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## Effect of Canarium Schweinfurthii (Africa Black Olive) Oil on Restraint Stressed Induced Renal Dysfunction in Wistar Rat

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### Abstract

#### Background

Chronic stress regardless of its origin has server repercussion on health, notably impacting the renal system. the kidney is vulnerable to prolonged stress, during stress the renal tissue are exposed to oxidative damage. This study aims to evaluate the protective effect of canarium schweinfurhii oil on renal dysfunction of Wistar rats subjected to restraint stress on the following biomarkers Creatinine, Urea, Blood urea nitrogen and Total protein respectively.

#### Methodology

A total of twenty-eight (28) apparently healthy adult male Wistar rat weighing between 150-170g were used for this study. They were allocated into four groups of seven rat (n=7 rat/group). Group I: control group and administered with 10ml/kg/bw of water, Group II: stress group + 10ml/kg/bw of water, Group III: this group under goes stress and treated with 10ml/kg (1ml/100g) of canarium schweinfurhii oil, Group IV: this group under goes stress and treated with 20ml/kg (2ml/100g) of canarium schweinfurhii oil. All group were treated for 21 days. All data were expressed as mean  $\pm$  SEM. The data obtained were statistically analyzed using analysis of variance (ANOVA) with Tukey's multiple comparison post hoc tests to compare the level of significance between control and experimental groups. All statistical analysis were evaluated using SPSS version 17.0 software. The values of  $P < 0.05$  were considered as significant.

#### Result

Results showed a significant increase ( $p < 0.05$ ) in creatinine, urea, and BUN levels in Group B compared to the control. Group C showed a significant decrease in these parameters compared to Group B, suggesting a protective effect of Canarium schweinfurthii oil.

#### Conclusion

The findings concluded canarium schweinfurhii oil at a dose of 10ml/kg (1ml/100g) provided some protection against stress-induced renal dysfunction

**Keywords:** Stress, Renal Dysfunction, Canarium Schweinfurhii

#### Abbreviations

CSO: canarium schweinfurhii oil

BUN: blood urea nitrogen

EDTA: ethylenediaminetetraacetic acid

## Introduction

Day-to-day activities in human life often involve various forms of stress. Students are frequently subjected to academic stress from exams, lectures, and assignments, while adults navigate the complexities of work-related and relationship stress [1]. Chronic stress, regardless of its origin, can have severe repercussions on health, notably impacting the renal system. The kidneys, which are responsible for filtering blood, regulating fluid balance, and maintaining electrolyte levels, are especially vulnerable to prolonged stress. Elevated stress hormones like cortisol can lead to hypertension and other risk factors for kidney disease. During stress, renal tissues are vulnerable to oxidative damage [2]. The excessive production of ROS can lead to mitochondrial dysfunction, cellular injury and dysfunction leading to renal failure [3]. Stress is a biological response that occurs in response to any external or internal stimulus [3]. The body's reaction to these stressors, or stimuli, is known as the stress response. The stress response is a set of physiological changes that help the body adapt to the stressor and return back to homeostasis [4]. Restraint stress refers to the application of physical force or restraints on experimental animals, which leads to a state of stress and agitation. It is often used as a model to induce anxiety and depression-like behaviors in experimental animals for studying the physiological and behavioral responses associated with stress [5]. One of the physiological responses observed during stress is the activation of the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is responsible for regulating the body's response to stress through the release of stress hormones such as corticotrophin-releasing hormone (CRH) by the hypothalamus, adrenocorticotropic hormone (ACTH) by the pituitary gland, and cortisol by the adrenal glands [6]. Restraint stress rapidly increases the release of CRH and ACTH, which in turn triggers the release of cortisol, which is the primary stress hormone [7]. *Canarium scweinfurthii* (Africa black olive), an African olive tree species, is known for its rich phytochemical composition, which includes antioxidant compounds such as flavonoids and phenolic compounds. These bioactive compounds have been reported to possess various health-promoting properties, including anti-inflammatory and antioxidant effects [8]. With these properties, *Canarium scweinfurthii* has the potency to mitigate the effect of stress [9].

