SYSTEMIC TOXICITY FROM TOPICALLY APPLIED VIRGIN COCONUT OIL IN BURNED EXPERIMENTAL ANIMALS

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SUMMARY

The healing properties and potential benefits of virgin coconut oil (VCO) for treating burned skin lesions have been demonstrated. Nevertheless, prolonged topical use can lead to systemic effects, especially when applied to open wounds. Currently, the systemic toxicity of topical VCO in burned experimental animals has yet to be fully elucidated. ***Objective****: The present study investigated the systemic toxicity from topically applied VCO in burned experimental animals.* ***Methods:*** *All animals, except the normal control group, were subjected to thermal burns on the back of each rat by using a standard burning technique. Following the burning, each animal was placed in a separate cage, and the affected areas were covered with VCO* 0.2 or 0.4 ml/day *twice a day for 21 consecutive days. Hematological and biochemical analyses were determined. Macroscopic and histopathological examinations* of the liver and kidney were conducte*d at the end of the treatment period.* ***Results:*** *Our results indicated that topical administration of VCO caused no significant change in animals' general status, hematological parameters, and renal and hepatic functions. Additionally, it did not alter the liver and kidney histology in animals.* ***Conclusion:*** *These findings suggest that the topical application of VCO was considered safe for the wounded animals.*

Keywords*: Virgin coconut oil, burn, systemic toxicity, experimental animals.*

1. INTRODUCTION

The skin, the most significant human organ, is the primary mechanical barrier between the body and the external environment. It protects against harmful agents, regulates temperature, and maintains water and electrolyte balance [1]. According to the World Health Organization, millions of people with pathological wounds require medical attention each year. Currently, there are several treatments for skin injuries, comprising surgical procedures, non-surgical therapies, and pharmacologic agents, with costs of $12 billion annually [2]. Virgin coconut oil (VCO) is a natural oil extracted from the mature kernel of the coconut fruit through different mechanical and natural methods. VCO has been traditionally used as a moisturizer for centuries in the tropical regions. It offers numerous health benefits due to its retained vitamins, antioxidants, and antimicrobial and antiviral properties [3].

We have demonstrated the beneficial effects and potentials of VCO in terms of healing effects in burned skin lesions [4]. However, long-term topical application can also have systemic effects, mainly when applied to open wounds [5]. Additionally, evaluating the possible toxic effects of herbal products, including toxicity tests in experimental animal models, is essential before conducting clinical studies in humans. To date, the systemic toxicity of topical VCO in burned experimental animals has not been fully elucidated. Thus, the present study investigated the systemic toxicity from topically applied VCO in burned experimental animals.

2. MATERIALS AND METHODS

2.1. Preparation of VCO

VCO was produced by Vi Dieu Nam Medicine and Pharmacy Limited Company, reaching the manufacturer's standard. VCO was made by cold-pressing the liquid from the solid endosperm of mature coconut (*Cocos nucifera* L.). The 120 ml bottle of VCO adheres to ISO 22000:2018 standards.

2.2. Experimental animals

Healthy *Wistar albino* rats of either sex, weighing 180±20 gam, were used. Animals were acclimatized to the laboratory conditions (constant temperature of 24±1^{o}C, with a 12-light/dark cycle) for seven days before the study commenced. All experiments, as well as animal care and handling, followed the relevant guidelines and regulations.

2.3. Evaluation of systemic toxicity of topical administration of VCO in burned animals

The 30 rats were randomly divided into three groups of 10 animals. The rats were anesthetized with a single intraperitoneal injection of 250 mg/kg chloralhydrate. As preparation, they were shaven at the dorsum with an electric shaver and later sterilized with 70% alcohol. All animals, except the normal control group, were subjected to thermal burns on the back of each rat by using a standard burning technique [6]. Burn wounds were formed by pressurelessly applying a 200-gram cylindrical stainless-steel rod (2.5 cm diameter), which was pre-heated to 100°C in boiling water with the thermal equilibrium confirmed by a monitoring thermometer, onto the shaven skin for 35 seconds. All animals were resuscitated immediately with Lactated Ringer's solution (2 ml/100 g body weight) intraperitoneally. Following the burning, each animal was placed in a separate cage, and the affected areas were covered with VCO 0.2 or 0.4 ml/day twice a day for 21 days. The vehicle-treated burned rats topically received sterile distilled water.

