Chemo-Protective Effects of *Anacardium Occidentale* Nutshell Hexane Extract on Catalase and Tyrosinase Activities in UV-Exposed Skin in Wistar Rats

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ABSTRACT

Anacardium occidentale (cashew) nutshell hexane liquid (CNSHL) has been traditionally used in parts of Africa, South America, and Asia for the treatment of various skin conditions, including wounds, dermatitis, and cancerous ulcers. Chronic exposure to ultraviolet (UV) radiation is known to induce skin damage, oxidative stress, and may promote carcinogenesis. This study aimed to evaluate the potential protective effect of CNSHL on biochemical parameters associated with oxidative stress in Wistar rats exposed to UV radiation. Thirty (30) male Wistar rats weighing between 160–200g were randomly assigned into six groups (n = 5). Group A (negative control) was not exposed to UV radiation. Group B (positive control) was exposed to UV light but received no treatment. Group C was exposed to UV light and treated topically with CNSHL. Group D was exposed to UV light and treated with a standard drug. Group E and F were not exposed to UV light but treated with Hexane extract and standard drug respectively. Catalase and tyrosinase activities in serum were analyzed as markers of oxidative stress and melanogenesis. The results showed that Group C (CNSHL-treated) exhibited significant improvements in catalase activity and favorable modulation of tyrosinase levels compared to the untreated group. The biochemical effects were comparable to those observed in the standard drug-treated group, suggesting that CNSHL possesses potential chemo preventive properties against early-stage UV-induced skin damage. These findings not only validate the traditional use of cashew nutshell extracts but also establish their promise as a potent, affordable, and natural topical agent for the prevention and management of UV-induced skin damage, with potential for development into a standardized dermatological formulation.

Keywords: Anacardium occidentale, ultraviolet, CNSHL, skin.

INTRODUCTION

The incidence of skin cancer in people has been increasing day by day. The main reason for skin cancer is due to UV exposure because large amounts of UV-radiation reach earth's surface due to depletion of ozone layer (Ramya et al., 2013). Skin cancer can be of two types mainly, malignant melanoma and non-malignant melanoma which is divided into basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Ultraviolet-radiation (UV-radiation), mainly occur due to chronic exposure of ultraviolet-radiation (UV-radiation), UV sunlight. Malignant melanoma may be due to intense sun exposure and history of sun burn. 80-85 % of non-melanoma skin cancers are basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). (Ramya et al., 2013). Squamous cell carcinoma (SCC) is more dangerous and is responsible for many deaths. (Ramya et al., 2013). Skin cancer in early stage can be cured easily by simple procedures or techniques but advanced skin cancer cannot be treated effectively by any medications. (Ramya et al., 2013). So, there is a need to detect and treat disease at early stage. Overall, 80 % of skin cancers are BCC, 16 % are SCC and 4 % are melanoma (Ramya

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et al., 2013). Cashew was introduced into Nigeria by the Portuguese traders around the 16th century (Adeigbe *et al.,* 2015). It was first planted in Agege, Lagos State, from where it spread to a few other parts of the country through transfer of nuts by man. For over 400 years after introduction, cashew trees were exploited mainly for appple; no commercial value was attached to the nuts (Aliyu, 2012). Many of the trees flourished in the wild while being utilized for aforestation and erosion control scheme particularly in the escarpment areas of Udi in Anambra state. The first commercial cashew planting in Nigeria was in the mid 1950 at Ogbe, Oji, Udi and Mbala by the defunct Eastern Nigeria Development Corporation (ENDC) and Iwo, Eruwa and Upper Ogun by the defunct Western Nigeria Development Corporation (WNDC) (Asogwa *et al.,* 2009). The Cashew Nutshell contains a viscous and dark liquid, known as cashew nut shell liquid (CNSL), which is extremely caustic. It is contained in the thin honeycomb structure between the soft out skin of the nut herder inner shell. The CNSL contain of the raw of nut varies from 20-25 percent. Cashew nutshell liquid (CNSL) is a mixture of anacordic acid, cardanol, cordal, and 2-methylcardal. Cashew nutshell, which are otherwise ago waste of cashew nut has many biochemical potentials, impressive research work has been performed toward transforming the agro of cashew wastes (cashew nut shell) into valuable biological active and useful product. CNSL component, exhibits multifunctional roles and has been used as a bioactive agent. The extract has also been used for the synthesis of various analogues for a variety of possible biological applications such as antibacterial, antioxidant antitumor, anticholinesterase, anti-gout properties and anti-viral activity (Warts) and substitute for biodiesel which are important for new pharmaceutical products. This research was designed to evaluate the potential therapeutic effects of Anacardium occidentale nut shell hexane liquid on the early stage of skin cancer UV-light by determining the effect of *Anacardium occidentale* nutshell hexane liquid on catalase activity in serum of the experimental rats, the effect of *Anacardium occidentale* nutshell hexane liquid on tyrosinase activity in serum of the experimental rats and the effect of *Anacardium occidentale* nutshell hexane liquid on total protein concentration in serum of the experimental rats.

