was a significant ($P < 0.05$) weight gain at day 14 in the CPZ (5mg/kg) when compared with the control and Rauwolfia vomitoria (300mg/kg) groups (Figure 1). A significant ($P < 0.05$) increase in the brain weight was also recorded in the CPZ (5mg/kg) compared with the control, but a significant decrease in brain weight was observed in *R. vomitoria* (300mg/kg) exposed rats when compared with the control (Figure 2).

There was no significant ($P < 0.05$) difference in the measure of Spatial memory tasks (percentage alternations) assessment tests across all experimental groups (B, C, D and E) when compared with the control (Figure 3) suggesting that both CPZ and *R. vomitoria* have no untold effects

on spatial memory tasks in rats. The brain Ca^{2+} levels of rats administered 5 mg/kg of CPZ were significantly ($P < 0.05$) higher than the control. There was no significant ($P < 0.05$) difference in the brain Ca^{2+} level of all rats administered 10 mg/kg of CPZ, 150 mg/kg and 300 mg/kg of *R. vomitoria* when compared with the control. ACh levels of rats administered 150 mg/kg of *R. vomitoria* and 5 mg/kg of CPZ were significantly ($P < 0.05$) higher than the control rats (Table 1).

The brain Glutathione peroxidase (GPx) levels in the rats administered 5 mg/kg and 10 mg/kg of CPZ were significantly ($P < 0.05$) high when compared with the control rats and the *R. vomitoria* (150 and 300 mg/kg) exposed rats. High GPx levels were also recorded in the *R. vomitoria* (150 and 300 mg/kg) administered rats, but was only significant ($P < 0.05$) at 150 mg/kg (Table 1).

The histoarchitectural observations of the histomorphology of the pyramidal neurons in the internal pyramidal cell layer of the prefrontal cortices of the rats were normal in the control and *R. Vomitoria* (150 and 300 mg/kg) administered rats, but appeared distorted in shape in the CPZ (5 and 10 mg/kg) administered groups. Some vacuolations were observed around the soma, in what appears like necrosis, but there were no necrotic cells observed (Figure 4).

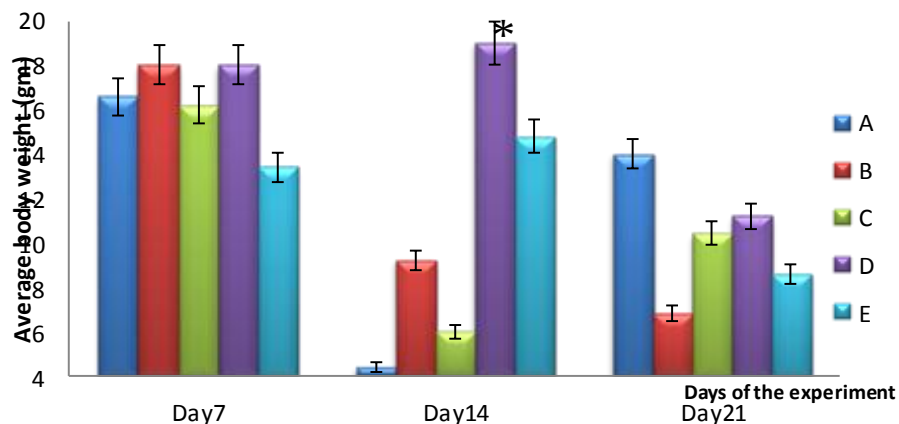


Figure 1: Showing the average weekly body weight of rats following oral administrations of chlorpromazine and *Rauwolfia vomitoria*. Values are plotted as Mean; symbol (*) indicates a significant difference at $P < 0.05$ from the control.

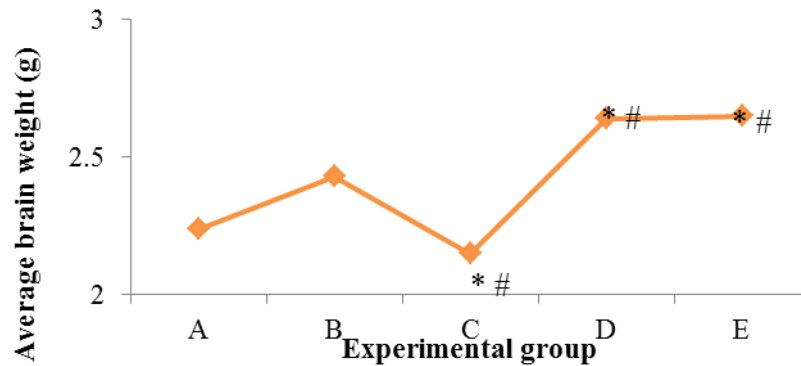


Figure 2: Showing the average brain weight of rats following oral administrations of chlorpromazine and *Rauwolfia vomitoria*. Values are plotted as Mean; symbol (*#) indicates a significant ($P < 0.05$) difference from the control and other groups.

