Effect of Herbal Supplements on Anxiety, Hyperactivity and Memory in a Rat Model

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Abstract

There are several psychological disorders with the basis of anxiety, hyperactivity and memory that utilize a number of drugs, often having ineffective or abusive, detrimental effects. It has been suggested that St. John's Wort and Ginko biloba, two herbal supplements, could effectively treat the symptoms and behaviors resulting from such disorders without contributing to unfavorable results often seen with standard medications. Behavioral tasks were utilized to assess the subjects' responses to treatments in adolescent *Rattus Norvegicus.* These included an open field placement, elevated plus maze and object discrimination task. From our findings, there were no statistical differences between the treated groups and control groups on the effectiveness of herbal supplements on behavioral responses to the three tasks. This suggests that while they may provide a healthier and less abusive alternative to the conventional drugs used today, the effectiveness of herbal supplements doesn't seem to reduce anxiety or hyperactivity or improve memory.

Keywords: St. John's Wort, Ginko Biloba, Anxiety, Hyperactivity, Memory.

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Anxiety, hyperactivity and memory loss are deficits that have shown prevalence in several psychological disorders. Anxiety spectrum disorders are currently among the most common neuropsychiatric disorders that have drastically affected people in developed countries, with an estimated 13.3% of the adult population (18-54) in America alone (Safi, Neuhausser-Wespy, Welzl & Lipp, 2006; Sartori, Landgraf & Singewald, 2011). From an evolutionary perspective, the behavioral responses elicited by anxiety are necessary to increase the chances of survival; however, there is a surplus of negative effects on health associated with chronic stress. Drugs used to treat anxiety disorders are shown to be effective in a fraction of patients, with around 40% of individuals having little or no response (Virok et al., 2011). The several disadvantages could possibly be eradicated by replacing the drugs with natural products (Walesiuk, Trofimiuk & Braszko, 2005). St. John's Wort is ranked in the top five sold herbal supplements manufactured to assist with a variety of problems ranging from treatment of depression to treatment of anxiety disorders. The adverse effects associate with St. John's Wort is 1-3%, which compared to those of placebos (Kaustubh, 2005). While there is weak evidence of significant effect of the treatment of anxiety disorders, chronic administration of St. John's Wort has been shown be effective in patients with somatoform disorder, significantly reducing overall anxiety, as well as reverse the anxiety behaviors associated with increased corticosterone levels (Crupi et al., 2004; Saeed, 2007).

'Maladaptive impulsivity been implicated in wide range of psychiatric and neurological disorders including Attention-Deficit/ Hyperactivity Disorder (ADHD)' (Wiskerke, Stoop, Schetters, Schoffelmeer & Pattij, 2011). ADHD is the most widely

diagnosed disorder among children and adolescents (4-17 years). Diagnostic prevalence rates in 2007 reached 9.5%, with 4.8% of those diagnosed prescribed medication (Visser, Bitsko, Danielson & Perou, 2010). The majority of medications are stimulant based, with amphetamines (adderall) and methylphenidate (Ritalin and concerta) being the most commonly prescribed (Stimulant ADHD medications: methylphenidate and amphetamines, 2008). Abuse of stimulants for 'performance enhacement' and recreational purposes contributes to the ranking of third among illicit drug abuse among 12th graders (Stimulant ADHD medications: methylphenidate and amphetamines, 2008). St. John Wort has been implicated to have benefits in reducing impulsivity and hyperactivity behavior. Studies have shown it to block locomotor hyperactivity and such stereotyped behaviors (Uzbay, 2008). Additionally, influences of this pharmacotherapy have shown an inhibition in reuptake of noradrenaline further adding to the beneficial effects on locomotor hyperactivity (Sarris, 2011). In contrast, research on a derivative of St. John's Wort for treatment of ADHD symptoms has shown no additional benefits than the placebo. This, however, was potentially attributed to the low levels of derivative, with higher percentages giving hope of better results (Weber, 2008).

