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# THE EFFECT OF FRESH COCONUT OIL ON GASTROINTESTINAL TRACT MICROBIOME, HEAMATOLOGICAL /BIOCHEMICAL INDICES OF WISTAR RATS

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**ABSTRACT**

Coconut oil has been claimed to have some beneficial health effects on the heamatological and biochemical indices, which is adjudged to the presence of medium-chain triglycerides. It was thus, intriguing to investigate its benefits in alleviating the prothrombotic tendency. This study was to investigate the effect of fresh coconut oil on the gastrointestinal tract (GIT) microbiota and hematological and biochemical indices of Wistar rats. Ten adult Wistar rats of comparable age weighing 94 -125g were used for the study (n-5). The animals were divided into two groups, Group one served as the control, and Group two was the test group which was given 0.5 ml/kg of coconut oil. Colony count was done on both the fecal and GIT content while the biochemical and hematological parameters were evaluated via whole blood samples following standard procedures. This study showed a decrease in hemoglobin, red blood cell count, white blood cell count, granulocyte, mean corpuscular hemoglobin concentration, platelet count, and mean platelet volume when compared to the Control Group. Coconut oil (0.5ml/kg body weight) had higher haematinic potency without posing a threat to the hepatocytes. There was no significant change in Na+, K+, Cl-, Cr, and HCO3 and a significant decrease in CHO, HDL, and LDL when compared to the control. However, the K+ concentration in the test Wistar rats showed a significant increase. The study shows that coconut oil has a vasodilator action on the aorta and the lipid profile was not affected. Hence, coconut oil could serve as an alternative haematinic agent.



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## Introduction

Natural products including medicinal plants, herbs, and spices, and their different constituents are normally considered safe due to their use with minimal detrimental impact [1]. Based on this fact, plants extract and essential oils have been used as alternatives to antibiotics because of their antimicrobial activities and favorable health benefits on the gastrointestinal system [2]. Coconut oil plays a very vital role in our daily diet as it is an important physiologically functional food with numerous health benefits that have been recognized in many parts of the world [3].

The microbiome displays a symbiotic relationship, contributes to the extraction of energy from food, synthesis of vitamins and amino acids, and helps forms barriers against pathogens and is considered essential for the maintenance of human health [4]. However, microbial community compositions vary over a human lifetime and geographic locations [5].

Also, the human gastrointestinal microbiome has been the major focus of studies as it contains the vast majority of microbial biomass [6] and can easily be sampled by the collection of fecal material. Additionally, it is involved in digestion, metabolite production, and cross-talk with the immune system [7] and has been implicated in various disease processes [8].

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The disruption of the gastrointestinal microbiome homeostasis called dysbiosis has been associated with inflammatory bowel disease (IBD) [9] irritable bowel syndrome (IBS) [10], Celiac disease, food allergies, type 1 diabetes [11], type 2 diabetes, cancer, and cardiovascular disease. Dysbiosis can be caused by environmental factors commonly encountered in societies, including diet, the standard of hygiene, and contamination of food and water [12].
Coconut oil has recently been the object of study and has been shown to possess a broad spectrum of antimicrobial properties [13]. Also, it has been found to contain bad fat alongside animal fat due to its saturated fatty properties. In contrast, it also possesses a unique liquid profile with proven health and heamatological benefits. These benefits include antifungal, antioxidant, and anti-inflammatory properties. However, one of the biggest problems facing modern society is the overuse of antibiotics. This problem has led to an interest in researching the use of natural products such as coconut oil to enhance the treatment of infectious diseases and boost the immune system. This research is to ascertain the effect of oral administration of fresh coconut oil on the gastrointestinal microbiome of Wistar rats and provide a better understanding of the biochemical/hematological parameters as well as the extraction method that will retain its essential components.

## Materials and Methods

*Materials*
Fresh coconut oil, blending machine**(**SBM-2977, OSAKA, JAPAN), measuring cylinder, feeding troughs, dissecting kit and board, top loading balance, needles and syringe, swap sticks, wooden cages, Petri dishes, lead acetate paper, beakers, aluminum foil, autoclave, test tubes, test tube racks, slides, lithium heparin, EDTA tubes, McCartney bottles, Nutrient agar, Nutrient broth, peptone water, sterile water, D-Glucose, sucrose (kermel), lactose, Simmons Citrate Agar(Titan Biotech),Urea broth, hydrogen perioxide, Kovac's reagent ,crystal violet, Lugol's iodine, alcohol, saffranin, immersion oil, Phenol red, broth of cultured organisms.

