

Evaluation of the Antioxidant Properties of *Vitellaria paradoxa* Seed Extract and Its Effect of Ultra Violet Radiation Induced Skin Damage

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Authors' contributions

This work was carried out in collaboration among all authors. Author FMI designed the study, supervised the laboratory experiment, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors URJ and HE carried out the laboratory experiment and managed the analyses of the study. Author OVA reviewed and proofread the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJBGMB/2020/v6i130141

Editor(s):

(1) Dr. Mangala Kohli, College in New Delhi, India.

Reviewers:

(1) Suman Bala, United BioSource Corporation, USA.

(2) Kavya Mathukumalli, University of Houston College of Pharmacy, USA.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/61297>

Received 20 July 2020

Accepted 26 September 2020

Published 04 November 2020

Original Research Article

ABSTRACT

Ultra violet radiation (UVR) is relevant in nature, but despite its importance it has been labeled as a causative agent in skin damage. This study was done to evaluate the antioxidant properties of shea butter and its role in the prevention of skin damage. Six groups of albino rats with three rats per group were used, group 2-5 were exposed to UVR at the dose of 180 mJ/cm² with group 1 serving as the normal control, group 2 (negative control), group 2-5 (treated groups) and group 6 (normal + treated). The treatment was done by topical application of *Vitellaria paradoxa* seed extract 5 minutes after exposure to UVR. The in-vitro antioxidant properties determined using DPPH radical scavenging activity revealed that the aqueous extract of *Vitellaria paradoxa* possesses high antioxidant activity. There was an increase in the concentration of GSH of the treated animals when compared to the negative control (p<0.05). The histopathology result shows the extract had a

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protective effect on the skin tissue of the experimental animals. This study suggests the extract has the potential to serve as a chemopreventive agent against UV-induced skin damage by neutralizing the effects of free radicals generated by ultraviolet radiation and as such can be recommended for use as UVR screen which could in turn reduce the risk of UVR skin damage.

Keywords: Skin damage; ultra violet radiation; *Vitellaria paradoxa* (Shea butter); antioxidant.

1. INTRODUCTION

Shea butter, a fat extracted from *Vitellaria paradoxa*'s kernels is reported to have been used to prevent skin cancer, which occurs as a result of irreparable damages on the cells of the skin [1]. Oxidative stress can arise as a result of imbalance of free radicals and high levels of xenobiotics. Once activated, reaction continues auto-catalytically; it has a progressive course, and its final result are structural and functional changes of substrate [2]. Free radicals' effect can be neutralized by the activities of antioxidants and antioxidant enzymes.

Shea butter contains compounds like cinnamate which serve as a good sunscreen, it plays a significant role in preventing the skin from UVR-induced damage [1]. Shea butter also contains antioxidants such as vitamin A, E and polyphenols which help in neutralizing the effect of free radicals, thereby preventing skin damage. Some of the isolated chemical constituents are reported to have anti-inflammatory, emollient, and humectant properties [3].

Antioxidants which are compounds known to possess the ability to inhibit oxidation and neutralize the effects of free radicals can be produced in the body, readily supplied through diet, supplement drugs or through the use of extracts from plants that has medicinal effects. However, several enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase etc. act as antioxidants to influence oxidative stress.

The exposure of the skin to ultraviolet radiation has a destructive effect by causing DNA damage that can lead to malignant transformation. If the skin has not been affected severely, the damaged cells can be repaired by Nucleotide Excision Repair (NER). In the absence of appropriate repair, skin cancer may develop. UV radiation (ultraviolet radiation) has been an important light ray both to humans and other components of the environment, but it has also been a major problem to man as it is one of the causes of cancer, most especially skin cancers.

The occurrence of non-melanoma and melanoma skin cancers have been increasing over the past decades. 2-3 million non-melanoma skin cancers and 132 000 melanoma skin cancers occur globally each year (WHO, 2017) [4]. About 90% percent of non-melanoma skin cancers are associated with exposure to ultraviolet radiation (UVR) from the sun [5].

2. MATERIALS AND METHODS

2.1 Sample

The sample (Aqueous extract of *Vitellaria paradoxa* Seed) was obtained from Raw Material Research and Development Council (RMRDC), Kwara State, Nigeria.