The signs and parameters were checked during the study:

- General conditions, body weight changes;
- Evaluation of hematological parameters: red blood cell count, hemoglobin, hematocrit, total white blood cells (WBC), and platelet count;
- Evaluation of liver damage through aspartate aminotransferase level (AST) and alanine aminotransferase level (ALT);
- Evaluation of liver function through total bilirubin, albumin, and total cholesterol;
- Evaluation of kidney function through creatinine level.

Follow-up parameters were checked at the time points before, after 10 and 21 days of applying VCO in thermal burn rats. At the end of the experiment, rats were euthanized after blood collection, and the internal organs (heart, liver, spleen, kidney, and lungs) were removed and observed for any gross lesions. The liver and kidney of 30% of the animals in each group were preserved in a 10% buffered formaldehyde solution for histopathological studies. The sections were stained with hematoxylin and eosin (H&E) and checked for histopathological assessment by the blinded researcher.

2.5. Statistical analysis

Sigmaplot 12.0 (SYSTA Software Inc, Richmond, CA, USA) was used for statistical analysis. Obtained data were expressed as the mean ± S.D and compared with either one-way-ANOVA followed by the post hoc Student-Newman-Keuls test for multiple comparisons. Statistically significant differences were considered when the p-value was less than 0.05.

2.6. Research location

The research was conducted at the Department of Pharmacology, Hanoi Medical University.

3. RESULTS

3.1. General condition and body weight changes

During the experiment, animals in all groups had normal locomotor activities, good feedings, agility, skin, fur colors, bright eyes, and dry stools. As shown in Figure 1, after 10 and 21 days of treatment, the body weight of treated rats increased compared to before treatment ($p<0.05$). There was no significant difference between the treated and control groups ($p>0.05$).

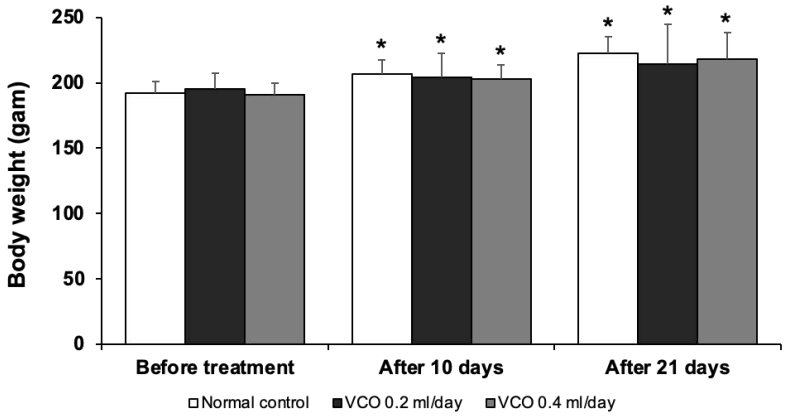


Figure 1. The effect of VCO on body weight changes

*p<0.05, **p<0.01, ***p<0.001: as compared with the time point "Before treatment"

3.2. Effect of VCO on hematological parameters

There were no significant differences in red blood cell count, hematocrit, hemoglobin level, or mean corpuscular volume between the VCO-treated groups and the normal control group ($p>0.05$) (Table 1).

Table 1. The effect of VCO on red blood cell count, hematocrit, hemoglobin level, mean corpuscular volume

Parameters	Group	Before treatment (1)	After treatment	
			10 days (2)	21 days (3)
Red blood cells count (T/L)	Normal control (a)	10.59 ± 0.87	11.18 ± 0.65	11.06 ± 0.96
	VCO 0.2 ml/day (b)	10.25 ± 0.80	10.86 ± 0.91	10.42 ± 0.87
	VCO 0.4 ml/day (c)	11.42 ± 1.37	11.35 ± 1.57	10.54 ± 1.37
	$p_{2-1} > 0,05$, $p_{3-1} > 0,05$, $p_{b-a} > 0,05$, $p_{c-a} > 0,05$			
Hemoglobin (g/dl)	Normal control (a)	12.47 ± 1.25	13.23 ± 1.30	13.07 ± 1.13
	VCO 0.2 ml/day (b)	12.39 ± 0.98	13.42 ± 1.14	12.63 ± 1.41
	VCO 0.4 ml/day (c)	13.39 ± 1.65	13.68 ± 1.21	12.73 ± 1.76
	$p_{2-1} > 0,05$, $p_{3-1} > 0,05$, $p_{b-a} > 0,05$, $p_{c-a} > 0,05$			
Hematocrit (%)	Normal control (a)	52.51 ± 8.14	55.26 ± 7.50	57.09 ± 8.92
	VCO 0.2 ml/day (b)	51.51 ± 4.60	54.48 ± 4.90	51.90 ± 4.75
	VCO 0.4 ml/day (c)	56.66 ± 7.53	57.26 ± 5.66	53.42 ± 7.35
	$p_{2-1} > 0,05$, $p_{3-1} > 0,05$, $p_{b-a} > 0,05$, $p_{c-a} > 0,05$			
Mean corpuscular volume (fL)	Normal control (a)	51.70 ± 2.11	52.60 ± 1.51	52.30 ± 1.25
	VCO 0.2 ml/day (b)	51.10 ± 1.10	51.60 ± 1.35	51.00 ± 2.21
	VCO 0.4 ml/day (c)	49.90 ± 2.69	50.80 ± 2.86	50.50 ± 3.44
	$p_{2-1} > 0,05$, $p_{3-1} > 0,05$, $p_{b-a} > 0,05$, $p_{c-a} > 0,05$			