Of the public health concerns affecting society, one of the most urgently researched as of late is the susceptibility of individuals to dementia associated with aging and neurodegenerative disorders such as Alzheimer's Disease (AD). Efforts to prevent or delay the cognitive impairments are of utmost importance. Ginko biloba is one of the most widely marketed herbal treatments used as a preventative to age-related cognitive decline (Snitz et al., 2009). Research has shown possibilities to prevent neuronal damage and inhibit amyloid aggregation that is common of AD (Harding, 1990; Snitz et al.,

2009). There is much inconsistency, however, in the research compiled. Several studies showed no differences in the amount of cognitive decline or facilitation of memory in adults elderly adults without impairment between Ginko biloba treated and control groups (Dodge, 2008; Snitz et al., 2009; Solomon, Adams, Silver, Zimmer & DeVeaux, 2002). Other research, however, has suggested improvement to working and spatial memory and delay of cognitive decline in chronic Ginko biloba treated individuals compared to controls (Silberstein, 2011; Trick, Boyle & Hindmarch, 2004) as well as prevention of beta-amyloid production and aggregation (Bate, Salmona & Williams, 2004; Tang, Nag, Shiu and Pang, 2002).

In the exploration of Herbal supplements' effects, I hypothesize that improvements will be made on anxiety, locomotion behavior and memory. Since St. John's Wort is currently getting attention for being able to improve ADHD symptoms and Ginko Biloba claiming to promote memory, treated rats should show a decrease in anxiety and hyperactivity as well as an increase in memory as tested through a variety of behavioral tasks.

Method

All procedures met the Austin College IACUC guidelines, and subjects were treated according to the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington, D.C., 1996) and the *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (National Academy Press, Washington D.C., 2003).

Subjects

Twenty adolescent male Long-Evans rats weighing ~100 g on arrival (~350 g on sacrifice) were obtained from Charles River Laboratories. Rats were housed in pairs under controlled conditions on a 12:12 hour Light:Dark cycle (0700 on). Access to food and water was *ad libitum.* Rats were handled for 5 minutes per day during the two weeks prior to use in behavioral procedures.

Drug Administration

St. John's Wort and Ginko Biloba, two Spring Valley herbal supplements bought from Walmart, were orally administered to the experimental group of rats (n=10) in a diluted media of 75% water and 25% apple juice. The water/apple juice solution was given to the control group of rats (n=10) at the same time as the experimental group. The St. John's Wort solution (~0.3 mg/mL supplement consumed/day) was given consecutively over one week to the experimental group before behavioral analysis. After a 2-day non-treatment, wash out period, the Ginko biloba solution was administered over 2 consecutive weeks to the experimental group, with the first week consisting of a low dose (~0.12 mg/mL supplement consumed/day) (the data for which was disregarded) and the second week consisting of a high dose (~0.36 mg/mL supplement consumed/day), before behavioral analysis.

Behavioral Tasks

Open Field Placement. The behavioral apparatus was a wooden enclosure (212cm long X 74cm wide) with a grid floor separated into 48 squares (18.5cm), 28 peripheral and 20 central. Rats were placed in the center of the grid and recorded for 5 minutes for the amount of time spent grooming and for the number of fecal droppings,

rears, and line crossings (external and total)—as indicated by two front paws over a gridline. The experimental group was treated with St. John's Wort.

Elevated Plus Maze. The behavioral apparatus was a 4-armed black, wooden structure (8.5cm wide X 120cm long X 78.5cm high) with two open arms (55.5cm long X 8.5cm wide) and two enclosed arms with removable Styrofoam walls (55.5cm long X 8.5cm wide X 23.2cm high). Open arms were arranged opposite of each other. Rats were placed in the center of the plus maze and recorded for 5 minutes for amount of time spent in the open and closed arms, as indicated by two front paws crossing the entrance. The experimental group was treated with St. John's Wort.

Object Discrimination Task. The behavioral apparatus was an open box (46cm wide X 50.5cm long X 41.5cm high) with a wooden floor and two identical objects on either end. Objects used were an assortment of bottles, blocks, and containers Rats were placed in the center of the box and recorded for 3 minutes for the amount of time spent exploring each object, defined as sniffing at, whisking at, or looking at the object from no more that 2 cm away. With a 1-hour and 1 week delay, the rats were reintroduced to the apparatus, this time with a new (novel) object replacing one of the old (familiar) objects. Rats were placed in the center and recorded for 3 minutes for the amount of time spent exploring the novel and familiar objects. The experimental group was treated with Ginko biloba.