2.2 Experimental Animals

The experimental animals (albino rats) were obtained from the Animal House, Department of Biochemistry, Kogi State University, Anyigba, Nigeria. The rats were acclimatized for 2 weeks prior to the experiment and exposed to 12 hours light and darkness while allowed access to food and water ad libitum.

2.3 Methods

2.3.1 1,1-Diphenyl-2-Picrylhydrazine (DPPH) scavenging activity

1 ml of various concentrations of the extracts in methanol was added to 4 ml of 0.1 mmol/L methanolic solution of DPPH. A blank probe was obtained by mixing 4 ml of 0.1 mmol/L methanolic solution of DPPH and 200 μ l of deionized distilled water (D.D. H₂O). After 30 minutes of incubation in the dark at room temperature, the absorbance was read at 517 nm against the prepared blank. Inhibition of free radicals by DPPH in percent (I %) was calculated using this formula:

$$\% \text{ Inhibition} = \frac{100 - (\text{ABS sample} - \text{ABS blank})}{\text{ABS control}} \times 100$$

2.3.2 Ultra violet radiativon induced skin damage

Eighteen (18) experimental animals were weighed, the fur on their back shaved and they were divided into six (6) groups according to the experimental design below. The induction was done according to a modified method by Wang et al. [6].

2.3.3 Experimental design

Group 1 (Normal Control), Group 2 (Negative control), Group 3 (500 mg/ml *Vitellaria paradoxa* extract), Group 4 (1000 mg/ml *Vitellaria paradoxa* extract), Group 5 (2000 mg/ml *Vitellaria paradoxa* extract), Group 6 (Normal + 500 mg/ml *Vitellaria paradoxa* extract).

2.3.4 Treatment of experimental animals

The experimental animals were treated alongside with the induction process following the details of their grouping above. The treatment of the animals were done physically by applying the extract on their shaved skin 5 minutes after exposure to the UV. At the end of the treatment, they were starved for 12 hours, weighed and anesthetized and sacrificed.

2.3.5 Collection of organs

The vital organs (heart, spleen, liver, lungs and kidney) of the animals were collected and weighed.

2.3.6 Preparation of serum sample

Blood were collected into sample bottles and was centrifuged at 3000 RPM (using a micro field centrifuge) for 15 minutes. The serums were decanted into a different sample bottle and was stored in the freezer for further analysis.

2.3.7 Preparation of tissue homogenate

The organs collected from each animal were rinsed in normal saline and homogenized in ice cold buffer with pH 7.4 using a homogenizer. The homogenates were centrifuged at 3000 RPM for 15 minutes and the supernatants were collected.

2.3.8 Determination of reduced glutathione (GSH)

Reduced glutathione (GSH) content was determined according to the procedure described by Griffith [7].

2.3.9 Histological studies

The skin and liver tissues of the experimental animals were stored respectively in 10% formalin for 24 hours. The formalin fixed specimens were embedded in paraffin and section (3-5µm) and stained with hematoxylin and eosin dye. The histological sections were evaluated by light microscopy.

2.3.10 Statistical analysis

The data was analyzed using SPSS version 16, p value < 0.05 were considered statistically significant.

3. RESULTS

3.1 The Effect of *Vitellaria paradoxa* Seed Extract (Shea Butter) on 1,1-Diphenyl-2-Picrylhydrazine (DPPH) Scavenging Activity

The determination of the effect of Shea butter using DPPH Scavenging activity is shown in Fig. 1. The activity of antioxidant is shown to have an increased level in low concentration of 10 mg/ml than when compared to higher concentrations of 20, 30, 40 and 50 mg/ml i.e. a higher level of antioxidant activity (% inhibition) is observed in the control sample, Butylated hydroxytoluene (BHT). The level of antioxidant in the shea butter decreases with increased concentrations with exception to the concentration of 10 mg/ml.

3.2 The Effect of *Vitellaria paradoxa* Seed Extract (Shea Butter) on the GSH Level of UVR Induced Skin Damage in Experimental Animals

The determination of the effect of shea butter on the GSH level of UVR-induced skin damage in the experimental animals is shown in Fig. 2. The study showed an increased level of glutathione in the skin of the normal control when compared to that of the serum or liver.