As shown in Figure 2, there were no significant differences in total WBC count and platelet count between the VCO-treated groups and the control group (p>0.05) (shown in Fig. 2).

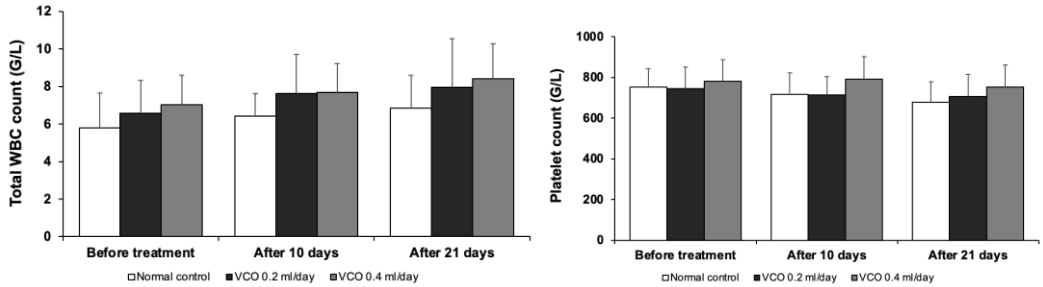


Figure 2. The effect of VCO on total WBC and platelet count

3.3. Effect of VCO on liver damage

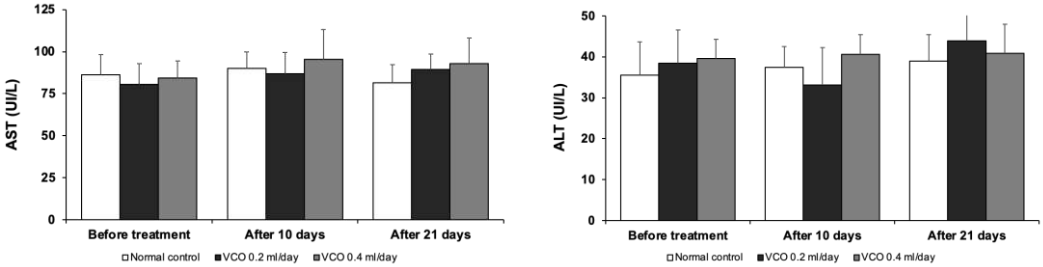


Figure 3. Effect of VCO on AST and ALT level

Figure 3 demonstrates that after 10 and 21 days of treatment, VCO at doses of 0.2 ml/day and 0.4 ml/day did not cause statistical differences in AST and ALT levels when comparing the treated groups to the control group (p>0.05).

3.4. Effect of VCO on liver function

Table 2 illustrates that after 10 and 21 days of treatment, there was no statistical difference in total bilirubin, albumin, and total cholesterol concentration in the all-treated groups compared to the control group (p>0.05).

