Effects of Chronic Consumption of *Cola nitida* on Melatonin and Enzyme Production in Albino Wistar Rats

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**ABSTRACT**

The effect of *Cola nitida* consumption on melatonin production in albino male Wistar rats was investigated. Wistar rats weighing 150 - 220g were fed for 30 days with different percentages of powdered *Cola nitida* (5%, 10%, 20%, 30% and 0% w/w). Serum concentrations of melatonin, ascorbic acid (AA), glutathione (GSH), serum alkaline phosphatase (ALP), serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of albino rats were determined using standard methods. Results show that melatonin level increased in the range of 14.8 - 23.8 ng/ml. As the concentration of *Cola nitida* in the exposed groups increased there was a decrease in melatonin concentration (9.3 ng/ml). There was a significant decrease (p<0.05) in the level of ascorbic acid (0.0085 to 0.0057 mg/dl) and glutathione (5.7 to 2.5 mg/dl) compared to the control group which showed 8.9 mg/dl and 6.7 mg/dl respectively. Similarly, aspartate aminotransferase (56.7 to 24.8 U/l) and alkaline phosphatase activities (54.5 to 25.3 Ul) decreased significantly (p<0.05) as the concentration of *Cola nitida* increased compared to control. However, alanine aminotransferase activity increased as the concentrations of *Cola nitida* increased between 20 to 30%. This study shows that *Cola nitida* reduce the levels of melatonin production in exposed rats and may consequently cause sleeping related disorders among consumers or exposed groups.

**Keywords**: *Cola nitida*, Diet, Melatonin, Ascorbic acid, Albino rats

**INTRODUCTION**

Nuts exist in nature, which are highly consumed as part of man’s cherished diet. However, its chemical constituents can favorably influence body physiology. *Cola nitida* is one of such nuts that its consumption stimulates the central nervous system, gives physical strength, and help to reduce hunger and fatigue in individuals consuming it [1,2].
Studies have shown that *Cola nitida* contains sugar, theobromine, caffeine, polyphenol and some proximate compounds such as water, ash, fats, and protein [3-5]. The phenolic contents of *Cola nitida* have also being reported to include d-catechin, l-epicatechin and kolanin [1]. Some of these chemical constituents are used in beverage drinks, as flavor, laxative, heart stimulator and sedatives [4-6]. Many professionals consume *Cola nitida* because of its stimulatory and fatigue resistant effects on them. This has led to increased addiction to *Cola nitida* for individuals consuming it. Results of the previous studies have consistently shown adverse effects of *Cola nitida* consumption to include increase contraction of cardiac muscle, increased secretion of gastric acid, and increased uptake of glucose in the skeletal muscles of dog [7-10]. Many researchers have attributed the biological and physiological effects of *Cola nitida* to its caffeine content [9,1,7]. Also toxicological studies on caffeine consumption have shown lots of clinical symptoms, such as; nervousness, insomnia to sensory disturbances, diuresis arrhythmia, elevated respiration and gastrointestinal disturbances [11]. Reproductive effects [5,12,13], psychoactive behavioral effects [14] and loss of body weight [15,16], have also been reported. The effects of *Cola nitida* on inhibition of enzymes have been attributed to the presence of catechin, epicatechin, apigenin and naringenin [17]. However, information on the effect of *Cola nitida* consumption on the neuroendocrine modulator parameter, such as melatonin level in humans is scarce. Melatonin is a major neuroendocrine modulator with a far-reaching biological influence over most hormonal and behavioral functions of human beings [18]. There are reports that melatonin is strongly affected by the length of the day, artificial illumination, electromagnetic energy, exercise, nutrition, and other factors [19]. There is no knowledge on the effects of nuts such as *Cola nitida* on melatonin secretion. Therefore, the aim of this study is to investigate the effect of *Cola nitida* on the melatonin level of albino rat.

**MATERIALS AND METHODS**

*Plant material collection and preparation*

Pods of *Cola nitida* were procured from the Ekeonunwa Market, Owerri, Imo State, Nigeria and were identified by a plant taxonomist at the Department of Biology, School of Science, Federal University of Technology, Owerri. The nuts were removed from the pods, air-dried for two weeks at room temperature and ground into a fine powder using a grinder.

*Experimental design*

Thirty (30) male Albino Wistar rats weighing between 120 – 200 grams were procured from Faculty of veterinary Medicine, University of Nigeria Nsukka and housed in the Research Laboratory of Department of Biology, Federal University of Technology Owerri Nigeria. The rats were housed in five standard cages with the sides made of closely meshed wire gauze to allow for enough ventilation. Each cage housed six rats for each group under hygienic condition at temperature (25 ± 2°C) and an appreciable relative humidity with a 12:12 hr. light/dark cycles.