*Procurement and Preparation of Coconut Oil*
*Animal Procurement*
Ten (10) adult Wistar rats of comparable age weighing between 94grams-146grams were used for the study. The animals were housed in wooden cages in the animal house and maintained in standard laboratory conditions of room temperature and relative humidity with a 12hours light/dark cycle with adequate ventilation for the duration of the experiment.
The animals were allowed for 2 weeks for acclimatization and during this process, they were given full access to food and water before being randomly divided into 2 (two) groups, namely
**Group 1:** (normal control) without coconut oil but were fed with food and water.
**Group 2:** (Test) was given 0.5ml/kg of coconut oil and also fed with food and water.

Fresh coconut oil was obtained from fresh and matured coconuts. Fresh coconut oil was extracted using the wet extraction methods proposed by Nevin and Rajamohan [14].

*Determination of Weight*
The weight of the Wistar rat was obtained by placing them on an analytical weighing balance. The weights obtained were recorded accordingly for both the control and test. It was carried out before and after the administration of the coconut oil.

*Preparation of Nutrient Broth*
The Nutrient broth (Titan Biotech Ltd Delhi, India) was prepared by dissolving 1.3g of nutrient broth into 100ml of distilled water. Thereafter, 10ml of nutrient broth was transferred into 4 labeled McCartney bottles. The bottles were sterilized by autoclaving at 15psi (121℃ ) for 15 minutes. It was allowed to cool at room temperature.
The fecal matter obtained from the Wistar rats (both control and test) was swabbed and it was inoculated into sterilized nutrient broth bottles. The broth bottles were incubated at 37℃ for 24 hours.

*Bacterial Count*
*Procedure for Serial Dilution*
Peptone water was prepared by dissolving 1.5g of peptone water in 100ml of distilled water. It was transferred into McCartney bottles and was sterilized by autoclaving at 15psi(121℃) for 15 minutes. Then, 9ml of the sterilized peptone water was transferred aseptically into 14 test tubes (7 test tubes labeled test and 7 test tubes labeled control).
Then 1ml of broth organism suspension was placed into the first test tube (T1). This was carried out serially until the last test tube (T7). Then, 1ml was discarded from the last test tube (T7). This procedure was repeated for test tubes labeled control

*Preparation of Culture Media*
*Preparation of MacConky Agar for Spread Plate Technique*
Approximately 4.7g of MacConkey agar (Titan Biotech Ltd, Delhi, India) was weighed accurately and dissolved in 100ml of distilled water. It was sterilized by autoclaving at 15psi (121℃) for 15 minutes. Ten (10ml) of the MacConkey agar was

measured and transferred aseptically into 4 Petri dishes, it was rocked properly for even distribution and was allowed to solidify.

About 0.1ml of the broth organism medium from the last test tube was measured and placed into the solidified MacConkey agar. A sterile glass rod was used to spread the organism on the agar plate

*Preparation of Nutrient Agar for Spread Plate Technique*

2.8g of Nutrient agar was weighed and dissolved in 100ml of distilled water. It was sterilized by autoclaving at 15psi (121℃) for 15 minutes. The same procedure for the spread plate was carried out and the agar plates were incubated at 37℃ for 24 hours.

*Preparation of Agar Slants*

Nutrient agar was prepared by dissolving 2.8g of nutrient agar in 100ml of distilled water and it was sterilized by autoclaving at 15psi (121℃) for 15 minutes.

Then, 10ml of nutrient agar was transferred aseptically into 4 sterile McCartney bottles which were placed in a slant position. It was allowed to cool and solidify at that angle. The slants were inoculated with the broth organism suspension and it was incubated at 37℃ for 24 hours.

*Haematological Evaluation*

It was determined using the Sysmex® Automated Hematology Analyzer Kx-2IN, Sysmex Corporation, Kobe-Japan.

*Fecal Matter Collection and Evaluation after Administration of Coconut Oil*

After the administration of the coconut oil, the fecal matter obtained from the Wistar rats (both control and test) was swabbed and it was inoculated into sterilized nutrient broth bottles. The broth bottles were incubated at 37℃ for 24 hours. Thereafter, the procedure for the viable bacterial count was appropriately followed and the labeled agar slants were inoculated with the broth organism suspension and it was incubated at 37℃ for 24 hours

*Gastrointestinal Tract Sample Collection and Evaluation*

The gastrointestinal tract (large intestine) of Wistar rats was swabbed and inoculated into sterilized nutrient broth bottles. The bottles were incubated at 37℃ for 24 hours and viable bacterial counts were done as described by Enwa *et al.* [15].

*Biochemical Test*

All biochemical analyses were done following the methods adopted by Enwa *et al.* [16].

**Results and Discussion**

**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 0.5ml/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

**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 0.5ml/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

**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

**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 |

Natural products are of great importance to the health of individuals and communities and their medicinal values lie in some chemical substances that produce definite physiological actions in the human body [17].