Table 2. The effect of VCO on liver function

Parameters	Group	Before treatment (1)	After treatment	
			10 days (2)	21 days (3)
Total bilirubin (mmol/l)	Normal control (a)	8.65 ± 0.52	8.74 ± 0.65	9.04 ± 0.57
	VCO 0.2 ml/day (b)	8.73 ± 0.55	9.23 ± 0.60	9.30 ± 0.75
	VCO 0.4 ml/day (c)	8.54 ± 0.47	9.01 ± 0.90	9.11 ± 0.87
	$p_{2-1} > 0,05$, $p_{3-1} > 0,05$, $p_{b-a} > 0,05$, $p_{c-a} > 0,05$			
Albumin concentration (g/dl)	Normal control (a)	3.17 ± 0.33	3.30 ± 0.21	3.46 ± 0.28
	VCO 0.2 ml/day (b)	3.18 ± 0.26	3.11 ± 0.34	3.24 ± 0.31
	VCO 0.4 ml/day (c)	3.30 ± 0.21	3.12 ± 0.19	3.27 ± 0.29
	$p_{2-1} > 0,05$, $p_{3-1} > 0,05$, $p_{b-a} > 0,05$, $p_{c-a} > 0,05$			
Total cholesterol concentration (mmol/l)	Normal control (a)	1.40 ± 0.16	1.34 ± 0.20	1.37 ± 0.17
	VCO 0.2 ml/day (b)	1.44 ± 0.14	1.36 ± 0.15	1.42 ± 0.10
	VCO 0.4 ml/day (c)	1.46 ± 0.11	1.42 ± 0.13	1.36 ± 0.14
	$p_{2-1} > 0,05$, $p_{3-1} > 0,05$, $p_{b-a} > 0,05$, $p_{c-a} > 0,05$			

3.5. Effect of VCO on kidney function

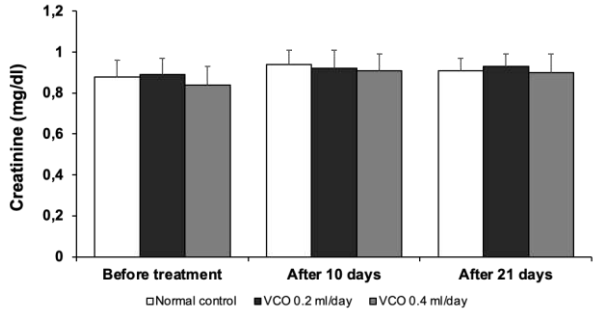


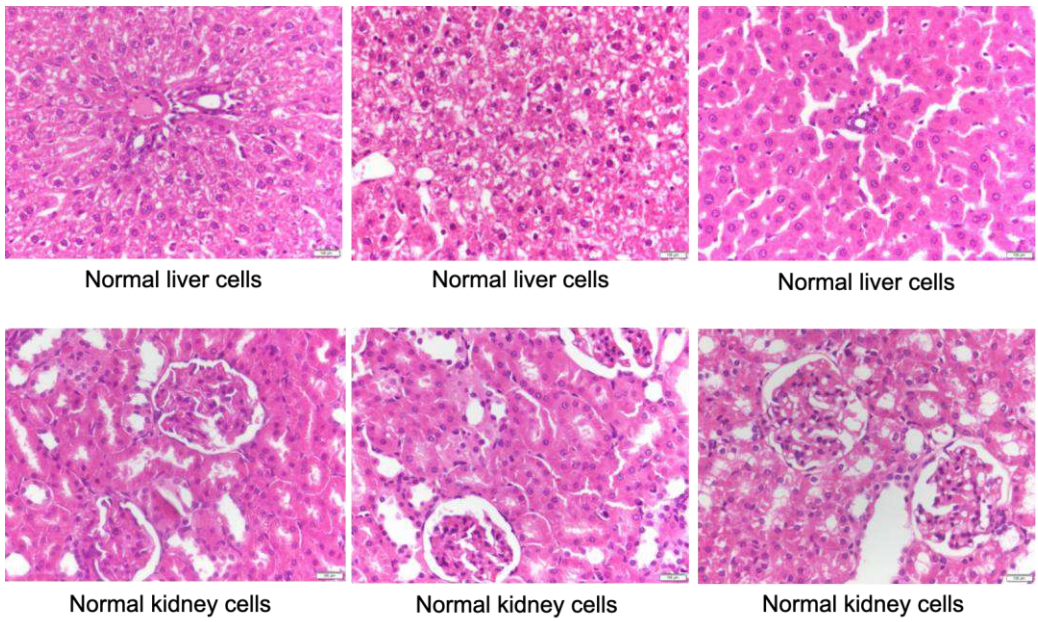
Figure 4. Effect of VCO on creatinine level

Figure 4 demonstrates that after 10 and 21 days of treatment, VCO at doses of 0.2 and 0.4 ml/day did not cause a statistical difference in creatinine levels between the treated and the control groups ($p>0.05$).

3.6. Histopathological examination

No gross lesions or changes in size were observed when all experimental rats were subjected to a complete gross necropsy, which examined the hearts, livers, lungs, kidneys, and abdominal cavities.

There were no significant differences between VCO-treated rats and the control group in histopathological examinations of livers and kidneys.



