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## Time-Dependent Influence of *Sphenocentrum jollyanum* Pierre on Neuro-Hepatic Mitochondrial Metabolizing Enzymes in Male Wistar Rats

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### Abstract

**Background:** The use of herbal concoctions as a means of medication with no regard for quality assurance and toxicological study increases daily. Meanwhile, cases of accidental poisoning due to consumption of herbal concoctions had been previously reported. The present study aims to evaluate the effect of *Sphenocentrum jollyanum* Pierre methanolic leaf extract on mitochondrial metabolizing enzymes in the brain and liver

**Methods:** Fresh leaf of *Sphenocentrum jollyanum* Pierre was collected at Igbajo, South West, Nigeria. The methanolic extract of the leave was administered at varying doses of 200, 400, and 600 mg/kg for 14, 28, and 42 days respectively. The liver and brain were excised from the rat after the last administration, and mitochondrial metabolizing enzymes were assessed in the brain and liver samples of the rat

**Results:** The Hepatic Succinate dehydrogenase (SDH) activity was up-regulated at 200mg/kg-600mg/kg (28days) and 200-600mg/kg (42days), while other doses had no significant SDH activity. Malate dehydrogenase (MDH) activity increased at all doses, with its peak activity observed at 600mg/kg (42days). Increased hepatic Complex I+III activity was observed at all doses, with its peak at 200mg/kg(42days). Furthermore, the activity of hepatic Complex II+III was upregulated at all doses of administration. Liver Complex IV activity significantly increased at 400mg/kg (28days) instead of the declined activity recorded at other doses. Neural SDH activity increased at all doses. MDH activity significantly decreased at all doses except at 200mg/kg (28days) and 600mg/kg (42days). There was an observed up-regulation of neural Complex I+III activity at all doses; Complex II+III activity increased at all doses. Neural Complex IV activity increased significantly at 400-600mg/kg (42days). All data are relative to control.

**Conclusion:** Data from this study indicate perturbations (increase and decrease) in the neuro-hepatic mitochondrial metabolizing enzymes

**Keywords:** *Sphenocentrum jollyanum* Pierre, mitochondria, perturbation, herbal concoction, metabolizing enzymes

## 1.0 INTRODUCTION

Herbal medicine has been proven to be very effective against many diseases [1]. As such, many people living in developing countries majorly depend on herbal medicine as an alternative to the modern health care delivery system [2, 3]. This is partly due to poverty and the non-availability of the modern health care system within reach, among other reasons [4].

One such medicinal plant used to manage various ailments is *Sphenocentrum jollyanum* Pierre. It is commonly known as "akerejupon" among the Yoruba people of southwestern Nigeria. *S. jollyanum* is a perennial tree plant. A fully grown *S. jollyanum* has an average height of 1.5 meters, with very few branches. The leaf is about 5 – 12 cm wide and is wedge-shaped [5, 6]. The *S. jollyanum* seeds usually occur as a cluster containing one large oval-shaped seed [7].

Previous phytochemical analysis on various parts of the plant revealed that *S. jollyanum* Pierre is rich in bioactive compounds such as saponin, tannins, flavonoids, terpenoids, and alkaloids; the most prominent bioactive compound [8,9,10]. These bioactive chemical constituents have been attributed to the plant's biological and pharmacological activity [3]. Interestingly, angiogenic [9], antioxidant [11,12] antimalarial [13], antidepressant [14], antipyretic [15], antidiabetic [16], antibacterial [17] anti-inflammatory [18], antiviral [19], anti-allergy [20] hypolipidemic [16] analgesic [15], hepatoprotective [21] and anxiogenic [22] potentials of *S. jollyanum* Pierre had been reported. Mitochondria are fundamental to many cellular functions; such functions include energy (ATP) production and the maintenance of calcium homeostasis in the cell [23,24,25,26].