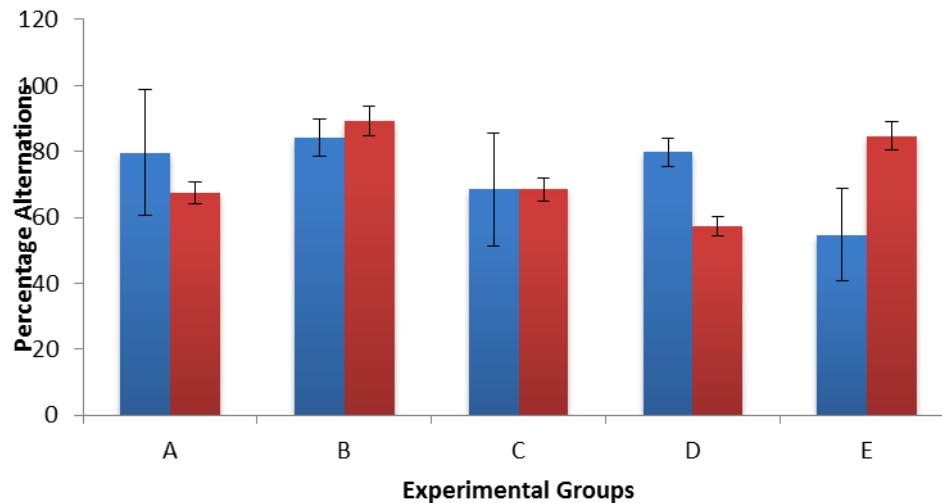


Figure 3: Showing the average percentage alternation in rats at days 14 and 21 of chlorpromazine and *Rauwolfia vomitoria* administrations. Values are plotted as Mean $\pm$ SEM; ANOVA analysis show no significant ($P < 0.05$) difference between the groups.

Table 1: Showing the brain tissue levels of Calcium ion (Ca^{2+}), Acetylcholine (ACh) and Glutathione peroxidase (GPx) following oral administrations of chlorpromazine and *Rauwolfia vomitoria*.

Groups	Ca^{2+} (mM/mg tissue)	ACh (mM/mg tissue)	GPx (mM/mg tissue)
A	2.62 ± 0.025	20.0 ± 2.0	4320.5 ± 25.8
B	3.06 ± 0.09	33.5 ± 0.5^a	5272.5 ± 42.5^a
C	2.60 ± 0.11^d	26.5 ± 0.5	4474.5 ± 35.5
D	2.85 ± 0.05^a	30.0 ± 1.0^a	$6154.0 \pm 61.0^{a,b,c}$
E	3.09 ± 0.11	29.0 ± 2.0	$6267.0 \pm 47.0^{a,b,c}$

Values are presented as Mean $\pm$ SEM; Superscripts (a, b, c, and d) indicate significant ($P < 0.05$) difference from groups A, B, C or D using ANOVA and "Bonferroni's Multiple Comparison Test.