Normal control (rat #02, HE x 400) **VCO 0.2 ml/day** (rat #13, HE x 400) **VCO 0.4 ml/day** (rat #28, HE x 400)

Figure 5. Histopathological morphology of liver and kidney

4. DISCUSSION

VCO was used in traditional medicine based on theories, beliefs, and experiences. We have demonstrated the potential of topical VCO in terms of healing effects in burned skin lesions [4]. However, long-term topical application can also affect the systemic effects, mainly applied to open wounds [5]. Currently, there is no adequate data about the safety of VCO. In this study, we evaluated the systemic toxicity after applying topical VCO on thermal burns. Overall, the findings of this study indicated that topical administration of VCO caused

no significant change in the general status, hematological parameters, or renal and hepatic functions. Additionally, they did not alter the liver and kidney histology in animals.

Toxicity refers to unwanted effects on biological systems. To evaluate biological toxicity, it is essential to choose the correct system since no effects may otherwise be seen [7]. The toxicity study provides information on the undesirable effects of continuous or repeated exposure to plant extracts or compounds over a portion of the average life span of experimental animals [8]. Regarding the assessment of repeated-dose toxicity tests, they were performed to investigate the general conditions (body weight, appearance, and behavior) and the potential toxic effects on the animals' hematology, biochemical parameters, and histopathology.

The changes in body weight are the most basic index to reflect the general health status of animals and the combined effects of xenobiotics on the body [8]. Our study observed weight gains in all animals topically administered with VCO. In addition, none of the *Wistar* animals in all treated groups showed any macroscopic or gross pathological changes compared to the control group. It can be stated that VCO did not interfere with the normal metabolism of animals, as corroborated by the non-significant difference in the control group. The World Health Organization identifies hematological parameters as sensitive and reliable indicators for assessing the toxicity of herbal extracts [8]. The blood circulatory system performs essential functions. The hematopoietic system is one of the most sensitive targets of toxic compounds and is a critical parameter in animal physiological and pathological status [9]. Furthermore, analyzing changes in the hematological system is essential for risk evaluation, as these changes can provide valuable predictions about human toxicity. Our results showed that, after 21 days of treatment, there were no significant differences in total red blood cells, hematocrit, hemoglobin level, platelet count, and total WBC count between the VCO-treated groups and the normal control group. All parameters were within the normal reference range of the species [10]. Thus, it can be concluded that the VCO does not affect the hematological system.

Additionally, analysis of the liver is critical in the toxicity evaluation of drug tests as it is necessary for the survival of an organism. The biochemistry analyses evaluated the possible alterations in hepatic function influenced by the product [11]. Total bilirubin, albumin, and total cholesterol are valuable indices of the excretory function of the liver. Besides, ALT and AST are valuable indices for identifying inflammation and necrosis of the liver. Accordingly, the liver releases AST and ALT, and an elevation in plasma concentration indicates liver damage. Moreover, the kidneys are the body's excretory organs, and renal parenchyma cells are highly vulnerable to endogenous and exogenous substances. When a compound is introduced into the body, it can induce toxicity and damage the kidneys, affecting kidney function. Assessment of renal function after taking a drug is usually achieved by a quantitative blood creatinine test [12]. Creatinine is the most stable protein in the blood, almost independent of diet or physiological changes but only dependent on the ability of the kidneys to excrete it. When the glomeruli are damaged, blood creatinine levels rise earlier than urea. Blood creatinine is a more reliable and essential indicator than blood urea, which is currently used to assess and monitor kidney function [13]. Our results demonstrated that the blood biochemistry levels of VCO-treated groups presented no significant differences compared to the control group. This evidence shows that VCO did not affect liver and kidney functions. Furthermore, histopathological

examination of the liver and kidney of the control and all treated groups did not reveal any morphological differences. This is consistent with the results of biochemical parameters in VCO-treated groups.

Our findings showed that VCO caused no significant differences in general conditions, hematological parameters, liver and kidney function tests, and histopathological examination compared to untreated rats. A previous study evaluated VCO's safety in some Southeast Asian countries. The acute oral toxicity was assessed at the dose of 5000 mg/kg, and the sub-chronic and chronic oral toxicity was evaluated at the doses of 175, 550, and 2000 mg/kg [14]. The results revealed that the treated rats were safe with the research doses. Overall, our results showed the safety of topical VCO in burned animals.

5. CONCLUSION

VCO at doses of 0.2 and 0.4 ml/kg/day did not cause detrimental effects on the body weight, hematological and biochemical parameters, as well as macromorphology and micromorphology of the livers and kidneys of the treated animals compared to the control group.

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