The neuro-physiological effect of *Stevia rebaudiana* to Imprinted Control Mice (ICR) mice

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**Abstract**

The use of alternative medicine becomes popular once more in the Philippines and worldwide because nowadays, alternative medicines like herbal medicine are used to treat certain ailments and for disease prevention. *Stevia rebaudiana* which is a herbal food supplement contains high level of sweetening compounds known as steviol glycosides and it is used by people especially diabetic people as an alternative sugar. The treatment groups used were \( T_0 = \) Water; \( T_1 = 0.3 \text{ mg/ml of powdered } S. \text{ rebaudiana } T_2 = 0.6 \text{ mg/ml of powdered } S. \text{ rebaudiana, } T_3 = 0.9 \text{ mg/ml of powdered } S. \text{ rebaudiana, } T_4 \text{ is twice a day (T1), } T_5 \text{ is twice a day (T2), } T_6 \text{ is twice a day (T3), } T_7 \text{ is thrice a day (T1), } T_8 \text{ is thrice a day (T2) and } T_9 \text{ is thrice a day (T3) to determine if it causes a stimulant or depressant effect to the Central Nervous System (CNS) and Peripheral Nervous System (PNS) of ICR mice. Using one way ANOVA and two-sample t-test, the results in the physiological and behavioral test for CNS shows that the three doses including the frequencies administered to the ICR mice have no significant differences which indicates that it has the same depressant effect. However, in the hot plate method for PNS the ICR mice belonging to \( T_9 \) have longest time duration of reaction when the mice were subjected to heat and while in the dose, 0.9g of Stevia exhibited the greatest effect therefore powdered *S. rebaudiana* was proved to depress the CNS and to reduce sensitization to the PNS.

**Keywords:** *Stevia rebaudiana*, Imprinted Control Mice, Depressant, Stimulant, Neuro-Physiological effects

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**Introduction**

The use of alternative medicine becomes popular once more in the Philippines and worldwide because nowadays, alternative medicines like herbal medicine are used to treat certain ailments and for disease prevention (Sincerity, 2006). The chemical constituents present in herbal medicine are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. A lot of people believe that herbs are much safer than synthetic drugs because herbs are natural but this is not always true. Not all herbs are created equally because each herb can cause a different effect in the body system and in the mind. Misuse of herbal medicines is toxic and can cause serious side effect and health problems like stomach cramps, trembling, dizziness, nausea and vomiting, malnutrition, liver and renal dysfunction, pulmonary hypertension, shortness of breath and impaired abilities (Sincerity, 2006).

*Stevia rebaudiana* commonly known as Stevia, serves as an example of a herbal medicine. Stevia extracts is not just known to have therapeutic properties
but it comprises high level of sweetening compounds known as steviol glycosides, which are thought to possess antioxidant, antimicrobial and antifungal activity. The compound interest of the study is the two main sweetening, stevioside and rebaudioside A. Due to the increasing demand of consumer for herbal foods, *S. rebaudiana* can be considered that it has a great potential as a new agricultural crop and the proximate analysis for this study shown that Stevia comprises folic acids, vitamin C and all the indispensable amino acids with exception of tryptophan. It is important in human nutrition because it is an excellent source of carbohydrates, protein, crude fibre, minerals, dispensable and indispensable amino acids. Stevia leaf has the presence of biologically important secondary plant products which contributes to its medicinal value because they exhibit physiological activity (Sofowara, 1993).

The *S.rebaudiana* belongs to the 230 members of the genus, *Stevia* and only *Stevia rebaudiana* and *Stevia phlebophylla* have the capability of producing sweet steviol glycosides (Brandle and Telmer, 2007). It is a natural sweetener and a herbaceous perennial plant of the Asteraceae family. Its origin is in North and South America. *S.rebaudiana* is renowned for having a high content of sweet diterpene which is about 4-20% matter (Ghanta et al., 2007). It also supplies a quantity of sweet *ent*-kaurenediterpenoid glycosides (Prakash et al., 2008), and it also contains *stevia* glycosides for the sweet taste. The purpose of this study is to determine the neuro-physiological effects of *S.rebaudiana* leaves. The researchers will study the nervous system of the male ICR mice which contains all nerve tissues present in the body in which this nerve tissue is responsible for receiving and transmitting stimuli to nervous centers, initiating a response and interpreting it (Ophardt, 2003) because (King, 2011) stated that the Stevia contains beta-caryophyllene and caryophyllene oxide, compounds that may depress the activity of the central nervous system and may also have an effect on the transmission of nerve impulses to and from the person’s central nervous system and may slow down nerve conduction velocity.