As a result of its high degree of metabolic process vis a vis its constant need for an adequate supply of ATP, Neurons depend majorly on mitochondria to meet their energy need. As such, perturbation in mitochondria's morphology, quantity, and function in the nerve cells is expected to obstruct the normal process of neuronal transmission and its attendant consequences [23]. Despite this wide array of studies on *S. jollyanum* Pierre, an extensive literature search revealed that the effects of the plant on mitochondrial energy metabolizing enzymes have not been studied to date. The present study, therefore, was aimed at exploring these.

## 2.0 METHODOLOGY

### 2.1 Reagents

The diagnostic kit for lactate dehydrogenase was a product of CYPRESS® Diagnostics, Langdrop, Belgium. Mannitol, sorbitol, sucrose, glucose-6-phosphate dehydrogenase, ethylenediaminetetraacetic acid (EDTA), nicotinamide adenine dinucleotide (NADH), fructose-1,6-bisphosphate, succinate, phosphoenolpyruvate, oxaloacetate, rotenone, cytochrome C, and tris (hydroxymethyl) aminomethane (Trizma base) were products of Carlroth GMBH, Karlsruhe, Germany. All other reagents used in this study were of the purest grade available and were purchased from British Drug House (BDH) Chemicals Limited, Poole, England, and Sigma-Aldrich, Missouri, U. S. A.

### 2.2 Plant Material

Fresh *S. jollyanum* leaf was collected from Igbajo, South-West Nigeria, around June and was authenticated by Prof. A.T.J Ogunkunle of the Department of Pure and Applied Biology, Ladoké Akintola University of Technology, Ogbomoso, Nigeria. *Sphenocentrum jollyanum* leaves were washed with clean water and allowed to air-dry under room temperature, after which the dried leaves were milled to an excellent powder. The powdered leaf of *Sphenocentrum jollyanum* Pierre (2 kg) was exhaustively extracted with 6 liters of 70% methanol. The methanol filtrate was then concentrated with a rotary evaporator to obtain a dark-brown substance.

### 2.3 Animal Treatment

Sixty (60) male Wistar rats weighing between 180 and 235 g were purchased from the animal house, Department of Physiology, University of Ibadan, Nigeria, and were kept in the animal house of the Department of Biochemistry, Ladoké Akintola University of Technology, Ogbomoso, Nigeria. Animals were acclimatized for seven days before the commencement of treatment with *S. jollyanum* using the oral route of administration. The experimental rats were fed a standard pelletized diet purchased from Vita Feeds Nigeria Limited, Ibadan. The sixty (60) animals were randomly distributed into ten (10) groups of six (6) animals each. Group A (control) received distilled water, Group B-D, E-G and H-J received 200-600mg/kg of *S. jollyanum* methanolic extract for 14, 28, and 42 days. The control group served for all the time intervals. The animals were allowed free access to food and water ad libitum.

#### 2.4 Sacrificing of Experimental Animals and Collection of Tissues/organs

At the end of *S. jollyanum* treatment for 14, 28, and 48 days, the liver and brain were excised from the Wistar rats using diethyl ether as anesthesia after an overnight fast for biochemical analyses.

#### 2.5 Determination of Hepatic and Neural Succinate Dehydrogenase (SDH) Activity

The activity of SDH was determined following the procedure described by Veeger [27]. Succinate dehydrogenase catalyzes the conversion of succinate to fumarate

#### 2.6 Determination of Hepatic and Neural Malate Dehydrogenase (MDH) Activity

The interconversion of L-malate and oxaloacetate using nicotinamide adenine dinucleotide (NAD) as a coenzyme is catalyzed by MDH. The activity of MDH was estimated in the brain and liver using the procedure described by Thorne [28]. This was done by measuring the decrease in absorbance at 340nm resulting from NADH oxidation.