3.3 Determination of the Effect of *Vitellaria paradoxa* Seed Extract on the Skin and Liver Tissues of the Experimental Animals

Fig. 3 shows photomicrograph of skin and liver tissues of the various groups studied to determine the effect of *Vitellaria paradoxa* seed extract on skin and liver tissues in UVR initiation

of skin cancer in the experimental animals. The normal control, the group treated with 500 mg/ml, 1000 mg/ml and normal + 500 mg/ml of the seed extract showed essentially normal skin tissues and hepatocytes. On the other hand, the negative control showed severe seborrheic

dermatitis and actinic keratosis on the skin tissues, and marked interphase hepatitis. The group treated with 2000mg/ml of the seed extract also showed mild seborrheic dermatitis and actinic keratosis on the skin tissue, although the hepatocytes appeared essentially normal.

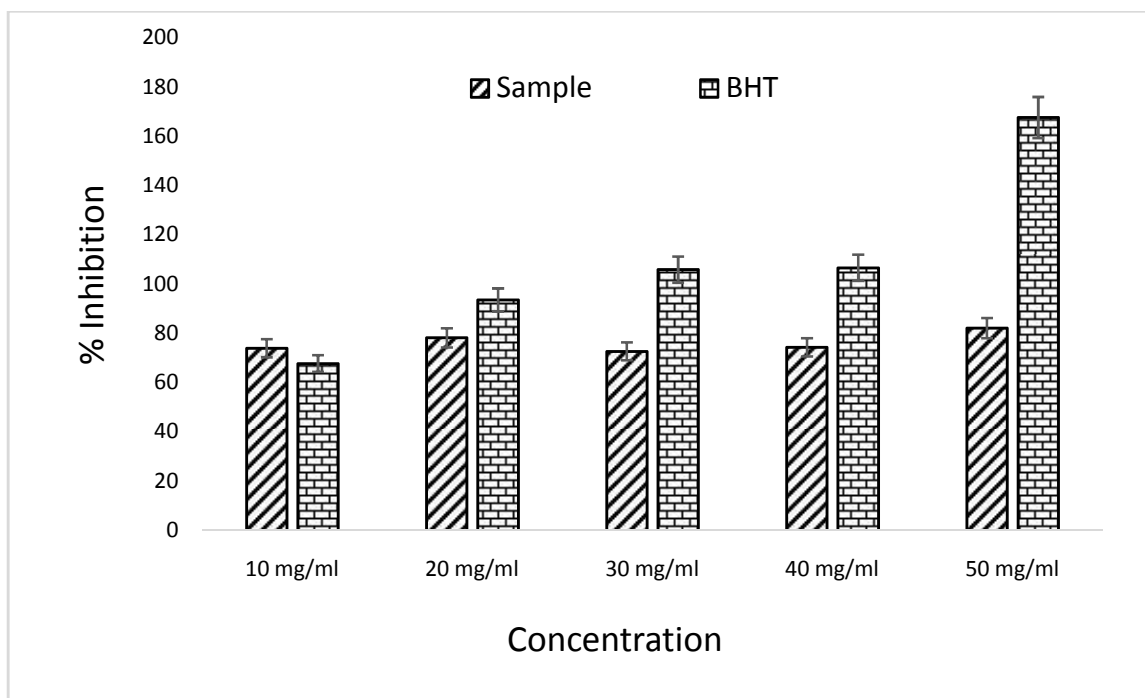


Fig. 1. The effect of *Vitellaria paradoxa* seed extract (Shea Butter) 1,1-Diphenyl-2-Picrylhydrazine (DPPH) scavenging activity