**Materials and Methods**

*Research design*

Nine treatment groups including one control group and the frequencies administered were used in the experiment. Three dose of *Stevia rebaudiana* were given. Three frequencies were administered with three replications in each. The forty-eight male ICR mice weighing 25-30 g were purchased from Research Institute for Tropical Medicine, Alabang, Muntinlupa City.

*Procurement and preparation of the different treatments*

Powdered *Stevia rebaudiana* were purchased from Mercury Drug Store in Dasmarñas City. The treatment groups (T1, T2, T3) has 0.3, 0.6 and 0.9 g weight of administered *S. rebaudiana* respectively and diluted to 1 ml of water. Water and rat food were obtained for T0. The doses were based on the body weight of male ICR mice. The researcher prepared four treatment groups (T0, T1, T2, T3) with different amount of doses and six treatment with frequency applied (T4, T5, T6, T7, T8, T9) that helped to determine treatment that has the highest indication that the powdered *S. rebaudiana* affects the central and peripheral nervous system such as depression or stimulant and pain perception respectively. The treatment groups that were administered to the ICR mice were based on the study of Al-Yousuf et al. (2002).
Acclimatization and maintenance of laboratory mice

The ICR mice were placed in one of the researcher’s dormitory and it is where one-week acclimatization and maintenance of male ICR mice was done. The four cages were kept in good condition with a well-ventilated room temperature of 26-30°C. Each cage was spacious enough to be able for the mice to roam around and for the researcher to observe their behavior. The place and cages were regularly sanitized and the mice were given sufficient food pellets and mineral water supply every other day. Daily monitoring was also conducted and dead mice were able to be taken out immediately.

Administration of powdered Stevia rebaudiana

The nine treatment groups were administered which includes the three different frequencies (once a day, twice a day and thrice a day). The administration was conducted every other week to give time for the examination in latter part.

Physiological and behavioral examination: After one week of administration of powdered S. rebaudiana, its behavior was observed and examined using the suggested method of Bernas et al. (2005). Different directions were conducted to test the different parameters in the said exam. The exam is a score-based test in each parameter and each score is being tabulated. The examination was done at the PCH laboratory in De La Salle University-Dasmariñas, Cavite.

Hot plate method

After one week of administration, after physiological and behavioral examination, hot plate method (Eddy and Leimback, 1953) was done. In this method, male ICR mice pain perception was tested. Mice were subjected to heat using the hot plate with beaker and the time was recorded until it jumped or licked its feet. Procedure was done with first examination.

Termination of laboratory activities

Laboratory activities were properly terminated in the third week of October based on the agreement done with the Bioethics Committee of the university.

Data analysis

The behavior of the mice was tabulated and the time recorded for pain perception was analyzed by getting its average. Two statistical analyses (One way ANOVA and two sample t-test) were done after the initial data to be able to compare the differences among the treatment and frequency applied.

Results

Physiological and behavioral evaluation

After data were tabulated, the treated mice shows sign of depression since the tabulated score in table 4.1 shows that the total scored values of the powdered Stevia are higher in the CNS depressant compared with the CNS stimulant which yielded low scored values. Frequent sleeping and decrease in motor activity were also observed which indicates powdered Stevia has possible depressant effect. The tabulated data also shows Statistical analysis was done by using the two sample t-test in order to make a comparison of the greatest effects among treatment groups and frequencies. The results obtained shows that there is a significant difference between the treated and untreated ones and there is no significant difference among the three treatment groups (T1, T2 and T3) which means that it both elicits the same CNS depressant effects on the ICR mice and threshold was already reached. Also there is a possibility that if the doses were increase in more than 0.9 g, greatest effect can be observed.
Table 1. A Summary of Mean Scores of the responses of the ICR mice to various doses

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment Groups*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0 T1 T2 T3 T4 T5 T6 T7 T8 T9</td>
</tr>
<tr>
<td>Startle reaction</td>
<td>36 16 13 14 6 6 6 6 6 4</td>
</tr>
<tr>
<td>Motor activity</td>
<td>54 25 15 12 12 6 0 5 2 1</td>
</tr>
<tr>
<td>Fine body tremors</td>
<td>0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Coarse body tremors</td>
<td>0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Fasciculations</td>
<td>0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Convulsions</td>
<td>0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Respiratory rate and depth</td>
<td>0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Total</td>
<td>90 41 28 26 18 12 6 11 8 5</td>
</tr>
</tbody>
</table>

