Specialty Journal of Pharmacognosy, Phytochemistry, and Biotechnology
ISSN: 3062-441X
2022, Volume 2, Page No: 1-7

Available online at: www.galaxypub.co/page/journals

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Impact of Fresh Coconut Oil on the Gastrointestinal Microbiome and Hematological/Biochemical Parameters in Wistar Rats

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Received: 19 November 2021; **Revised:** 28 January 2022; **Accepted:** 29 January 2022

ABSTRACT

Fresh coconut oil is known for its potential health benefits, particularly on hematological and biochemical markers, which may be attributed to its medium-chain triglycerides content. This prompted a closer examination of its effects in managing prothrombotic tendencies. This study aimed to investigate the effect of fresh coconut oil on the gastrointestinal tract microbiota, as well as hematological and biochemical markers, in Wistar rats. A total of ten adult Wistar rats, each weighing between 94 and 125 grams, were selected for the study (n = 5). The rats were divided into two groups: a control group and an experimental group that received 0.5 ml/kg of coconut oil. Colony counting was performed on both fecal and gastrointestinal samples, while hematological and biochemical markers were assessed through standard blood testing methods. The findings showed a reduction in hemoglobin, red blood cell count, white blood cell count, granulocyte count, mean corpuscular hemoglobin concentration, platelet count, and mean platelet volume in the coconut oil-treated group compared to the control group. Coconut oil (0.5 ml/kg) showed stronger hematinic properties without causing significant damage to the liver cells. There were no significant differences in the levels of Na+, K+, Cl-, Cr, or HCO3; however, there was a significant decrease in CHO, HDL, and LDL levels when compared to the control. Notably, the potassium (K+) concentration was significantly elevated in the experimental group. The results also showed that coconut oil produced a vasodilatory effect on the aorta, and no adverse effects on the lipid profiles were observed. Consequently, coconut oil may act as an alternative hematinic agent.

Keywords: Biochemical, Coconut oil, Microbiome, GIT, Heamatological

How to Cite This Article: Machate DJ, Figueiredo PS, Marcelino G, Guimarães RDCA, Hiane PA, Bogo D, et al. Impact of Fresh Coconut Oil on the Gastrointestinal Microbiome and Hematological/Biochemical Parameters in Wistar Rats. Spec J Pharmacogn Phytochem Biotechnol. 2022;2:1-7. https://doi.org/10.51847/q69gWhtCds

Introduction

Natural products such as herbs, spices, and medicinal plants, along with their various components, are widely regarded as safe due to their long-standing use with minimal harmful effects [1]. As a result, extracts from plants and essential oils have gained popularity as alternatives to antibiotics because of their antimicrobial properties and positive influence on the gastrointestinal system [2]. Coconut oil, which plays a vital role in daily nutrition, is considered a functional food with many recognized health benefits across various regions of the world [3].

The human microbiome, particularly within the gastrointestinal tract, plays a critical role in health by aiding in energy extraction from food, synthesizing essential nutrients like vitamins and amino acids, and defending against harmful microorganisms [4]. The composition of this microbiome can vary throughout an individual's life and

across different geographic regions [5]. The gastrointestinal microbiome, which contains a significant proportion of the body's microbial biomass [6], is frequently studied due to its role in digestion, metabolite production, interactions with the immune system [7], and its involvement in a variety of disease conditions [8].

When the delicate balance of the gastrointestinal microbiome is disrupted, a condition known as dysbiosis may develop, which has been linked to diseases such as inflammatory bowel disease (IBD) [9], irritable bowel syndrome (IBS) [10], celiac disease, food allergies, type 1 and type 2 diabetes [11], cancer, and cardiovascular disease. This imbalance can be triggered by factors such as diet, hygiene practices, and contamination of food and water [12].

Coconut oil has been the subject of growing interest due to its broad spectrum of antimicrobial effects [13]. Despite containing saturated fats similar to those found in animal products, it also has a unique composition that confers health benefits, including antioxidant, anti-inflammatory, and antifungal properties. With the increasing problem of antibiotic overuse, there has been a surge in research into natural alternatives like coconut oil to treat infections and support immune function. This study aims to investigate the effects of oral coconut oil on the gastrointestinal microbiome of Wistar rats, while also assessing its impact on various biochemical and hematological parameters, and determining the most effective extraction method to retain its essential components.