#### 2.7 Determination of Hepatic and Neural Combined Complexes I+III (NADH Cytochrome C Oxidoreductase) Activity

The activity of Complex I+III was assessed by Medja *et al.*, [29]. This activity was assessed following the increase in absorbance of reduced cytochrome C at 550nm. These complexes transfer electrons from NADH which is oxidized to NAD, to cytochrome C

#### 2.8 Determination of Hepatic and Neural Combined Complexes II+III (Succinate Cytochrome C Oxidoreductase) Activity

Complex II+III catalyzes an electron transfer from succinate to cytochrome C. The activity of Complex II+III was determined following the increase of absorbance of reduced cytochrome C at 550nm. Subsequent oxidation of the reduced cytochrome C was inhibited by adding cyanide to the reaction medium. The method used was described by Medja *et al.*, [29].

#### 2.9 Determination of Hepatic and Neural Combined Complexes IV (Cytochrome C Oxidase) Activity

Complex IV transfers an electron from reduced cytochrome C to oxygen in the respiratory chain. The activity of Complex IV was determined following the decrease in absorbance of reduced cytochrome C at 550 nm using the method described by Medja *et al.*, [29].

#### 2.9 Statistical Analysis

Results from the study were expressed as mean  $\pm$  SEM. Data were analyzed by subjecting data obtained to F-test (ANOVA) using Graphpad Prism® (V 8.01). *P* values less than 0.05 were considered statistically significant

### 3 RESULTS

Data from this study indicate perturbations (increase and decrease) in the neuro-hepatic mitochondrial metabolizing enzymes. As shown in figure 1a, the activity of hepatic SDH was up-regulated at varying doses of 200-600 mg/kg (28 days) and 200-600 mg/kg (42 days) compared to control the non-activity of the enzyme and other doses. Furthermore, administration of methanolic extract of *S. jollyanum* at all doses resulted in a significant increase of hepatic MDH activity relative to control. This is shown in figure 1b.

As indicated in figure 2a, hepatic complex I+III activity increased at all doses with its peak at 200mg/kg (42days) compared with control. As shown in figure 2b, liver complex II+III activity was upregulated at all doses instead of the downregulation of its activity observed at 200mg/kg (14days) relative to control. Figure 2c showed a decrease in hepatic complex IV activity at varying doses compared to the control, while an increase in activity was observed at 400mg/kg (28days).

Neural SDH activity was upregulated at all doses compared with the control, shown in figure 3a. Brain malate dehydrogenase activity significantly decreases at all doses except 200mg/kg (28days) and 600mg/kg (42days) relative to control, as shown in figure 3b.

As demonstrated in figure 4a, neural complex I+III activity significantly increased at all doses, with the peak of the enzyme activity observed at 200mg/kg compared to control. At varying doses (200-600mg/kg/14days, 28days, and 42days), the activity of neural complex II+III was upregulated relative to control. This is shown in figure 4b. As shown in figure 4c, the activity of neural complex IV significantly increased at 400-600mg/kg (42days) with a significant decrease at all other doses compared to control. At 200mg/kg (14days), the enzyme activity had no significant difference to control.

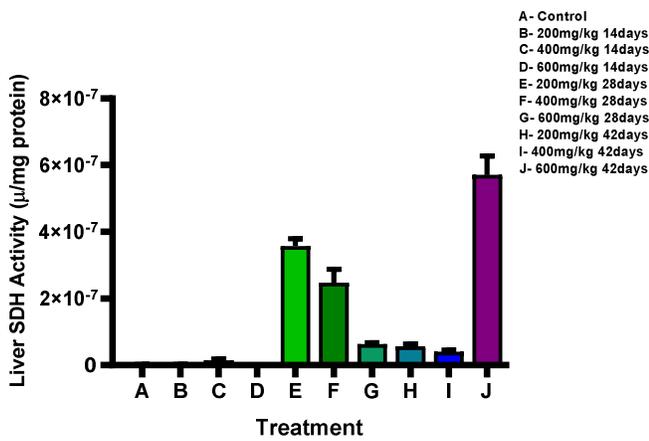


Figure 1a. Effect of *S. jollyanum* Pierre on hepatic succinate dehydrogenase activity. Each bar represents the mean ± SEM of six animals in each group.