*T0= control; T1= 0.3g Stevia; T2= 0.6g Stevia; T3= 0.9g Stevia; T4= twice a day (T1); T5= twice a day (T2); T6= twice a day (T3); T7= thrice a day (T1); T8= thrice a day (T2); T9= thrice a day (T3)

Table 2. A summary of the average time (seconds) of reaction to the hot plate method

<table>
<thead>
<tr>
<th>Treatment Groups*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>ICR mice</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Average time</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>T0 T1 T2 T3 T4 T5 T6 T7 T8 T9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.10 7.15 4.71 7.70 8.55 8.71 10.46 11.11 10.16 19.62</td>
</tr>
<tr>
<td></td>
<td>2.51 5.98 6.00 9.54 8.35 10.66 10.94 15.13 6.14 40.27</td>
</tr>
<tr>
<td></td>
<td>3.56 4.55 6.37 9.70 6.61 7.52 14.59 12.64 12.56 54.98</td>
</tr>
<tr>
<td></td>
<td>4.21 3.34 8.25 8.20 8.62 7.24 17.00 14.44 8.95 32.83</td>
</tr>
<tr>
<td></td>
<td>3.23 4.60 6.86 8.10 9.06 6.81 16.46 9.90 15.63 34.10</td>
</tr>
<tr>
<td></td>
<td>2.96 5.52 6.07 8.75 8.14 8.58 16.61 11.87 12.09 36.32</td>
</tr>
</tbody>
</table>

*T0= control; T1= 0.3g Stevia; T2= 0.6g Stevia; T3= 0.9g Stevia; T4= twice a day (T1); T5= twice a day (T2); T6= twice a day (T3); T7= thrice a day (T1); T8= thrice a day (T2); T9= thrice a day (T3)

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In addition, the comparison among the different frequencies indicates that there is no significant difference among the frequency of the dose applied, this means that even though the frequency were increased, it will still elicit same effect to CNS.

**Hot plate method evaluation**

Using the mean recorded time, results show that T9 which has an average time of 36.32 seconds, has the longest time recorded among the treatment groups (Table 2). Based on the results, as the researchers increased the doses of *Stevia* per treatment, the longer the time that the mice reacted to heat applied. It shows that powdered Stevia can cause analgesic effect to the pain perception of the ICR mice. Furthermore, results show that the average time takes longer when the dose increases. This means that the body was able to absorb the concentration and distribute it to the PNS pathways. Since heat was applied during the experimentation, pain perception was tested and the results showed that it causes numbness and a possibility that certain chemical molecules have been blocked or inhibited by powdered Stevia. The results acquired also indicates that the three treatment groups (T1, T2, T3) have significant differences indicating that there is an effect among the group but only differs to the degree of effects on the ICR mice. Based on the statistical analysis done by the authors, the results obtained indicates that once and twice administration has no significant differences among the treatment groups for the reason that there is a possibility that the liver of ICR mice were able to metabolize the first and second doses completely and not the third administration. As the dose increases, the average time of reaction of the ICR to the hot plate method also increases. Once a day and twice a day administration have only small increase which indicates that Stevia was possibly absorbed completely and was able to distribute to the system before the second administration in twice a day and was able take its effect. A greater effect was exhibited on the third administration for the possible reason that the pharmacodynamics effect of Stevia did not reached yet its half-life when another administration was done and the body was not able to metabolize the added concentration.