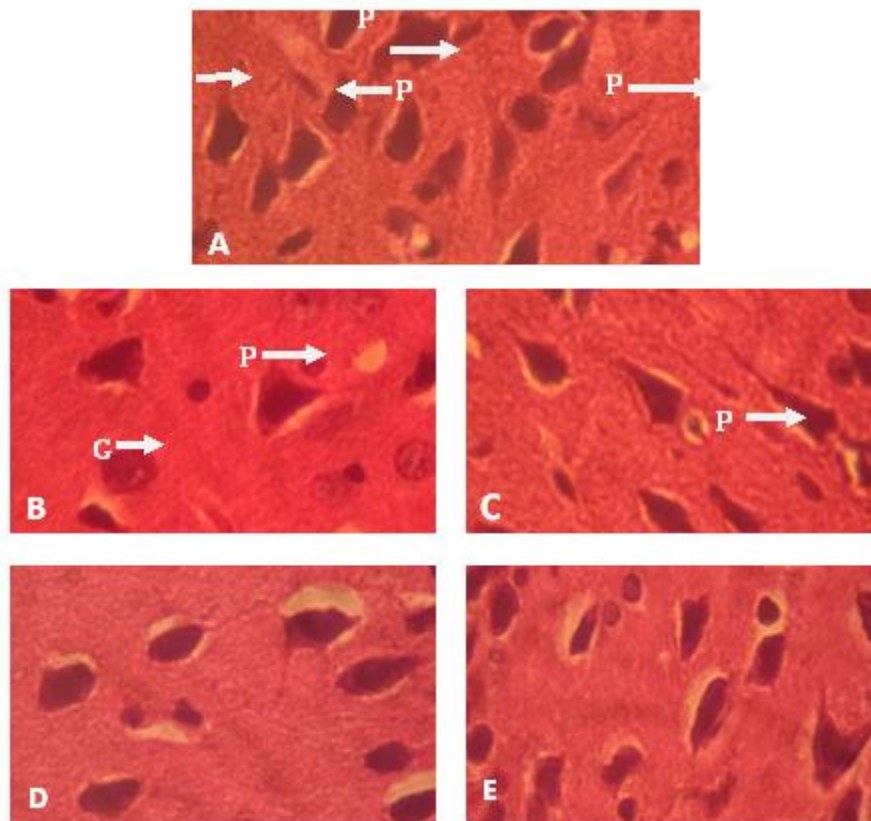


Figure 4: Representative photomicrographs of rats' groups A, B, C, D and E showing the pyramidal neurons (P) and granular cells in the internal pyramidal layer (V) of the prefrontal cortices of rats. (Haematoxylin-Eosin stain, Magnification $\times 400$)

Discussion

Clinical trials in the use of antipsychotic agents have been shown to improve the positive behavioural symptoms such as delusions and hallucinations among sufferers of psychosis, but most of the negative symptoms of the illness such as alogia and depression are in most cases not abated and often generate greater concerns (Alvin *et al.*, 2008). The main challenge today in the significant management of psychotic disorders are in the restriction of cognitive functions among the sufferers which most available drugs in

circulation including chlorpromazine have failed to adequately address (Green *et al.*, 2000). The present study looks at the comparative effects of chlorpromazine and *Rauwolfia vomitoria* on the central cholinergic system, spatial memory performance and oxidative stress.

Morphometric Data

The NHIS data in 1989 revealed that 42% of psychotic patients have a BMI > 27 compared to approximately 25% of the general population. Psychotropic weight gains are often associated with increased fat deposition in the visceral adipose tissue (Zhang *et al.*, 2004). Morphometric data which are estimated measures of metabolic rate and sometime body functions shows from the present study that Chlorpromazine potentially increased body weight at day 14 of the administration, and was especially significant ($P > 0.05$) at dose 5 mg/kg. This findings was strengthened by the reports of Eder *et al.*, 2001; Gupta *et al.*, 1998; Henderson *et al.*, 2000; and and Hummer *et al.*, 1995 where they recorded significant weight gains during treatment of psychosis using Chlorzapine and Olanzapine.

However, the increased body weight gain observed in the chlorpromazine administered rats at day 14 of the experiment declined progressively at day 21 and was not different from the control. This is an indication that the weight gains associated with antipsychotic agents are transient and reversible. The reasons for this observation and reports in various studies were not properly understood. The thought mechanism of action could be initial water retention of the body that may be lost after a couple of days or tolerance development by the body to the drugs.

Rauwolfia vomitoria decreases body weight significantly especially at 300 mg/kg, this is assumed to be caused by the Reserpine constituent which increases cholinergic activity in gastric tissue thereby reducing nutrients absorption (Quevauviller *et al.*, 1972). This finding was consistent with the observation of Eluwa *et al.* (2009) who reported a significant weight decrease with *R. vomitoria* in rats. The findings from the present study strengthen the belief that *Rauwolfia vomitoria* could be more effective in the prevention of overweight associated with psychosis and other antipsychotic medications.

Spatial Memory Task

The repeated acquisition approach (14th and 21st days) to Y-maze test was chosen specifically for the purpose of evaluating the effects of *Rauwolfia vomitoria* and chlorpromazine on learning/encoding and retrieval over time. This is more so because performance of the Y-MAZE test in rodents relies heavily on “spatial working memory” (Olton and Papas, 1979) which is commonly disrupted in psychotic individuals across a variety of test procedures (Keefe *et al.*, 1995 and Park *et al.*, 1992). Learning potential (i.e., as determined in multiple administrations of neurocognitive tests) predicts work skill attainment in psychosis, a factor known to predict the rehabilitation outcome in such individuals as reported by Sergi *et al.*, 2005. The present study observed that both *Rauwolfia vomitoria* and chlorpromazine may not adversely affect information encoding and learning potential since both extract and medicine do not have significant effects on the measure of spatial learning (% alternation) in the rats. The significant findings during the experiment were an increased right triplet (ABC or CAB) decisions in the *Rauwolfia vomitoria* exposed rats when compared to those of the chlorpromazine groups.