Materials and Methods

Materials

The materials used in this study include fresh coconut oil, a blending machine (model SBM-2977, OSAKA, JAPAN), measuring cylinders, feeding containers, dissecting tools, wooden cages, lead acetate paper, Petri dishes, beakers, aluminum foil, autoclave, lithium heparin, test tubes, slides, EDTA tubes, McCartney bottles, various culture media (Nutrient agar, Nutrient broth, peptone water), sterile water, D-glucose, sucrose (Kermel), lactose, Simmons citrate agar (Titan Biotech), Urea broth, hydrogen peroxide, Kovac's reagent, crystal violet, alcohol, Lugol's iodine, saffranin, immersion oil, and Phenol red, among others.

Coconut oil procurement and animal preparation

Animal procurement

A total of ten adult Wistar rats, each weighing between 94 g and 146 g, were utilized for the experiment. These rats were housed in wooden cages under controlled environmental conditions, with a 12-hour light/dark cycle, stable room temperature, and relative humidity. They were given two weeks to acclimatize before the study, with free access to food and water. Afterward, the rats were randomly assigned into two groups:

Control group (group 1): Rats were given food and water, without coconut oil administration.

Test group (group 2): Rats received a dose of 0.5ml/kg of fresh coconut oil, along with food and water.

The fresh coconut oil was sourced from mature coconuts and extracted using the wet extraction technique as outlined by Nevin and Rajamohan [14].

Weight measurement

Rats were weighed using an analytical balance before and after the administration of coconut oil. The data were recorded for both the control and test groups.

Preparation of nutrient broth

A standard nutrient broth (Titan Biotech Ltd, Delhi, India) was made by dissolving 1.3 g in 100 ml of distilled water. A volume of 10 ml was transferred into four separate McCartney bottles, which were sterilized by autoclaving at 15psi (121 °C) for 15 minutes. Once cooled to room temperature, these bottles were inoculated with fecal samples from both the control and test rats, and incubated at 37 °C for 24 hours.

Bacterial count procedure

Serial dilution process

Peptone water was prepared by dissolving 1.5 g of peptone in 100 ml of distilled water, followed by sterilization in McCartney bottles at 15psi (121 °C) for 15 minutes. Nine milliliters of sterilized peptone water was transferred

aseptically into fourteen test tubes (seven for the test group and seven for the control group). One milliliter of the organism suspension was added to the first test tube (T1), and subsequent dilutions were made by transferring 1 ml from one tube to the next, continuing to the last tube (T7), from which 1 ml was discarded. This process was repeated for both the test and control groups.

Culture media preparation

MacConkey agar for spread plate technique

To prepare MacConkey agar, 4.7 g was dissolved in 100ml of distilled water, sterilized by autoclaving at 15psi (121 °C) for 15 minutes, and poured aseptically into four Petri dishes. After rocking the plates to ensure even distribution, the agar was allowed to solidify. A volume of 0.1 ml from the final test tube of broth suspension was spread onto the MacConkey agar plate using a sterile glass rod.

Nutrient agar for spread plate technique

Similarly, 2.8 g of Nutrient agar was dissolved in 100 ml of distilled water, sterilized by autoclaving, and poured into Petri dishes. The spread plate technique was used to inoculate the solidified nutrient agar, which was then incubated at 37 °C for 24 hours.

Preparation of agar slants

To prepare the nutrient agar, 2.8 g was dissolved in one hundred ml of distilled water and sterilized under autoclave conditions at 15psi (121 °C) for 15 minutes. Aseptic techniques were used to transfer 10 ml of the sterile agar solution into four sterile McCartney bottles, which were then placed in a slanted position to cool and solidify. Once the agar had solidified, each slant was inoculated with a broth culture and incubated at 37 °C for 24 hours.

Hematological analysis

Hematological analysis was conducted using the Sysmex® Automated Hematology Analyzer Kx-2IN from Sysmex Corporation, Kobe, Japan.

Collection of fecal samples and post-coconut oil evaluation

After coconut oil administration, fecal samples from both control and test groups were collected. The samples were swabbed and transferred into sterilized nutrient broth bottles, which were then incubated at 37 °C for 24 hours. Following incubation, bacterial counts were performed, and the agar slants were inoculated with the cultured broth and incubated at 37 °C for an additional 24 hours.