**Discussion**

**Central Nervous System**

Depression is a significant contributor to the global burden of disease and affects people in all communities across the world. Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, decreased energy, feeling of guilt or low self-worth, disturbed sleep or appetite, inhibition or dampening of the respiratory centers occurs, and poor concentration. Moreover, depression often comes with symptoms of anxiety (World Health Organization, 2012). Using the stimulation test suggested by Bernas, the results proved that the Stevia causes depression in the CNS since the researchers observed that the motor activity of the ICR mice decreased and move spontaneously, but will move slowly when handled. In addition, in testing the rating for ataxia, the ICR mice only has constant coordination while moving and its respiratory rate and depth shows a definite decrease which is according to World Health Organization is a common symptom during mental depression where respiratory rate and depth decreases, and PCO$_2$ increases. Furthermore, when the reflex of corneal and pineal of the ICR mice was tested it only made a sluggish response and while testing for grip of the ICR mice to a particular screen, the ICR mice falls of when the screen is inverted.
Using the statistical analysis, it was known that the three treatment groups (T1, T2, T3) and frequencies has no significant difference and produce only same depressant effect to the CNS because Stevia extracts containing 50% stevioside were fed to rats at doses up to 1 g/day for 56 days without detectable effects on biochemical or pathological endpoints, except for a slight depression in hepatic lactate dehydrogenase activity (Lee et al., 1982). A Stevia extract containing 75% stevioside and 16% rebaudioside A was fed to rats for up to 24 months at doses up to 550 mg/kg/day. The only effect noted was slight growth retardation (Yamada, 1982). The Stevia may affect the mechanism of MAO-B, and it may be the cause of the oxidation of hydrogen peroxide and produced free radicals which can cause the malfunction in how MAO works because of the build-up of hydrogen peroxide in the brain which are extremely toxic to brain tissue and can cause immediate damage in the dopaminergic pathways and can lead to depression, panic disorder, or chronic schizophrenia and can also cause Parkinson’s disease, Alzheimer’s disease, and epilepsy.

Some drugs are considered as dopamine agonists and bind to dopamine receptors in place of dopamine and directly stimulate those receptors. When dopamine or dopamine agonists constantly stimulate dopamine receptors it decreases the number of receptors and the receptors become less sensitive to dopamine. While dopamine antagonists are drugs that bind but does not stimulate dopamine receptors (Erickson, 2012). Antagonists can prevent the actions of dopamine by keeping dopamine from binding to receptors and there is a possibility that when the Stevia were administered to the ICR mice the prolonged exposure to dopamine inactivated a regulatory protein in the brain known as, Protein Kinase B (PKB) or Akt and causes the ICR mice to be in a depressed condition. In addition, when PKB/Akt regulatory protein in the brain is inactivated that causes the mice to become desensitized to certain drugs (WebMD, 2005).

The two main systems in the brain that involve dopaminergic neurons are nigrostriatal system and the mesolimbic system. The nigrostriatal system, have an effect on parts of the brain that are responsible for the control of voluntary movement. It can result to a loss of movement control like Parkinson's disease if these neurons are damaged. The mesolimbic system consists of neurons that end in the nucleus accumbens and also includes connections with the amygdala, which is responsible for emotional responding, and while the hippocampus, is responsible for memory processing, Stevia may have an effect to function of the dopaminergic neurons particularly in the mesolimbic system because it alters the emotional responding of the mice by depressing the CNS (Carriere, 2012).

One of the observations noticed to the ICR mice is that it has a habit of sleeping frequently which may indicate that Stevia has possibility of having an antihistamine effect. Histamine facilitates variety of the physiological reactions on the peripheral tissue as well on the central nervous system through the three types of receptor for histamine which is H1, H2 and H3 (Schwartz et al., 1991). With the presence of histamine antagonist or antihistamine, it may not only inhibit histamine-H1 receptor interactions but also antagonize the muscarinic, serotonergic and dopaminergic actions (Yanai et al., 1990) which may tend to the decrease motor activity and causes the sleepiness of the mice. There is also a possibility that due to the different doses
of powdered Stevia, certain molecules are able to enter the bloodstream and outnumber the histamine and able to act to certain allergies or inflammation mediated by H1-receptor and yields sedative effects (Millwork, 2013). However, the mechanisms mentioned are not explored in this study and needs further investigation.

Peripheral Nervous System

The longer average recorded time for the application of hot plate method to the ICR mice indicates that Stevia has analgesic or sedative effect to the PNS and may be related to an effect on the cyclo-oxygenase or prostaglandin pathway which may inhibits the action of cyclooxygenase 1 and 2 which is essential to the production of pain (Ricciotti and FitzGerald, 2012). Another factor that can be the basis of the said result is that since the response of mice to the hot-plate paw-licking is due to the supraspinally integrated response (Suh et al., 2011), there is a possibility that the antinociceptive result of hot plate indicates in that Stevia may causes inhibition on supraspinal centers (Dewey et al., 1970). However, such mechanism is not explored in this study and needs further investigation.

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