Although the results of the study strongly indicate an improvement in spatial memory and working memory following administration of *Rauwolfia vomitoria* since chlorpromazine has been shown to impair memory retention (Alvin *et al.*, 2008), it is at variance with the report of Rosengarten and Quartermain in 2002 where they report no significant difference often following administration of other antipsychotic agents like olanzapine.

Oxidative Stress Marker

Most of the known antipsychotic agents such as haloperidol are noted to cause a significant reduction in the activities of antioxidant enzymes thereby resulting to lipid membrane peroxidation (Miljevic *et al.*, 2010), especially in the cerebral cortex (Seet *et al.*, 2010) with a consequent increase in the oxidative stress of the neurons. However, the present study shows that chlorpromazine at 5 mg/kgbw and 10 mg/kg.bw improves cerebral oxidation by increasing the glutathione peroxidase level, contrasting the previous reports of glutathione peroxidase reductions in haloperidol

(Miljevic *et al.*, 2010) and clozapine (Ciobica *et al.*, 2011) treatments. The present study also showed that *Rauwolfia vomitoria* at 150 mg/kgbw, like chlorpromazine improved oxidative activities by the increasing glutathione peroxidase level. *Rauwolfia* can therefore be implicated as a potential antipsychotic agent capable of treating impaired antioxidant defense by reversing the reduced glutathione peroxidase level associated with psychosis (Padurairu *et al.*, 2010; Dakhale *et al.*, 2004; Reddy *et al.*, 1991).

Central Cholinergic Markers

In many brain areas the dopaminergic system is antagonised by the cholinergic system. Muscarinic receptor numbers may be reduced in psychosis (Readler, *et al.*, 2003). Antipsychotics have been shown to increase the efflux of ACh in the rat medial prefrontal cortex and hippocampus (Parada *et al.*, 1997; Shirazi-Southall *et al.*, 2002; Chung *et al.*, 2004). Acetylcholine had been linked to memory storage since elderly people present a progressive decline in brain acetylcholine levels. Acetylcholine has been implicated to function in learning, training, memory, motor activity and control of sleep, especially as drugs that deplete acetylcholine or compete for its receptor sites to reduce performance of memory tasks. Given the observed effects of muscarinic agonists on cognitive behaviors in a variety of animal models, it is highly likely that the increased efflux of ACh is relevant to the ability of the antipsychotics to improve cognition. In the present study, Acetylcholine level in the brain tissue significantly increased in the experimental groups, but much increase was recorded in the *Rauwolfia vomitoria* (150 mg/kg) administered animals. This result indicates that both chlorpromazine and *R. vomitoria* have no negative effects on brain Acetylcholine level, but their increased level in the *Rauwolfia vomitoria* administered animals suggested that it is more effective in improving brain functions when compared with chlorpromazine. From the histochemical result, it can be inferred that both CPZ and *R. vomitoria* can down regulate oxidative stress since decrease in glutathione concentration has been reported in psychotic conditions (Padurairu *et al.*, 2010; Weiden *et al.*, 2004 and Makanjuola, 1987).

Histoarchitectural analysis

Histomorphological observations of the pyramidal cells and some granular cells in the internal pyramidal cortical cell layer of the prefrontal cortices of rats show no morphological alterations with oral administrations of *R. vomitoria* (Figure 2), but some malformations are observed in the histomorphology of the pyramidal neuron in the internal pyramidal cortical layer of the prefrontal cortices of rats given chlorpromazine.

Conclusions

In conclusion, the results of this study in rats indicate that oral administrations of chlorpromazine can impair the performance of tasks designed to assess spatial learning and working memory. These results suggest that chlorpromazine can exert negative effects on specific components of cognition that are affected in psychosis (Terry and Mahadik 2007) and *R. vomitoria* could be a better complementary antipsychotic with lesser or no deteriorating effects on spatial working memory and the central cholinergic systems as impaired information encoding and retrieval capacity are commonly reported in psychosis (Gur, *et al.*, 2000; Cairo *et al.*, 2006).

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