*Animal grouping and feed administration*

The animals were fed with water and normal feed *ad libitum*. The five groups of different experimental groups were fed with different concentrations of feed mixed with ground *Cola nitida* seed thus; the group (1) received normal rat feed and clean drinking water freely. The four other groups received different quantities of ground kola nut mixed with normal rat chow feed as follows: group two (2) five grams (5 g) of kola nut with ninety five grams (95 g) of rat feed, group three (3) received ten grams (10 g) of ground kola with ninety (90 g) of rat chow feed, and group four (4) received twenty grams (20 g) of ground kola nut.
with eighty grams (80g) of normal rat feed, group five (5) received thirty grams of kola nut with seventy (70) of rat chow feed in the percentage concentration of 5%, 10%, 20% 30% and 0% respectively. Mortar and pestle were used to blend the mixture to form the kola nut-diet. The kola nut-diet was administered to the four groups on daily basis for 30 days (Table 1).

Table 1: The treatment regime for each group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Diet Received</th>
<th>Percentage Quantity of Kola Nut in Diet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Kola nut-diet + water</td>
<td>5%</td>
</tr>
<tr>
<td>Group 2</td>
<td>Kola nut-diet + water</td>
<td>10%</td>
</tr>
<tr>
<td>Group 3</td>
<td>Kola nut-diet + water</td>
<td>20%</td>
</tr>
<tr>
<td>Group 4</td>
<td>Kola nut-diet + water</td>
<td>30%</td>
</tr>
<tr>
<td>Group 5</td>
<td>Normal rat feed + water</td>
<td>0% (control)</td>
</tr>
</tbody>
</table>

Collection of blood samples

Rats were anesthetized by exposing them in a desiccator containing cotton wool soaked with chloroform for about three minutes. After that the rat were placed on the dissecting board sacrificed, and carefully dissected to expose the heart region. Blood samples were collected using 5 ml hypodermic syringe and needle and each blood sample collected was discharged equally into an EDTA bottle. Blood samples were allowed to clot and centrifuged at 3000 rpm for 10 mins. The serum was separated using micropipettes and then used for biochemical analysis.

Biochemical analysis

Melatonin concentration was determined using the method described by Kayo and Borilek [20]. Ascorbic acid, Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined by the method of Duncan et al., [21]. Reduced glutathione level in serum was determined by the method of Jollow et al., [22], this method is based on the formation of a relatively stable chromophoric product on reacting with sulphur hydryl compound (GSH) with Elman’s reagent.

Statistical analysis

Each reading was taken in triplicates and all data were expressed as means and standard deviation and analyzed statistically using Analysis of Variance (ANOVA), the values were considered significant at $p \leq 0.05$.

RESULTS

The oral administration of *Cola nitida* increased the production of serum melatonin of albino Wistar rat. Results obtained showed that the highest level of melatonin production was observed in albino Wistar rats in Group 4 (30% concentration of *Cola nitida* in its diet). The lowest level was obtained from albino Wistar rats in control, which were not fed with *Cola nitida*. The levels of
melatonin production in all the groups fed with *Cola nitida* were higher than that obtained from the control (Figure 1). The results of levels of melatonin obtained from the rat fed with different concentrations of *Cola nitida* were significantly different from the control.

![Figure 1: Melatonin concentration (ng/ml) of albino Wistar rats exposed to different concentrations of *Cola nitida*. Bars are mean ± standard deviation. Bars bearing different alphabet letters are statistically significant (p<0.05).](image)

The glutathione concentrations showed decrease in all they albino Wistar rat fed with different concentrations of *Cola nitida* (Figure 2). The lowest concentration of glutathione was obtained from Group 4 (30% Concentrations of *Cola nitida*), followed by Group 3 (20% concentrations of *Cola nitida*). The levels of Glutathione of those fed with 20 and 30% of *Cola nitida* were significantly different from those obtained from 10% concentration (control).

![Figure 2: Glutathione concentration (mg/dl) of albino Wistar rats exposed to different concentrations of *Cola nitida* Bars are mean ± standard deviation. Bars bearing different alphabet letters are statistically significant (p<0.05).](image)

The result of concentration of ascorbic acid in albino Wistar rat fed with different concentrations of *Cola nitida* showed a significant decrease. Ascorbic acid reduced in the rats fed with *Cola nitida* compared to the control. The result also showed that as the concentration of *Cola nitida* increased in the diet of the rat, the concentration of ascorbic acid decreased. The highest ascorbic acid was obtained in the control (Zero % concentration of *Cola nitida*) group while the lowest value was obtained in group 3 (20% concentration of *Cola nitida*) and group 4 (30% concentration of *Cola nitida*) (Figure 3).
The result of alkaline phosphatase activity of albino rats fed with different concentrations of *Cola nitida* showed that the alkaline phosphatase level in rats reduced as the concentration of *Cola nitida* increased in the feeds given to the rats. The highest alkaline phosphatase was obtained in group one (control) while the lowest value was obtained from group 4 (30% concentration of *Cola nitida*). The values obtained from rats fed with *Cola nitida* were significantly different (p<0.05) from that of the control (Figure 4).