MATERIALS AND METHODS

Preparation of Plant Extracts

Anacardium occidentale seeds were obtained from Ochadamu-Ofu, Kogi state, Nigeria. The nutshell of *Anacardium occidentale* were washed in cold water. The nuts that floated were taken out and the nuts that did not float were air-dried. The kernels were shelled from the nuts and cut into smaller sizes. Extraction of *Anacardium occidentale* shell was carried out using soxhlet extraction method, with hexane as solvent. The sample to be extracted was weighed to a thimble and loaded into the chamber of the soxhlet extractor. 200 ml of the solvent was poured into a round bottom flask attached to the soxhlet extractor enough to siphon at least twice into the flask, the temperature for extraction was 69°C for hexane and heated at reflux. This cycle was repeated over and over again over six hours using hexane solvent extract (James *et al.,* 2014).

Experimental animals

The eighteen (30) male albino rats with average weight of 160-200 g were obtained from the animal house section of Salem University Lokoja, Kogi State. The animals were acclimatized in the experimental room for two weeks. The experimental animals were allowed access to standard food and water at libitum and handled according to guidelines on the use of experimental animals by the Department of Biosciences, Salem University Lokoja.

Animal study

This study focused on UV-induced skin damage and cancer initiation. The experimental animals were divided into six groups, each consisting of six rats. Prior to the experiment, the rats were weighed both before and after the skin on their backs was shaved. They were then allocated to the six groups according to the experimental design outlined below. Ultraviolet radiation (UVR) exposure, specifically in the UVB range (290–320 nm), was administered daily for 14 consecutive days using a modified protocol.

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Experimental design

Group 1- Negative control treated Distilled water (not exposed)

Group 2- Positive control (exposed but untreated)

Group 3- Exposed and treated with Hexane extract

Group 4- Exposed and treated with standard drug (Aldara)

Group 5- Not exposed but treated with Hexane extract

Group 6- Not exposed but treated with standard drug (Aldara)

Treatment of experimental animals

Prior to the commencement of treatment, the experimental animals were fasted for 12 hours and weighed. The treatment lasted for 14 consecutive days, during which the animals were exposed to UV radiation as described previously. Following UV exposure each day, the assigned plant extract was topically applied to the shaved dorsal skin of the animals, according to their respective groupings. At the end of the treatment period, all animals were humanely sacrificed for further analysis.

Collection of organs

The skin and the blood of the animals were collected and was stored for further analysis the organs collected from each animal were rinsed using normal saline.

Preparation of serum sample

Blood was collected into sample bottles and was centrifuged at 3000 rpm (using a micro field centrifuge) for 10 min. The serum was decanted into a different sample bottle and was stored at $4^{o}C$ for further analysis.

Statistical analysis

All values were express as the mean standard error of mean (SEM). Data were analyzed using one-way analysis of variance (ANOVA) and significance means were separated. Statistical software package for social science (version 20.00) was used and values were expressed as means of the determination standard error of mean (SEM). Difference was considered significance at $p < 0.5$. Alphabets on bars (a, b, c, d, e and f) indicate the statistical difference.