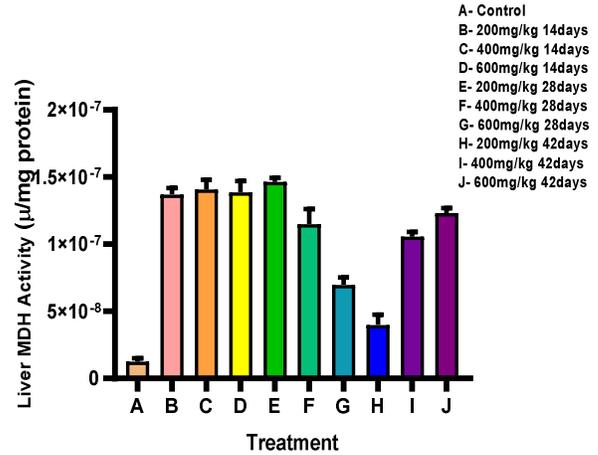


Figure 1b. Effect of *S. jollyanum* Pierre on hepatic malate dehydrogenase activity. Each bar represents the mean ± SEM of six animals in each group.

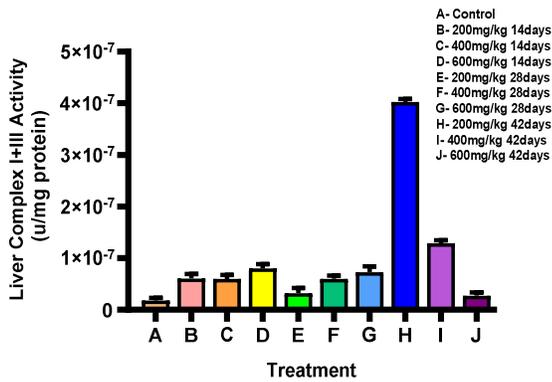


Figure 2a. Effect of *Sphenocentrum jollyanum* Pierre on hepatic complex I+III activity. Each bar represents the mean ± SEM of six animals in each group

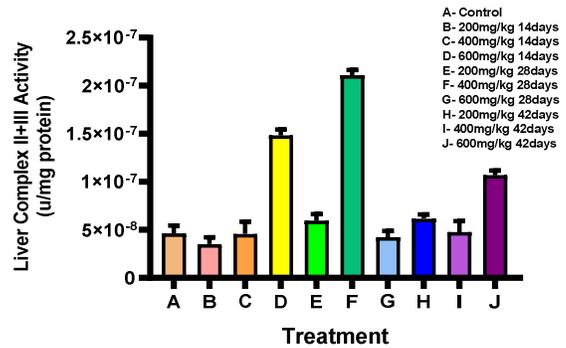


Figure 2b. : Effect of *Sphenocentrum jollyanum* Pierre on hepatic complex II+III activity. Each bar represents the mean ± SEM of six animals in each group.

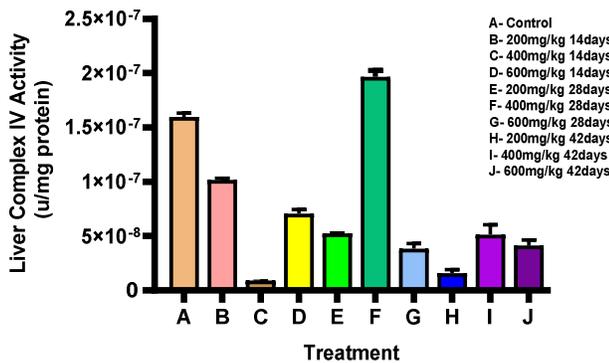


Figure 2c. Effect of *Sphenocentrum jollyanum* Pierre on hepatic complex IV activity. Each bar represents the mean ± SEM of six animals in each group.