The result of aspartate aminotransferase levels in rat fed with different concentration of *Cola nitida* is presented in Figure 5. The result showed increase in aspartate aminotransferase in control compared to the other groups. The lowest aspartate aminotransferase activities were lowest in group 3. There is no significant difference between the aspartate aminotransferase levels obtained in group 2 and 3 though the concentration of *Cola nitida* fed to the rats were different.
Figure 5: Aspartate aminotransferase activity (U/l) of albino Wistar rats exposed to different concentrations of *Cola nitida*. Bars are mean ± standard deviation. Bars bearing different alphabet letters are statistically significant (p<0.05).

The result of alanine aminotransferase activity in albino Wistar rats exposed to different concentrations of *Cola nitida* is shown in Figure 6. There is no significant difference between Alanine aminotransferase activity in the control (0% concentration of *Cola nitida*) and group 1 (10% concentration of *Cola nitida*). However, there were increase in alanine aminotransferase level in rat fed with 20%, 30% and 40% concentration of *Cola nitida* and there was no significant difference in the result obtained in the three groups.

DISCUSSION

The result obtained showed that melatonin level in rat in all the groups decreased significantly (p<0.001) when compared with the control. This signifies that chronic consumption of *Cola nitida* in low doses lowers the rate of melatonin. The reduction in melatonin could be attributed the caffeine content of *Cola nitida* which has been reported to inhibits melatonin synthesis through...
its interaction with adenosine [23]. It could also be that the caffeine had synergistic effects with other components of *Cola nitida*. The result showed the increase level of *Cola nitida* in diet reduced significantly melatonin levels showing that the higher the consumption rates the higher the reduction rate as well. Melatonin has been reported as a major neuroendocrine modulator of annual and circadian biorhythms in the body, and has a far-reaching biological influence over most of the autonomic, hormonal, and behavioral functions of the human organism [24].

The result showed the increase level of *Cola nitida* in diet reduced significantly melatonin levels showing that the higher the consumption rates the higher the reduction rate as well. Melatonin has been reported as a major neuroendocrine modulator of annual and circadian biorhythms in the body, and has a far-reaching biological influence over most of the autonomic, hormonal, and behavioral functions of the human organism [24]. The bulk of melatonin seen in circulating blood is derived from pineal gland regardless of the fact that pineal melatonin is just one of the many sites of melatonin production.

Glutathione level in rat in different groups significantly decreased (p<0.01) when compared with the group meaning. This could be due to the decrease in the melatonin level, which could have caused a non-stimulatory effect on glutathione, though the organism involved was still able to produce the antioxidant even with the reduction in melatonin level. Rodriguez *et al.*, [25] reported that melatonin antioxidative effect is one of the many functions of melatonin. Its ability to enhance the activities of varieties of antioxidative enzymes its stimulatory actions on the synthesis of another important intracellular antioxidant, glutathione has also been reported [26]. The result obtained, from 20% and 30% groups showing significant reduction when compared with the control and the reduction level in melatonin, present was sufficient to have sustained the glutathione production and hence reaffirming the fact that melatonin has stimulating effect on glutathione [26].

Vitamin C level in the rat group showed a significant decrease (p<0.05) when compared with the group, which means that the decrease in melatonin level had inhibitory effect on stimulation of vitamin C in the rats. The Vitamin C level in other groups showed significant difference when compared with that of the control and thus indicating that the melatonin level in them was not sufficient to have sustained a good level of vitamin C production, this finding is contrasting with the finding of [25]. Ascorbic acid is a good free radical scavenger due to its chemical properties [27]. It is also involved in many physiological changes in living organisms. Ascorbic acid has been reported to attenuate changes in the level of some enzymes (catalase and superoxide dismutase [28].

The study also shows that the rat alkaline phosphatase level enzyme activities were decreased at the 30 days of exposure of to *Cola nitida*. Similar result was also reported by Ogunmefun *et al.* [29], when he fed albino wistar rat with kolanut. The decrease in alkaline phosphatase was a proof of no damage to the liver; however it could be attributed to inhibition of enzymes synthesis as a result of exposure [30]. Effects of *Cola nitida* on serum enzyme activity, aspartate aminotransferase and alkaline aminotransferase increased these enzyme levels in rat. This could be as a result of over secretion of levels of these enzymes. This is an indication of harmful effects on the cardiac or hepatic tissues [31].

**CONCLUSION**

In conclusion, the *Cola nitida* introduced to diets fed to albino rats had the ability to inhibit melatonin production which in turn led to reduction in glutathione, vitamin C, and other antioxidant in the rats exposed. Disruption of the secretion of melatonin can lead to predisposition to many diseases and aggravation of many illnesses.

**REFERENCES**


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