Gastrointestinal tract sampling and evaluation

The large intestine of the Wistar rats was swabbed, and the swabs were transferred to sterilized nutrient broth bottles, which were incubated at 37 °C for 24 hours. Viable bacterial counts were then performed as described by Oghenemaro *et al*. [15].

Biochemical testing procedures

The biochemical tests were carried out using the methodologies described by Enwa *et al*. [16].

Results and Discussion

Natural products play a crucial role in enhancing both individual and community health, with their medicinal benefits stemming from various chemical compounds that have defined physiological effects on the human body [17]. This investigation demonstrated a significant ($P < 0.05$) difference in body weight between the treated and control Wistar rats **(Table 1)**. This change is attributed to the reduction in Low-Density Lipoprotein (LDL) in the treated rats, which shows a noticeable contrast compared to the control. High LDL levels contribute to weight gain and obesity, and a reduction in LDL correlates with decreased body weight. This finding suggests that coconut oil may be beneficial in managing weight. These results align with Nevin and Rajamohan [18], who reported that coconut oil facilitates weight reduction, enhances digestion, and has hypoglycemic effects.

Table 1. The effect of fresh coconut oil on body weight of treated and control Wistar rats.

Wistar rats	Weight
Group 1: control without treatment	119.00 ± 13.32
Group 2: treat with 0.5 ml/dl	111.80 ± 10.31

Values are expressed as mean ± SEM; ANOVA followed by LSD's multiple range tests; values not sharing a common superscript differ significantly at $P < 0.05$

A noticeable reduction in the hemoglobin levels of the experimental rats was observed when compared to the control group **(Table 2)**. Coconut oil is rich in essential amino acids and iron, and the decrease in hemoglobin could be linked to these components. Since iron is integral to hemoglobin function and oxygen transport, the lower levels of iron may contribute to the reduction in hemoglobin. This observation is consistent with Javadifar *et al*. [19], who found that incorporating coconut oil into infant foods affected iron levels significantly.

Table 2. The effect of fresh coconut oil on heamatological parameters of Wistar rats.

Name of sample	WBC 10^9/L	LYM 10^9/L	MID 10^9/L	GRA 10^9/L	MCV 10^9/L	MPV	RBC 10^9/L	HGB 10^9/L	HCT 10^9/L	MCH	MCHC	PLT
Group 1: Control without treatment	12.49 ± 1.798	7.576 ± 0.445	2.154 ± 0.568	2.208 ± 0.891	59.20 ± 5.805	10.16 ± 1.756	6.734 ± 3.017	13.76 ± 1.013	43.29 ± 18.49	16.16 ± 1.383	27.16 ± 0.923	594.80 ±268.72
Group 2: Treat with 0.5 ml/dl	8.498 ± 4.518	5.242 ± 2.226	1.152 ± 0.576	1.288 ± 1.008	67.60 ± 9.737	9.60 ± 1.377	6.400 ± 0.900	12.68 ± 1.118	52.53 ± 4.041	16.48 ± 0.925	26.14 ± 3.356	549.00 ±305.03

Values are expressed as mean ± SEM; ANOVA followed by LSD's multiple range tests; values not sharing a common superscript differ significantly at $P < 0.05$

The white blood cell count showed a significant reduction, which suggests that lower white blood cell levels might help reduce the risk of heart attacks, as there is a known relationship between white blood cells and cardiovascular events [20]. Elevated white blood cells can lead to artery blockage, increasing the risk of heart disease, stroke, and cardiovascular-related mortality. Therefore, reducing white blood cell counts may improve blood circulation and lower the risk of these conditions. Importantly, the reduction in white blood cells remained within the normal range of 4-11×10⁹/L. This suggests that coconut oil may aid in promoting healthy blood flow and enhancing the activity of lymphocytes, the main immune system cells [21].

After seven days of treatment, a significant reduction in red blood cell (RBC) count was noted in the coconut oil-treated rats compared to the control group. This decrease may be due to the reduced iron content resulting from coconut oil administration. Additionally, the oil may have induced RBC membrane lysis, contributing to the reduced RBC count.

Platelet counts also significantly decreased in the treated rats relative to the control group, with this change being both time- and dose-dependent. This suggests that coconut oil may negatively affect platelet counts, potentially by inhibiting the release of thrombopoietin, which regulates platelet production, or by interfering with vitamin K, a critical component in blood clotting. A reduction in platelets could impair the blood's ability to form clots [21].