Determination of catalase activity in serum

Catalase activity was measured following the method of Goth (1991) as modified by Hadwan and Abed (2016).

Procedure:

The reaction mixture comprised of 10 µL of sample, 100 µL of H_2O_2 (20 mM) in 50 mmol/L potassium phosphate buffer, pH 7.4 for test; 10 µL of distilled water, 100 µL of H_2O_2 (20 mM) in 50 mmol/L potassium phosphate buffer, pH 7.4 for control-test. Mixed and incubated at 37 °C for 3 min. After that, 400 µL ammonium molybdate (32.4 mmol/L) was added to stop the reaction. Hydrogen peroxide was used as standard. Absorbances for the test and control-test were recorded at 374 nm against the reagent blank; difference in absorbance between that of the test and control-test was calculated using the formula below.

Catalase activity (kU) = $((2.303/t) \times \text{Log}(S0/(S-M)) \times Vt/Vs)/1$

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t = incubation time

S0 = standard absorbance

S = Absorbance of test

M = Absorbance of control (correction factor)

Vt = total volume of reagents

Vs = volume of sample

Determination of tyrosinase activity in serum

Tyrosinase activity: Tyrosinase, also commonly called polyphenol oxidase, has two catalytic activities; o-hydroxylation of monophenols and aerobic oxidation of o-diphenols. Measurement of tyrosinase activity was performed using the LAMBDA 475nM UV/Vis Spectrophotometer and UV Lab software. Rapid acquirement of spectra and good sensitivity were obtained and the software was used to quantify and to process the data efficiently

Principle

Tyrosinase, a copper-containing oxidoreductase, catalyzes the orthohydroxylation of monophenols and the aerobic oxidation of catechols. The enzyme activity will be assayed by monitoring the oxidation of 3, 4-dihydroxyphenylalanine (dopa) to the red-colored dopachrome.

Procedure

1. Prepare the 0.1 M sodium phosphate buffer.

2. Dissolve tyrosinase and L-dopa in 0.1 M sodium phosphate buffer.

3. Determine the amount of tyrosinase. 3-1.

4. Pick 350µL of a sample (tyrosinase) Q

5. Add 480µL of 0.1ml phosphate buffer (PB) containing tyrosinase

6. Mixed it gently and take the reading at every 30 seconds for 3 minute.

Determination of total protein concentration in serum

Total protein concentration was determined with the use of Randox kit (TP 245). The assay was measured according to the method described in the kit.

Procedure:

Sample or standard (0.02 ml) and 1.0 ml of solution 1 were pipette into test tubes, mixed well, and incubated for 30 mins at 20^{o}C to 25^{o}C. Absorbance of sample (Asam) and the standard (Astd) were measured at 546 nm against the reagent blank. The concentration of total protein was calculated as follows:

Total protein (mg/l) = Asam/Astd × 5.81 (standard concentration)

Where Asam or Astd = absorbance of sample or standard

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RESULTS

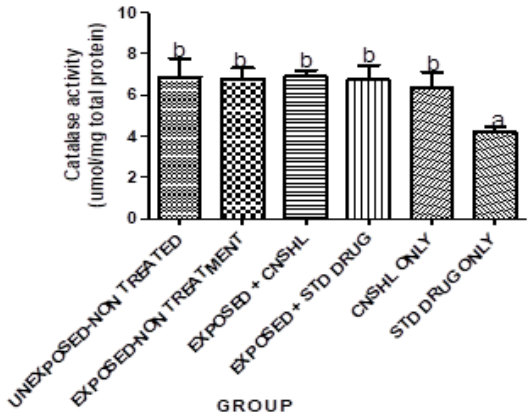

Figure 1: Effect of Anacardium occidentale nutshell hexane liquid on catalase activity in serum. Different alphabets represent significant differences (P <0.05).