## Material and Methods

### Plant Materials

Fresh "*Canarium scweinfurthii* (Africa black olive)" fruit was obtained from Pankshin in Plateau State, Nigeria. The identification of the plant was done at the department of plant and environmental biology, Prince Abubaker Audu University, Anyigba, Kogi State, Nigeria given the Voucher number: KSU-PT-B-066.

### Animals Materials and Management

A total of 28 apparently healthy adult male Wistar rats weighing between 150g -170g were used for this study. The animals were purchased from the Animal House of College of Health Sciences, Benue State University, Benue State, Nigeria. The animals were kept in cages under normal environmental temperature and were fed with standard pellet diet and water given ad-libitum. The rats were allowed to acclimatize to the laboratory environment for two weeks before the commencement of the experiment.

### Chemicals and Reagents

All chemicals and reagents used for this study were obtained commercially which includes; ketamine was purchased from Nigorate, Anyigba Kogi State, Urease reagent, Jaffe reagent, biuret reagent and buffer solution was purchased from Medex supply Nigeria Ltd, Ilorin Kwara state.

### Methods Animal Grouping

The animals were grouped into 4 groups containing 7 rats each and drug dosage was determined using the method as described by Oghenesuvwe et al., 2014 [10].

Group I (n = 7): this group serves as the Control group which will not undergo restraint stress, all animals in this group will be administered with 10ml/kg of water.

Group II (n= 7): this group serves as the stress group without administration of *Canarium scweinfurthii* (Africa black olive) oil, all animals in this group will be administered with 10ml/kg of water.

Group III (n= 7): this group undergoes restraint stress and treated with 10 ml/kg (1ml/100g) of *Canarium scweinfurthii* (Africa black olive) oil.

Group IV (n= 7) this group undergoes restraint stress and treated with 20ml/kg (2ml/100g) of *Canarium scweinfurthii* (Africa black olive) oil.

### Restraint Stress Induction

The Wistar rats were restrained using a locally constructed wooden restraint cage with dimensions of 30 cm (L) × 3 cm (B) × 3 cm (H). The restraint stress induction was carried out following the method as described by Ezekiel et al., (2016) with little modification [11]. Each Wistar rat was housed individually in a multi-compartment cage. The Wistar rats were exposed to chronic restraint stress for 6 hours (8am-2pm) per day for 21 days by keeping them in the restraint cages without food and water during the time of the experiment. The un-restrained rats were left undisturbed in their home

cages but without access to food or water during the time of the experiment [11].

### Sample Collection

At the end of the three weeks study the animals were anaesthetized with ketamine inter-peritoneal. And was humanely sacrificed. 5ml syringe were used to collect blood samples via cardiac puncture method into and EDTA sample bottles. The bloods sample were centrifuged at 2012.4 xg for 10 minutes in the EDTA tube to obtain plasma.

### Renal Function Analysis

Creatinine, urea, blood urea nitrogen, and total protein were evaluated with commercially available kits from Medex supply Nigeria Ltd, Ilorin Kwara state. via spectrophotometry.

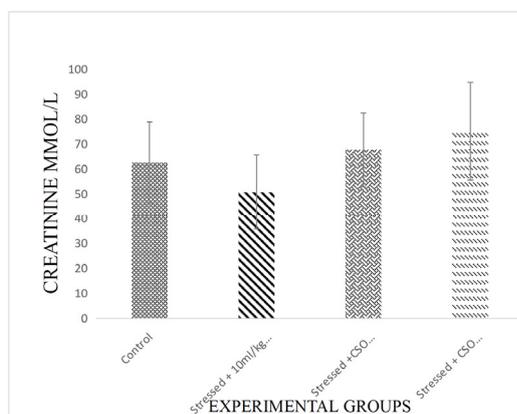
### Statistical Analysis

All data were expressed as mean  $\pm$  SEM. The data obtained were statistically analyzed using analysis of variance (ANOVA) with Tukey's multiple comparison post hoc tests to compare the level of significance between control and experimental groups. All statistical analysis were evaluated using SPSS version 17.0 software. The values of  $P < 0.05$  were considered as significant.