Statistical Analysis

The statistical significance of the results was computed by independent samples t-tests using *SPSS 12.0*. The treatments, St. John's Wort and Ginko biloba, were the independent variables, and the dependent variables were the number of lines crossed

(external and total), the number of fecal droppings, the number of rears, the time spent grooming, time spent in the open arms, number of open arm entries, percentage of time spent with the novel object, and total time spent with the objects during acquisition and recall.

Results

The St. John's Wort treated group showed no statistical differences in their behavioral responses to the open field placement in comparison to the control treated group. This included the number of lines crossed (external [$t(18) = -0.39$, $P = .35$] or total [$t(18) = -0.99$, $P = .17$]) (see *Figure A*), number of fecal droppings [$t(18) = 0.09$, $P = .47$] and number of rears [$t(18) = -0.08$, $P = .47$] (see *Figure B*), and time spent grooming [$t(18) = 0.97$, $P = .17$] (see *Figure C*). Additionally, there were no significant differences between groups on behavioral responses to the elevated plus maze: time spent in the open arm [$t(18) = 0.84$, $P = .21$] (see *Figure D*) or the number of open arm entries [$t(18) = -0.41$, $P = .34$] (see *Figure E*).

The Ginko biloba treated group showed no statistical differences in their behavioral responses to the object discrimination task in comparison to the control treated group. This included the percentage of time spent with novel object [$t(18) = 0.15$, $P = .44$] (see *Figure F*) and the total time spent interacting with the object at acquisition [$t(18) = 0.33$, $P = .37$] and recall [$t(18) = -0.46$, $P = .33$] (see *Figure G*).

Discussion

The main purpose of this study was to determine if herbal supplements (St. John's Wort and Ginko biloba) could work effectively on anxiety, hyperactivity and memory related behaviors in rat models in efforts to offer alternatives to the defective medications

available today. The open field placement is a common behavioral apparatus used to assess the anxiety-like behavioral responses of subjects forcefully confronted with threatening or stressful situations (Prut & Belzung, 2002; An et al., 2011). Anxiety was correlated to the amount of time spent in the central squares of the grid, with riskiness or a lack of fear being associated with the amount of time venturing to the peripheral squares (Aumatell et al., 2011; Crupi et al., 2011). Another common application is for evaluation of environmental interest and spontaneous motor activity (hyperactivity) as designated by the subjects' amounts of locomotor activity—indicated by the total number of gridline crosses (Choleris, Thomas, Kavaliers & Prato, 2011; An et al., 2011). *Figure A* indicated that there was no significant difference in the amount of total or external line crosses between both St. John's Wort treated or control treated groups. Because the total number of line crosses are an indication of hyperactivity and interest in the environment, our data suggest that both the experimentally treated and control treated groups were equally as interested in their environment, or equally as hyperactive. In other words, we can infer that St. John's Wort had no effect on the behavioral responses of the subjects in the open field placement task. The number of external line crosses, associated with riskiness or lack of anxiety, indicated that both the St. John's Wort treated and control treated groups were equally as risky to venture to the outer perimeter, or equally as nervous to stay in the center (*Figure A).* Because we can designate increased amounts of defecation and time spent grooming in the open field placement task as anxiety-like behaviors, our results (*Figures B & C*) would suggest that St. John's Wort had no effect on the anxiety-like behavioral responses elicited from being forced into threatening or stressful situations. *Figure B* additionally assesses the relative amount of curiosity of the

environment, or lack of anxiety, as designated by the average number of rears. The results suggest that the St. John's Wort treated and control treated groups did not differ in the levels of curiosity. This implies that St. John's Wort treatment had no effect on the relative levels of anxiety of rats in the open field placement. The results we saw from the open field placement task would propose that St. John's Wort may not be an ideal alternative to treatment of anxiety or hyperactivity disorders; however, more studies should be conducted with larger populations and perhaps a higher dosage. Buchholzer, Dvorak, Chatterjee and Klein (2002) reported that high dosages (10mg/kg) of hyperforin, a constitute of St. John's Wort, showed significant decreases in the observed locomotor activity whereas the low dosages were ineffective.