A: Unexposed-non treated

B: Exposed-non treatment

C: Exposed + cashew nutshell hexane liquid (CNSHL)

D: Exposed + standard drug

E: Cashew nut shell hexane liquid (CNSHL) only

F: Standard drug only

Figure 1 revealed that groups B, C, D and E had no significant difference (P > 0.05) in catalase activity of serum compared to group A. However, group F showed a significant reduction (P < 0.05) in the activity of catalase when compared with groups A and B.

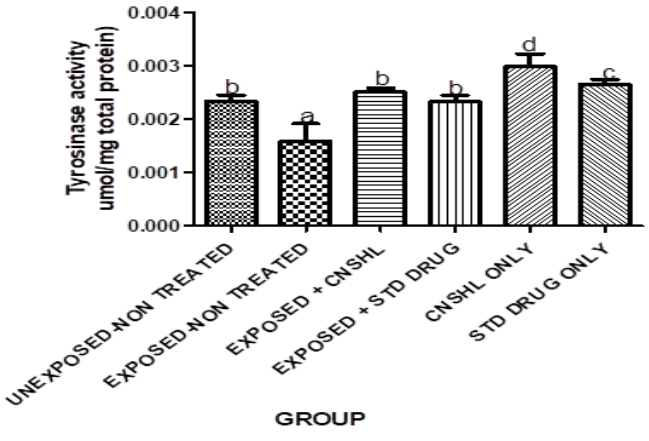

Figure 2: Effect of Anacardium occidentale nut shell hexane liquid on tyrosinase activity in serum. Different alphabets represent significant differences (P < 0.05).

A: Unexposed-non treated

B: Exposed-non treatment

C: Exposed + cashew nut shell hexane liquid (CNSHL)

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue IV April 2025

D: Exposed + standard drug

E: Cashew nut shell hexane liquid (CNSHL) only

F: standard drug only

Figure 2 revealed that group B had a significant reduction ($P < 0.05$) in the activity of tyrosinase when compared with A. Groups C and D showed no significant difference ($P > 0.05$) in tyrosinase activity when compared with group A. Groups E and F showed a significant increase ($P < 0.05$) in tyrosinase activity when compared with groups A and B.

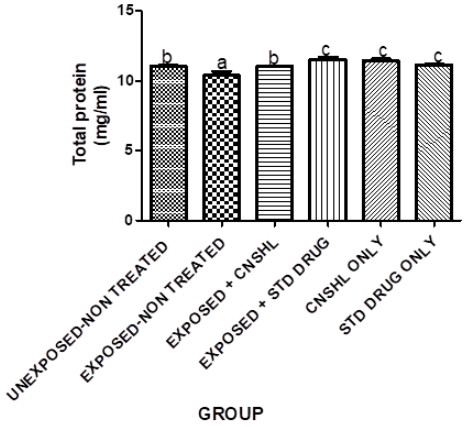

Figure 3: Effect of Anacardium occidentale nut shell hexane liquid on total protein concentration in serum. Different alphabets represent significant differences ($P < 0.05$).

A: Unexposed-non treated

B: Exposed-non treatment

C: Exposed + cashew nut shell hexane liquid (CNSHL)

D: Exposed + standard drug

E: Cashew nut shell hexane liquid (CNSHL) only

F: Standard drug only

Figure 3 revealed that group B had a significant reduction ($P < 0.05$) in the total protein concentration compared with group A. Group C showed no significant difference ($P > 0.05$) in total protein concentration when compared with group A. Groups D, E and F showed a significant increase ($P < 0.05$) in total protein concentration when compared with groups A and B.

DISCUSSION

The skin cancer is the most common form of cancer, accounting for nothing less than least 40% of cases globally. The most common type is non-melanoma skin cancer, which affects about 2-3 million people per year. Plant-based and traditional medicine systems will continue to play essential roles in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care.