This study shows a significantly different (P<0.05) in the body weight of treated and control Wistar rats **(Table 1)**. This is due to the decrease in Low-Density Lipoprotein (LDL) in the body weight of treated Wistar rats with significant change when compared to the control since high LDL results in to increase in weight leading to obesity. There is a direct association between body weight the cardiovascular risk factor, including high cholesterol. This means that as body weight decreases, so do LDL (bad fat) cholesterol decrease, an indication that coconut oil could be useful in weight management. These findings are in accordance with Nevin *et al.* [18] that coconut oil promotes weight loss, and improves digestion and hypoglycemic effects.

It was observed that there were significant decreases in the hemoglobin concentration of rats in the experimental groups when compared with the control **(Table 2)**. Coconut oil contains substantial amounts of essential amino acids and iron. The decrease in the hemoglobin concentration could be attributed to the presence of these amino acids and low iron content. Iron is an important component of hemoglobin and functions in the transport of oxygen to cells and tissues. This was found to be in line

with the works of Javadifar *et al.* [19] who incorporated coconut oil into infant weaning foods and observed a significant effect on the iron status.

There was a significant decrease in White blood cells which implies that reduced white blood cells will reduce the risk of heart attack as there is a correlation between White blood cells and heart attack as earlier reported by Mandl *et al.* [20]. An increase in white blood cells causes clogged or blocked arteries and results in coronary heart disease, ischemic stroke, and mortality from cardiovascular disease. Thus, its reduction enhances blood flow and reduces the risk of cardiovascular diseases. It is worthy of note that the reduction of white blood cells fell within the normal range of 4-11× $10^9$/L. This may also implies that the coconut oil extract promotes free flow of blood and exhibit stimulatory effect on the lymphocytes which are the main effectors cells of the immune system [21].

The administration of the coconut oil extract produced a significant decrease in the red blood cell (RBC) counts after the 7 days of treatment when compared with control groups. The reduction in the RBC count may be a result of the decrease in the iron content when the extract was given. It also means that the extracts when given acted antagonistically and lysed the membranes of the RBCs, leading to the observed reduction.

There was a significant decrease in platelet counts of the treated animals when compared to the control group and was observed to be time and dose-dependent. This shows that the extracts harmed the platelet counts of the animals. The outcome was the inhibition of the release of thrombopoietin, a regulator of thrombopoiesis, or as a result of the inhibition of vitamin k, which is an important factor in the blood coagulation process. A reduction in platelet count could reduce the ability of the blood to clot [21].

The Wistar rats treated with coconut oil showed a significant decrease ($P < 0.05$) in total cholesterol, triglyceride, and LDL level but showed a significant increase ($P < 0.05$) in HDL when compared to the control group **(Table 3)**. Eshiet *et al*. [22] had earlier used total cholesterol, LDL, high-density lipoprotein, and triglycerides to assess the risk of coronary heart disease. He opined that a significant reduction in LDL which is the bad cholesterol could cause atherosclerosis and increases the risk of cardiovascular diseases because it forms plaque deposit on the arteries and increment in HDL.

Surarong [23] has shown that coconut oil can reduce atherosclerosis which in turn reduces cardiovascular disease and improves the maintenance crew for the inner walls (endothelium) of the blood vessels as a result of a marginal increase in HDL.

In this work, potassium and sodium were measured and there was no significant difference ($P > 0.05$) in the sodium level but potassium concentration increased significantly ($P < 0.05$). Sodium is associated with blood pressure and in many hypertensive patients; a reduction in sodium intake lowers blood pressure. On the other hand, potassium, which is in the intra-cellular fluid, has been reported to be among the protective electrolytes against hypertension [24].

The various bacteria isolated and identified for pre-treated and post-treated Wistar rats were Aeromonas *hydrophila, Enterobacter Cloacae, Enterobacter aerogenes, Aeromonas faecalis, Proteus spp., Pseudomonas aeruginosa, Aeromonas hydrophila, and proteus vulgaris* respectively **(Tables 4-6)**. This indicates that coconut oil has potent antimicrobial properties that would have reduced the high bacteria microbiome to a low level after administration of the extract.

The microbial colony count of the fecal matter showed high values of bacterial load before the administration of coconut oil, but significantly reduced after administration of the extract.

## Conclusion

Conclusively, this work showed that coconut oil can be used for weight management, especially in cases of the obese for reduction of LDL which in turn will decrease the risk of cardiovascular disease.

Also, the consumption of coconut oil can influence different hematological parameters, especially the white blood cell which reduces the risk of heart disease like heart attack and can decrease the microbial load because of its antimicrobial activities. Therefore, coconut oil is efficacious in weight management, as an antimicrobial agent, haematinic agent (haemo-protective), and immune protective.

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**Ethics statement:** Ethical approval was obtained from the Faculty of Pharmacy Ethical Committee. Approval reference number- PHC/PMB/ 2021/07/11.

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