### Result

#### Effect of *Canarium Schweinfurthii* (Africa Black Olive) Oil on Restraint Stress-Induced Renal Dysfunction in Wistar Rat on Creatinine Level

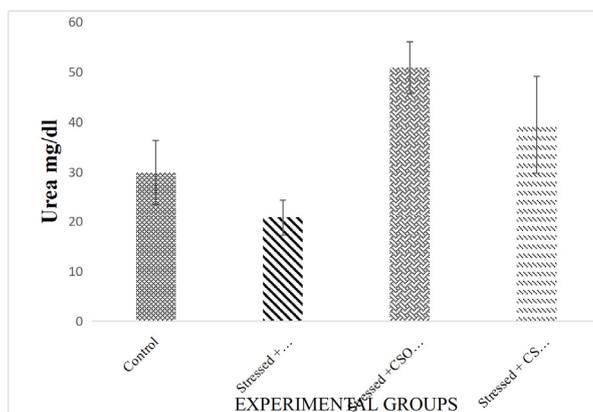
The effect of *canarium schweinfurthii* (Africa black olive) oil on restraint stress-induced renal dysfunction in Wistar rat on creatinine level is shown in figure 1. Creatinine level for the four groups was as follows: positive control,  $62.69 \pm 16.18$ ; Stressed + DW,  $50.71 \pm 14.95$ ; 10 ml/kg of C.S (Group C),  $67.75 \pm 14.75$ ; 20ml/kg of C.S (Group D),  $75.12 \pm 19.51$ , respectively. There was no significant difference between the groups ( $p > 0.05$ ).



**Figure 1: Creatinine Level in the Blood Following 21 Days of Oral Administration of *Canarium Schweinfurthii* (Africa Black Olive) Oil in Different Dosage (10ml/kg and 20ml/kg). All the Error Bars are SEMs. DW (Distilled Water), CSO (*Canarium Schweinfurthii* Oil)**

#### Effect of *Canarium Schweinfurthii* (Africa Black Olive) Oil on Restraint Stress-Induced Renal Dysfunction in Wistar Rat on Urea Level

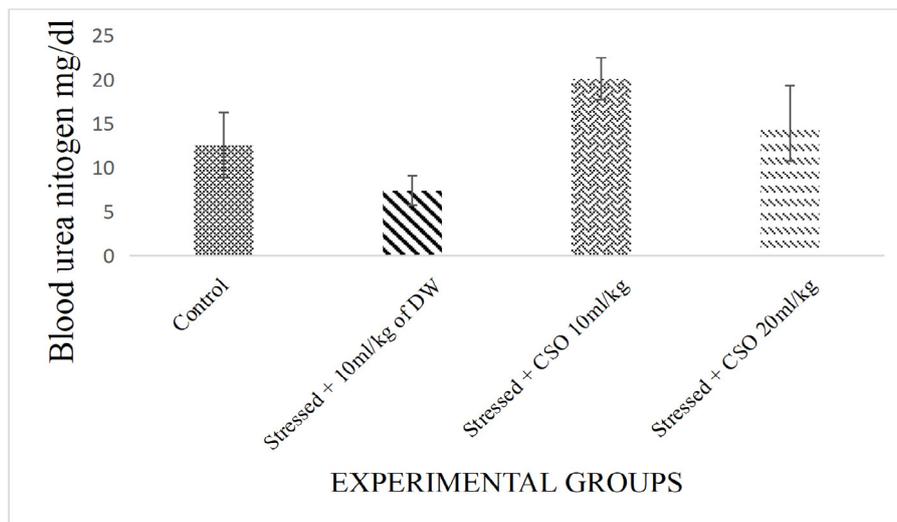
The effect of *canarium schweinfurthii* (Africa black olive) oil on restraint stress-induced renal dysfunction in Wistar rat on urea level is shown in figure 2. Urea level for the four groups was as follows: positive control (Group A),  $26.94 \pm 6.36$ ; Negative control (Group B),  $15.84 \pm 3.57$ ; 10 ml/kg of C.S (Group C),  $70.91 \pm 5.15$ ; 20ml/kg of C.S (Group D),  $51.50 \pm 9.65$ , respectively. There was no significant difference between the groups ( $p > 0.05$ ).