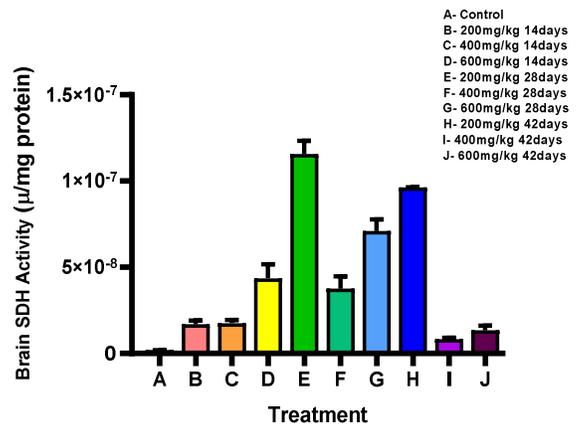
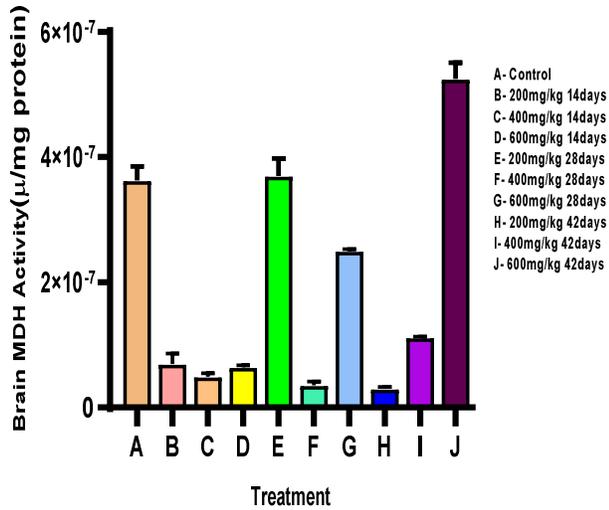
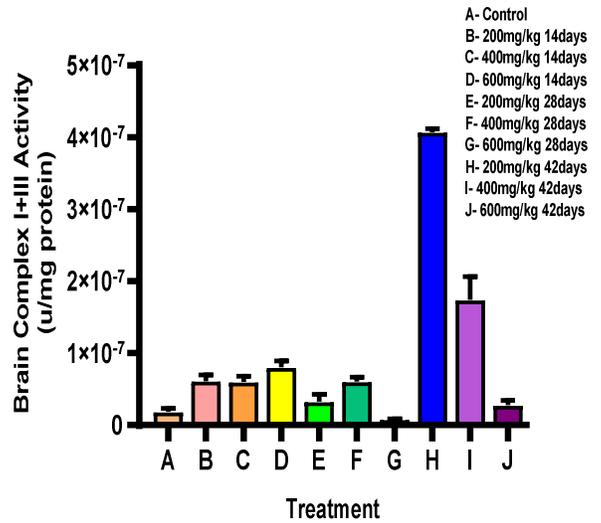


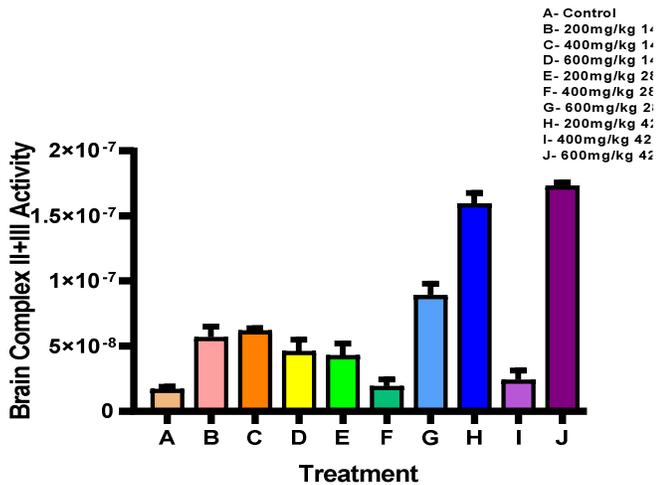
Figure 3a. Effect of *S. jollyanum* Pierre on brain succinate dehydrogenase activity. Each bar represents the mean ± SEM of six animals in each group.