The elevated plus maze is commonly known as a validated measure of both unconditioned and conditioned anxiety or fear (Aumatell et al., 2011), with the time spent in the closed arms indicative of anxiety-like behavior and time spent in the open arm suggestive of lower anxiety or fear (An et al., 2011). *Figure D & E* suggest that the amount of time spent in the open arms and the average number of open arm entries did not differ among the St. John's Wort treated and control treated groups. This indicated that the treatment did not successfully decrease the anxiety-like behaviors exhibited in comparison to our controls.

The object discrimination task is used to assess the exploratory behavior of subjects to novel objects more than familiar objects. Their tendency to explore the new objects indicates retention of memory to the familiar objects (Walesiuk, Trofimiuk & Braszko, 2005). This is hippocampal to an extent in its declaraive-like nature, and more time spent with the novel object suggests remembrance of the less interesting, familiar

object. *Figure G* showed that the amount of time of exploratory behavior during the acquisition and recall phase were not significantly acted on by Ginko biloba treatment. The results would suggest that the treatment neither increased nor decreased the interactions of the subjects with their environments. *Figure F* demonstrated that the percentage of time the subjects interacted with the novel object did not differ significantly between treatment groups. This would suggest that Ginko biloba had no effect on the retention of memory of the familiar object as demonstrated through increased time of interaction with the novel object. More tests with a larger population should be conducted to see if the data becomes more favorable. Females have been shown to have higher exploratory drives than males (Aumatell et al., 2011); hence, more experiments including both genders may show different results. Interestingly, the literature shows Ginko biloba to normalize cognitive deficits see post-induced stress (Aumatell et al., 2011); therefore, the design of our experiment may have been addressing the wrong effects in Ginko biloba due to cortisol's involvement in hippocampal degredation.