**Figure 2: Urea Level in the Blood Following 21 Days of Oral Administration of *Canarium Schweinfurthii* (Africa Black Olive) Oil in Different Dosage (10ml/kg and 20ml/kg). All the Error Bars are SEMs. DW (Distilled Water), CSO (*Canarium Schweinfurthii* Oil)**

### Effect of *Canarium Schweinfurthii* (Africa Black Olive) Oil on Restraint Stress-Induced Renal Dysfunction in Wistar Rat on Blood Urea Nitrogen Level

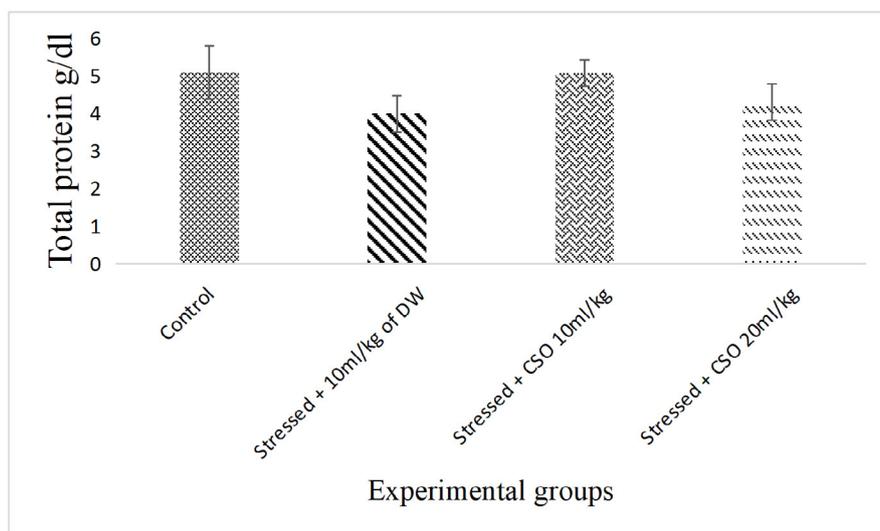
The effect of *canarium schweinfurthii* (Africa black olive) oil on restraint stress-induced renal dysfunction in Wistar rat on blood urea nitrogen level is shown in figure 3. blood Urea nitrogen level for the four groups was as follows: positive control (Group A),  $12.58 \pm 3.70$ ; Negative control (Group B),  $7.40 \pm 1.66$ ; 10 ml/kg of C.S (Group C),  $33.11 \pm 2.40$  20ml/kg of C.S (Group D),  $24.05 \pm 4.31$ , respectively. There was no significant difference between the groups ( $p > 0.05$ ).



**Figure 3: Blood Urea Nitrogen Level Following 21 Days of Oral Administration of *Canarium Schweinfurthii* (Africa Black Olive) Oil in Different Dosage (10ml/kg and 20ml/kg). All the Error Bars are SEMs. DW (Distilled Water), CSO (*Canarium Schweinfurthii* Oil)**

### Effect of *Canarium Schweinfurthii* (Africa Black Olive) Oil on Restraint Stress-Induced Renal Dysfunction in Wistar Rat on Total Protein Level

The effect of *canarium schweinfurthii* (Africa black olive) oil on restraint stress-induced renal dysfunction in Wistar rat on total protein level is shown in figure 4. total protein level for the four groups was as follows: positive control (Group A),  $5.09 \pm 0.71$ ; Negative control (Group B),  $4.60 \pm 0.48$ ; 10 ml/kg of C.S (Group C),  $4.99 \pm 0.35$ ; 20ml/kg of C.S (Group D),  $4.31 \pm 0.49$ , respectively. There was no significant difference between the groups ( $p > 0.05$ ).