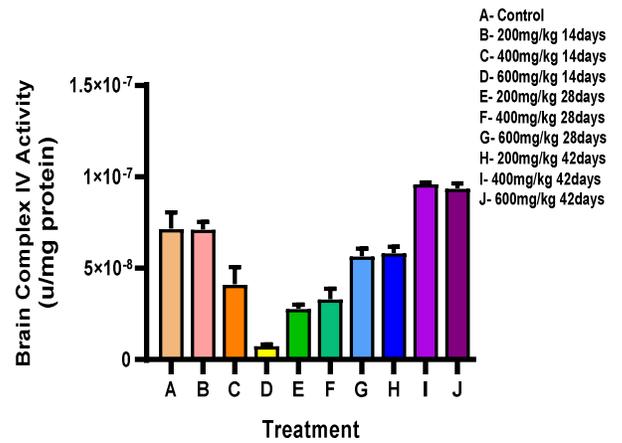
**Figure 3b.** Effect of *S. jollyanum* Pierre on brain malate dehydrogenase activity. Each bar represents the mean ± SEM of six animals in each group.



**Figure 4a.** Effect of *Sphenocentrum jollyanum* Pierre on brain complex I+III activity. Each bar represents the mean ± SEM of six animals in each group.



**Figure 4b.** Effect of *S. jollyanum* Pierre on brain complex II+III activity. Each bar represents the mean ± SEM of six animals in each group.



**Figure 4c.** Effect of *Sphenocentrum jollyanum* Pierre on brain complex IV activity. Each bar represents the mean ± SEM of six animals in each group.

#### 4.0 DISCUSSION

The findings of this study indicated that administration of methanolic extract of *Sphenocentrum jollyanum* Pierre leaf extract at all varying doses is associated with perturbations in activity mitochondrial metabolizing enzymes. These perturbations were characterized by an increase and decrease in the activities of these metabolizing enzymes in the liver and brain.

Numerous shreds of evidence show modifications of mitochondrial dynamics in organs involved in energy metabolism. The liver also plays a vital role in glucose homeostasis and the development of metabolic alterations. Furthermore, mitochondria also function in insulin signaling and participate in systemic glucose homeostasis [28].

Mitochondria are fundamental to many cellular functions; such functions include energy (ATP) production and the maintenance of calcium homeostasis in the cell. Meanwhile, mitochondria have been the foremost source of free radical production. Apoptosis is also triggered by the mitochondrial [27,28,29,30]. As a result of its high degree of metabolic process vis a vis its constant need for an adequate supply of ATP, Neurons depend majorly on mitochondria to meet their energy need. As such, perturbation in mitochondria's morphology, quantity, and/or function in the nerve cells is expected to obstruct the normal process of neuronal transmission and its attendant consequences [27].

The energy demand determines the number of mitochondria required to support its energy requirement. Because of its very high requirement for ATP, nerve cells contained numerous mitochondria. The process of electron transport in mitochondria is a significant pathway for the generation of reactive oxygen species (ROS) [29,30]. Meanwhile, the process of electron transport through the various complexes of the Electron Transport Chain (ETC) has been implicated as a significant risk factor in the incidence of several pathological conditions, such as neuropsychiatric [31] and neurodegenerative diseases [32,33].

Succinate dehydrogenase (SDH) is a key in intermediary metabolism and aerobic energy production in living cells. Succinate dehydrogenase catalyzes the oxidative conversion of succinate to fumarate during the process of the Krebs cycle with the concomitant reduction of ubiqui-

none to ubiquinol with flavin adenine dinucleotide (FAD) serving as the electron acceptor [34]. SDH plays a significant metabolic role both in the citric acid cycle and the electron transport chain. SDH deficiency has been implicated in various pathologies such as mitochondria encephalopathy, optic atrophy, Leigh syndrome, and Huntington's diseases [35].