The treatment with coconut oil led to a notable decrease ($P < 0.05$) in total cholesterol, triglycerides, and LDL levels, while HDL levels significantly increased ($P < 0.05$) in comparison to the control group **(Table 3)**. Eshiet *et al*. [22] used these markers to evaluate the risk of coronary heart disease, noting that a reduction in LDL (bad cholesterol) reduces the likelihood of atherosclerosis, which in turn reduces cardiovascular risk. Chinwong *et al*. [23] showed that coconut oil can reduce atherosclerosis and improve vascular health by raising HDL levels.

Table 3. The effect of fresh coconut oil on biochemical parameters on Wistar rats.

Sample name	Na+	K+	CL-	Urea	Cr	HCO3	CHO	TG	HDL	LDL
CONTROL	135.40 ±4.037	3.560 ± 0.152	95.40 ± 7.021	10.32 ± 0.460	0.560 ± 0.089	28.72 ± 3.409	127.40 ±37.12	61.20 ± 13.66	28.40 ± 2.073	73.92 ±24.33
Group 2: Treat with 0.5ml/dl	135.60 ±5.549	3.660 ± 0.304	95.80 ± 5.762	11.08 ± 0.912	0.590 ± 0.082	29.30 ± 2.588	122.20 ±31.74	45.00 ± 4.472	28.20 ± 4.382	69.40 ± 16.60

Values are expressed as mean ± SEM; ANOVA followed by LSD's multiple range tests; values not sharing a common superscript differ significantly at $P < 0.05$

Potassium and sodium levels were also measured, revealing no significant difference ($P > 0.05$) in sodium levels, but a significant increase in potassium concentration ($P < 0.05$). Sodium is linked to blood pressure regulation, and reducing sodium intake in hypertensive individuals can lower blood pressure. Conversely, potassium, found in intracellular fluid, has been identified as a protective electrolyte against hypertension [24].

The bacterial species isolated from the Wistar rats before and after coconut oil treatment included *Aeromonas hydrophila*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Aeromonas faecalis*, *Proteus* spp., *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, and *Proteus vulgaris* **(Tables 4-6)**. These findings suggest that coconut oil has strong antimicrobial properties, reducing the bacterial load in the microbiome after treatment. The microbial colony count of fecal samples was significantly higher before coconut oil administration but decreased following treatment, further supporting its antimicrobial effects.

Table 4. Viable colony counts of pre-treatment and post-treatment Wister rats (CFU/mL)

Culture plate identification code	Pretreatment colony count	Post-treatment colony count
NA feces C1	TNTC	110
NA feces C2	TNTC	133
MA feces C1	TNTC	100
MA feces C2	TNTC	102
NA feces T1	75	74
NA feces T1	59	45
NA feces T1	118	56
NA feces T1	74	59

Table 5. The post-treatment Wister rats' gastrointestinal intestinal tract colony count (CFU/mL)

Culture plate identification code	GIT Post-treatment colony count
MA GIT C1	99
NA GIT C1	123
MA GIT C2	95
NA GIT C2	100
MA GIT C2	48
NA GIT T1	42

Table 6. Incidence of bacteria colony count in the gastrointestinal tract of Wistar rats

Name of organism	Source	No of organism	% of incidence
Aeromonas hydrophilia	GIT	2	11.8
Proteus spp.	GIT	15	88.2

Aeromonas hydrophilia	Feces	8	32
Enterobacter cloaca	Feces	1	4
Enterobacter feacalis	Feces	1	4

Conclusion

In conclusion, the findings of this study highlight that coconut oil can be a valuable tool in managing body weight, particularly in individuals with obesity, by lowering LDL cholesterol and reducing the associated risk of cardiovascular diseases. Furthermore, regular intake of coconut oil positively affects several hematological factors, including a reduction in white blood cells, which could lower the risk of heart conditions like heart attacks. Additionally, coconut oil's antimicrobial properties help decrease microbial populations in the body. As such, coconut oil demonstrates efficacy in promoting weight loss, enhancing immune function, serving as a blood-protective agent, and providing antimicrobial benefits.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: Ethical approval for this study was granted by the Faculty of Pharmacy Ethical Committee, reference number PHC/PMB/2021/07/11.

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