**Figure 4: Total Protein Level in the Blood Following 21 Days of Oral Administration of *Canarium Schweinfurthii* (Africa Black Olive) Oil in Different Dosage (10ml/kg and 20ml/kg). All the Error Bars are SEMs. DW (Distilled Water), CSO (*Canarium Schweinfurthii* Oil)**

### Discussion

This study investigated the protective effects of *Canarium schweinfurthii* oil on renal dysfunction induced by restraint stress in Wistar rats. The findings showed that restraint stress significantly elevated the levels of serum creatinine, urea, and BUN, indicating impaired kidney function. These results align with previous study done by Ansari *et al.*, (2021) [12]. Who report that *Thymus serrulatus* essential oil has a protective effect on Cadmium-Induced Nephrotoxicity in

Rats, through Suppression of Oxidative Stress and Downregulation of NF- $\kappa$ B, iNOS, and Smad2 mRNA Expression suggesting that chronic physical stress can induce renal oxidative damage and impair filtration function.

Treatment with *Canarium schweinfurthii* oil resulted in a significant reduction in these biomarkers in the stressed rats, suggesting a nephroprotective effect. This may be attributed to the anti-inflammatory and antioxidant properties of CSO, which have been reported in other studies. The improvement in renal parameters in Group C (stress + CSO at 10 ml/kg) and Group E (stress + CSO at 20 ml/kg) indicates that CSO can mitigate stress-induced renal damage. . This result disagrees with the findings of Okwuosa *et al.*, (2010) who report that the stem bark extracts of *canarium schweinfurthii* significantly reduced plasma creatinine levels and preserving renal histoarchitecture. They concluded that stem bark extracts of *canarium schweinfurthii* produce a fall in plasma creatinine and urea in present of acetaminophen-induced nephroprotective in rat.

## Conclusion

In this study Group B (stressed + DW) showed the lowest levels of blood urea nitrogen (BUN) and total protein, indicating significant stress impact leading to increased protein catabolism and reduced protein synthesis. Group C (stressed + 10ml/kg of CSO) exhibited the highest BUN levels and elevated creatinine levels, suggesting increased protein breakdown, renal stress, and mild kidney impairment due to the low dose of CSO. Group D (stressed + 20 ml/kg of CSO) showed elevated but lower BUN and creatinine levels compared to Group C (stressed + 10ml/kg of CSO), implying that the higher dose of CSO was more effective in mitigating stress effects and supporting kidney function. Total protein levels increased in Group C (stressed + 10ml/kg of CSO) compared to Group B (stressed + DW), indicating partial protective effects of the low CSO dose, whereas Group D (stressed + 10ml/kg of CSO) displayed even higher total protein levels, suggesting a dose-dependent enhancement of protein synthesis and stress adaptation. Urea levels followed a similar trend, with Group B (stressed + DW) having the lowest levels, Group C (stressed + 10ml/kg of CSO) the highest, and Group D (stressed + 10ml/kg of CSO) showing elevated levels but lower than Group C (stressed + 10ml/kg of CSO). Overall, the findings suggest that while both doses of CSO provided some protection against stress-induced biochemical changes, the higher dose (20 ml/kg) was more effective in reducing stress impacts, supporting protein metabolism, and maintaining renal function.

## Recommendations

- Future similar study on the effect of *canarium schweinfurthii* on stress induced renal dysfunction should include measurement of uric acid levels and electrolyte such as chloride, bicarbonate etc following administration of different doses.
- Other future similar study on the effect of *canarium schweinfurthii* on stress induced renal dysfunction should be done on Wistar rat with metabolic disorder such as diabetes, obesity etc.
- Similar future study should include the effect of *canarium schweinfurthii* on stress induced Wistar rat should be done on important organs such as the lung, brain and heart that are capable of damaging during stress.

## Abbreviations

CSO: *canarium schweinfurthii* oil

BUN: blood urea nitrogen

EDTA: ethylenediaminetetraacetic acid

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