In the current study, the activity of hepatic SDH was significantly up-regulated at all doses, as shown in figure 1a, and at all doses of methanolic extract of *S. jollyanum* Pierre leaf. This might suggest a homeostatic response of the hepatocytes towards increased energy demand by the cell, causing an increase in the expression of the genes coding for these enzymes. Neural SDH activity was up-regulated at all doses, as described in figure 3a. taken together, it may be inferred that the mild enhancement and inhibition/modulation of SDH at different doses and duration of exposure are metabolic adaptations to metabolic stress induced by *S. jollyanum* Pierre on the living systems. Furthermore, malate dehydrogenase (MDH) is a citric acid cycle (Krebs cycle) enzyme. SDH catalyzes the inter-conversion of malate to oxaloacetate in the citric acid cycle. This reaction occurs through the oxidation of the hydroxyl group on malate and reduction of  $\text{NAD}^+$ . It should be noted that MDH has both cytosolic and mitochondria isoforms, but the mitochondrial isozyme is linked to the ETC as an electron donor in a process that culminates in adenosine triphosphate (ATP) synthesis [36]. Elevate activity of MDH has been used as an index for liver and heart damage together with lactate dehydrogenase [37]. In the present study, exposure to *Sphenocentrum jollyanum* Pierre resulted in a significant increase of hepatic MDH activity relative to control. In contrast, neural MDH activity significantly decreases at all doses except at 200mg/kg (28days) and 600mg/kg (42days), as shown in figure 3b. As expected, MDH, an  $\text{NAD}^+$  - linked enzyme, contributes to ATP production and inhibition of neural MDH by *S. jollyanum* Pierre by an unknown mechanism but which may be similar to that of LDH represent a potential catastrophe to the brain cells.

The ETC comprises a series of complexes that transfer electrons from electron donors to electron acceptors via a redox reaction. This electron transfer is coupled with protons ( $\text{H}^+$ ) transfer across a membrane. The electrochemical gradients of protons generated due to their movement

across the membrane are used to drive the process of ATP synthesis. This electron transport chain comprises four complexes: I, II, III, and IV. Electrons are harnessed from NAD<sup>+</sup>, and FAD-linked enzymes of the tricarboxylic acid (TCA) cycle and the proton-motive force generated is thus used to synthesize ATP. Electrons move from complex I directly to complex III via ubiquinone while electrons from FAD-linked SDH pass from complex II-III [38,39,40]. In this present study, TCA, ETC enzymes of brain and liver tissues responded differently to the administration of methanolic extract of *Sphenocentrum jollyanum* Pierre leaf extract.

Furthermore, hepatic combined complex I+III activity increased at all doses. Combined complex I+III supplies the electrons and (H<sup>+</sup>) to the respiratory chain; inhibition could lead to uncoupling of the electron transport chain from oxidative phosphorylation, leading to reduced ATP synthesis [41,42,43]. Combined Complex II+III activity was up-regulated at all doses except 200mg/kg (14days). Complex IV activity at 400mg/kg (28days) was up-regulated compared with control. (Figure 2a-2c). Neural combined complex I+III activity significantly increased at all doses, at varying doses (200-400mg/kg/14days, 28days, and 42days), the activity of combined complex II+III was upregulated (figure 4a&4b). Also, complex IV activity significantly increased at 400-600mg/kg (42days) while the enzyme activity was downregulated at other doses. Also, at 200mg/kg (14days), the enzyme activity had no significant difference to control. (Figure 4c). All observations are compared with control.

These observations showed that methanolic extract of *S. jollyanum* Pierre compromised mitochondrial transmembrane potential and possible uncoupling or electron transport chain from oxidative phosphorylation in different tissues through inhibition of hepatic and neural complex IV may be the hallmark of *S. jollyanum* Pierre toxicity.

### Conflicts of Interest

The authors declare no competing interests.

### Acknowledgment

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### Authors' Contributions

**JO**F conceived and designed the study, contributed to data collection, data analysis tools, statistical analysis and manuscript writing. **SA**K contributed to data collection, data analysis tools, analysis of data and manuscript writing. **OF** contributed to data collection, data analysis tools and analysis of data. **JA**B contributed to data collection and data analysis tools. All authors approved the final copy of the manuscript.

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