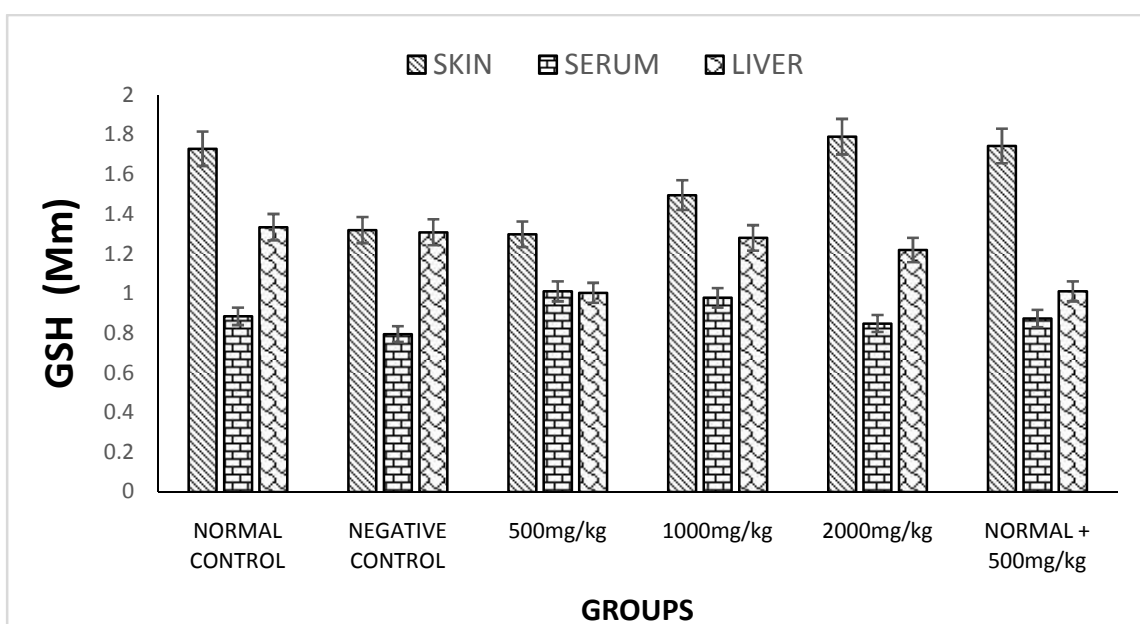


Fig. 2. The effect of *Vitellaria paradoxa* Seed Extract (Shea Butter) on the GSH level of UVR-induced skin damage experimental animals