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Figure A

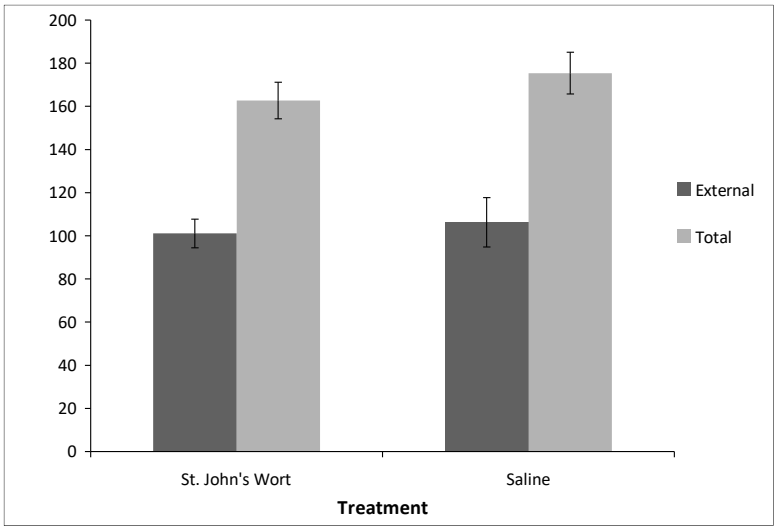

Figure B

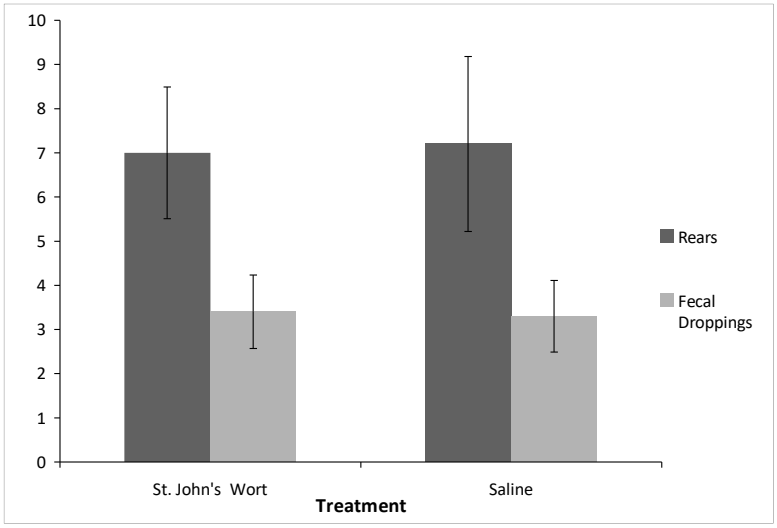

Figure A) Average numbers of total and external line crosses representing hyperactivity and risky behavior between both the St. John's Wort treated and control treated groups. *B)* Average number of rears, representing environmental curiosity, and fecal droppings, representing relative anxiety, between both the St. John's Wort treated and control treated groups. No significant differences were found between treatments. Standard errors are represented by error bars.

Figure C

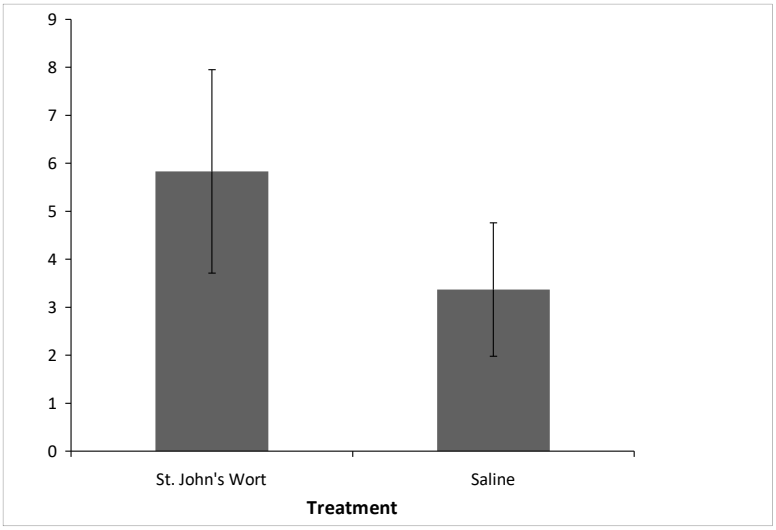

Figure C) Average amount of time spent grooming, representing relative anxiety, between both St. John's Wort treated and control treated groups. No significant differences were found between treatments. Standard errors are represented by error bars.