Catalase is a critical antioxidant enzyme that detoxifies hydrogen peroxide (H_2O_2), a reactive oxygen species elevated during oxidative stress. The results indicated that serum catalase activity in Groups C, D, and E did not differ significantly ($P > 0.05$) from the control groups (A and B), suggesting a protective effect of both the CNSHL n-hexane extract and Aldara in preserving antioxidant capacity post UV exposure. Notably, Groups C

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and D—both exposed to UV radiation and treated with either the CNSHL extract or Aldara—maintained catalase levels comparable to the positive control. This preservation of catalase activity may be attributed to the presence of bioactive compounds such as anacardic acid and cardanol in the extract, which possess known antioxidant and anticancer properties.

Interestingly, Group E, which was treated with the CNSHL extract without UV exposure, also showed no significant change in catalase activity. This might indicate minimal oxidative challenge in the absence of UV-induced H_2O_2 production, leading to limited catalase engagement. In contrast, Group F, which received Aldara without UV exposure, showed a significant decrease in catalase activity compared to Group A. This reduction may reflect an adverse effect of the drug when used in the absence of its intended therapeutic context, such as UV-induced skin damage, aligning with precautionary notes associated with Aldara's clinical use. Regarding tyrosinase activity—a key enzyme regulating melanogenesis—the study revealed a significant reduction ($P < 0.05$) in Group B compared to Group A. This was unexpected, as UV radiation typically stimulates tyrosinase expression and melanin production. Previous findings, such as those by Gilchrist et al. (2016), reported an upregulation of tyrosinase gene expression following UV exposure. The discrepancy observed in this study may be due to environmental or experimental differences, or possibly the presence of underlying skin conditions that influenced enzyme activity in Group B. Groups C and D, which received treatment following UV exposure, showed no significant change in tyrosinase activity compared to the positive control, suggesting that both the CNSHL extract and Aldara may modulate melanogenesis activity post-UV exposure, potentially contributing to skin protection or repair mechanisms.

This result correlates to that of Elmer-Rico, et al (2005). Their result showed that tyrosinase inhibitors were successfully detected using the standard method. In addition, their result showed that tyrosinase inhibitors from CNSHL showed an activity comparable to that of known tyrosinase inhibitors; cyanide and benzoic acid. Therefore, the CNSHL can as well be used in place of the standard tyrosinase inhibitors. Group E and F showed significant increase in tyrosinase activity when compared to group A and B. This is in contrast with Xiang-Ping et al. (2016). They studied the function of cardanol; a major phenolic component of CNSL. The study revealed the ability of cardanol to decrease the steady-state rate of tyrosinase activity efficiently. This contrast may be as a result of study area difference or difference in assay procedures.

It was observed that the total protein concentration in serum of group B showed significant reduction ($P < 0.05$) compared to group A. This could be as a result of the negative effects of UV radiation on protein which includes denaturing of protein structure, generation of reactive oxygen species resulting in protein thiol oxidation (Chan et al., 2006). Group C showed no significant difference ($P > 0.05$) when compared with group A (negative control). This implies that the hexane extract of cashew nut shell can remedy the effects caused by exposure to UVR as it concerns total protein concentration in serum. However, group D showed a significant increase ($P < 0.05$) in the total protein concentration compared to groups A (negative control) and B (positive control). This might be as a result of the bioactive components present in the standard drug and absent in the experimental drug. Groups E and F showed a significant increase ($P < 0.05$) when compared to groups A (negative control) and B (positive control). This result proves that the CNSHL is as good as the standard drug but not better as it relates to UVR exposure and its effect on total protein concentration in serum.

CONCLUSION

This study has shown that the N hexane extract of Anacardium occidentale has chemo preventive activity on early-stage UV radiation-induced skin cancer. However, further research is to be carried out to ascertain the mechanism of its action as an experimental drug for UV radiation induced skin cancer.

RECOMMENDATION

Further research is to be carried out to ascertain the mechanism of its action as an experimental drug for Ultra-Violet radiation induced skin cancer.

Declarations

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Declaration of Competing Interest

All authors declare zero financial or inter-personal conflict of interest that could have influenced the research work

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