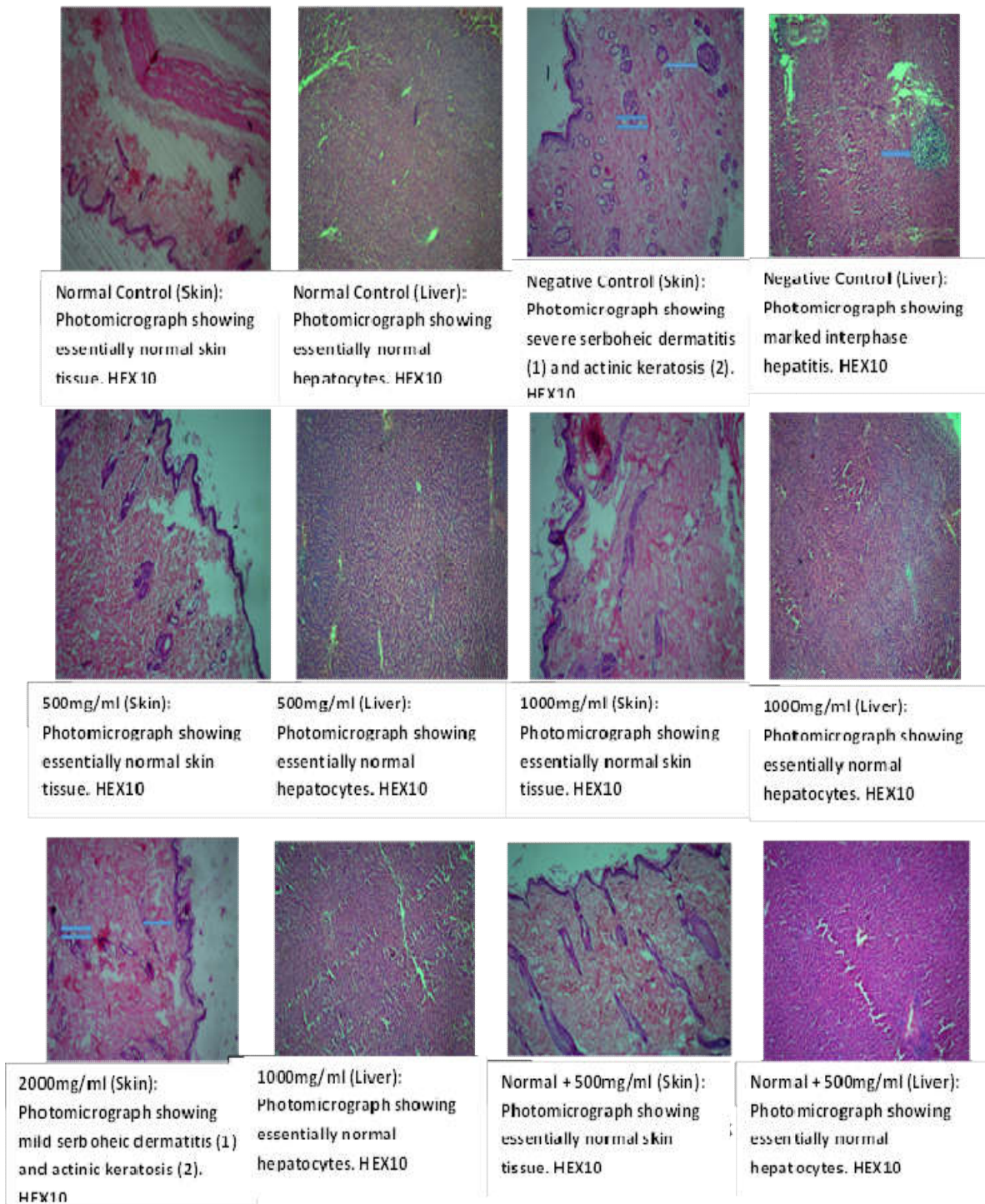


Fig. 3. Histological study of the effect of *Vitellaria paradoxa* seed extract on the skin and liver tissues in the experimental animals

4. DISCUSSION

Medicinal plants have been part of disease management in humans as well as animals, some animals are known to consume certain

herbs instinctively when they sense illness while humans for centuries have used different medicinal plants in treatment cocktails. The phytochemical contents and pharmacological actions of many plants having medicinal

potentials are yet to be identified by scientists [8]. These phytochemicals have potential for use as drugs, and the content and known pharmacological activity of these substances in medicinal plants is the scientific basis for their use in modern medicine, if scientifically confirmed. Modern research has been focused on evaluating the mechanism of action of these plants.

The skin is the major target of oxidative stress as a result of the highly reactive free radicals that it is frequently exposed to from the environment. This can lead to skin damage and in furtherance cancer [9]. Extreme exposure to ultraviolet radiation (UVR) from sunlight and its penetration into the skin cells can alter genetic codes [10-12]. *Vitellaria paradoxa* seed extract (shea butter) is known to contain cinnamate and other antioxidants [1]. These compounds especially cinnamic acid in the oil provides vital protection against harmful UVR [13]. The compounds also play a role in preventing skin damage caused by ultraviolet radiation.

This study reveals that shea butter possess high antioxidant capacity (Fig. 1) when subjected to 1,1-diphenyl-2-picrylhydrazine (DPPH) scavenging activity assay, this explains the action behind some of its role in disease management.

Fig. 2 shows that there is an elevated level of glutathione in the skin of the normal control when it is compared to that of the serum. The liver and skin of the negative control shows almost the same concentration, but this is not so for the serum; the group treated with shea butter had changes in the skin levels of GSH, when compared to liver and serum levels. The levels of GSH increases with increasing concentration of shea butter for the concentration of 2000 mg/ml, this is believed to be due to the high antioxidant activity of the extract.

The result from the histopathology (Fig. 3) shows that the extract was able to prevent ultra violet radiation from having a damaging effect on the skin.

5. CONCLUSION

The study suggests that *Vitellaria paradoxa* seed extracts has high antioxidant properties that plays a vital role in the prevention of skin damage caused by ultra violet radiation. Though further work is still required to fully determine the

mechanism by which the extract elicit its action, this study suggests that to reduce the risk of skin damage, shea butter can be incorporated into body creams or solely used as ultra violet radiation screen.

ETHICAL APPROVAL

The experimental animals were handled according to the guidelines set by the Research Ethical Committee of Kogi State University, Anyigba, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:

The peer review history for this paper can be accessed here:

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