Figure D

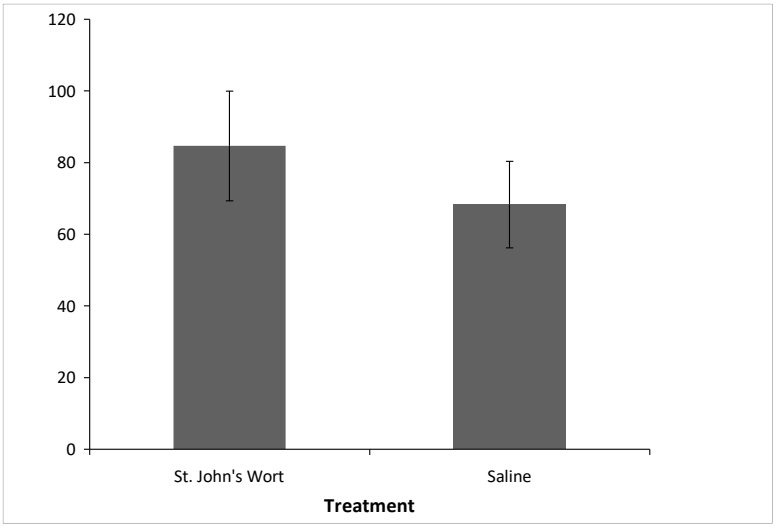

Figure E

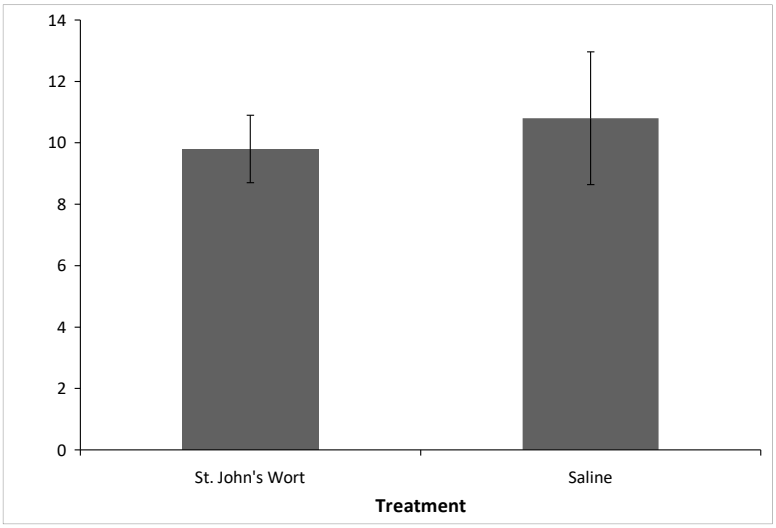

Figure D) Average amount of time spent in open arms and *E)* average number of open arm entries in the elevated plus maze representing relative lack of anxiety between both the St. John's Wort treated and control treated groups. No significant differences were found between treatments. Standard errors are represented by error bars.

Figure F

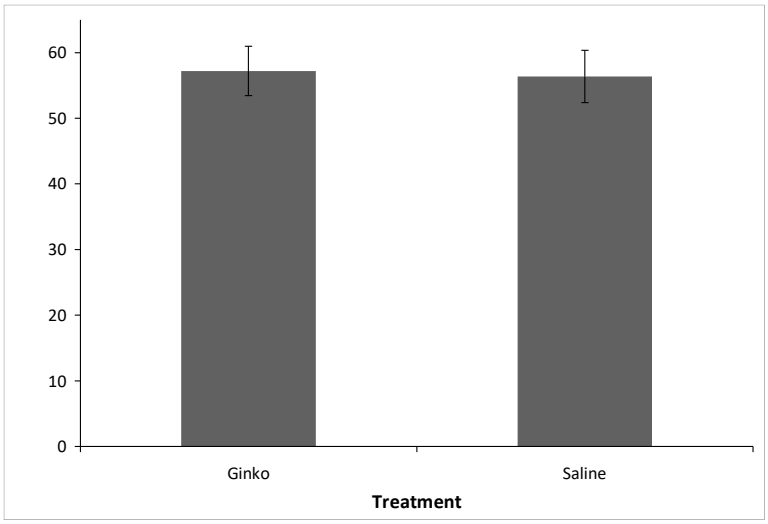

Figure G

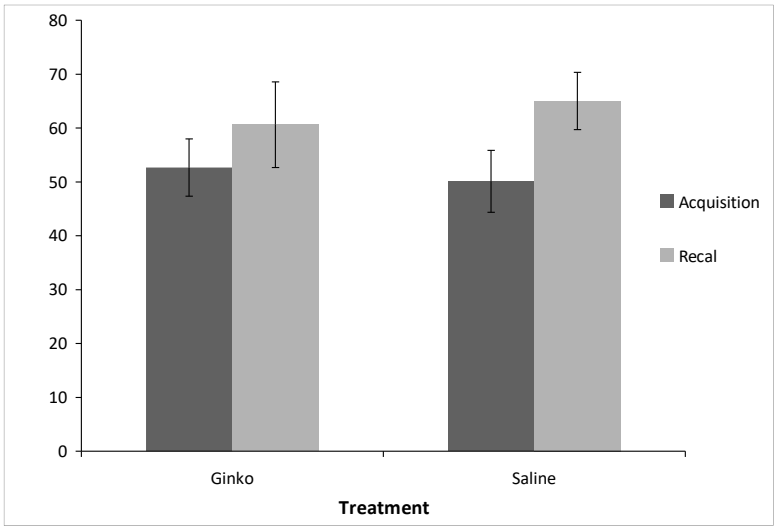

Figure F) Percentage of time spent interacting with the novel object, representing the familiarization or memory of the familiar object, and *G)* total time spent interacting with the objects during acquisition and recall, representing the amounts of exploratory behavior of the objects between both the Ginko biloba treated and control treated groups. No significant differences were found between treatments. Standard errors are represented by error bars.