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Effects of *Theobroma cacao* Pod Husk Extract on the Body Weight, Prothrombin Time and Fasting Blood Glucose of *Staphylococcus aureus*-induced Albino Rats

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Abstract

The increasing burden of *Staphylococcus aureus* related skin diseases, has driven interest in plant-derived compounds with therapeutic potential. *Theobroma cacao* pod husk is an agricultural by-product which has been traditionally explored for medicinal purposes. This study evaluated the effect of *Theobroma cacao* pod husk extract on the body weight, prothrombin time, and the fasting blood glucose in *Staphylococcus aureus*–induced albino rats. Amelonado cocoa pods were processed as ash and blend powder, then extracted with 70% ethanol at room temperature. Thirty-six albino rats were divided into four groups viz: treatment only group, infected and treated group, infection only group and control group respectively. Infection was induced using *Staphylococcus aureus*, after which varying concentrations of the extract were topically administered for consecutive 5 (five) weeks. A marked elevation in the body weight of the treatment only group (142.33±0.15) was observed, when compared with the infection only group (120.10±0.36). There was a significant decrease in prothrombin time at higher concentrations of treatment only group (63.40 ± 0.56) when compared to the control group (76.60 ± 0.35). Additionally, a significant reduction in the fasting blood glucose levels of the treated only group when compared to the infection only group (91.67 ± 0.12) was observed. These findings therefore suggest that the *Theobroma cacao* pod husk extract possess hematological- associated effects in *Staphylococcus aureus*–induced skin conditions.

Keywords: *Theobroma cacao* pod husk, *Staphylococcus aureus*, Prothrombin time, Fasting blood glucose

INTRODUCTION

The major contributor to global morbidity, especially in low and middle income countries are infectious diseases. One of the most prevalent pathogenic bacteria with the potential to cause diverse infections, is *Staphylococcus aureus* (SA). SA is Gram-positive bacteria with the potential of inducing severe infections like skin abscesses, bacteremia, pneumonia, heart valve issues, and bone infections in both humans and animals (Ghali-Mohammed *et al*., 2023). It is a type of pathogen that commonly resides on the skin, in the nares, and on the mucous membranes of healthy people. Ghalehnoo, (2018) reported that SA can cause various illnesses through either suppurative (pus-forming) or nonsuppurative (toxin-mediated) processes. According to Su *et al.,* (2023), SA is the main pathogen causing skin and soft tissue infections like pustules, folliculitis, boils, and abscesses. It ranks among the leading causes of

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human infection worldwide and easily gains antimicrobial resistance by mutation or by transferring genetic material from other bacteria (Linz *et al*., 2023).

SA infections can trigger inflammation that affects metabolism and blood clotting as well as impacting body weight, blood sugar, and clotting factors. The most common pathogen isolated in skin-and-soft-tissue infections is SA (Akinduti *et al*., 2022) and the infections caused are typically treated with a range of pharmaceutical products. However, there is a need for a natural, affordable and effective solution with minimal side effects To therefore address the infection-induced metabolic and hematological unbalancing, a search for therapeutic agents majorly from plant source is paramount.

It was reported by Rahayu *et al*. (2023), that herbal medications are endorsed by the World Health Organization for their natural health benefits. Plant-derived products are gaining attention as alternative or complementary therapies because of their bioactive compounds, accessibility and affordability. Approximately 422,000 flowering plant species have been documented globally, with over 50,000 of them employed in traditional and modern medicine (Uniyal *et al.,* 2006). Products derived from medicinal plants are regarded as natural, with a long history of use serving as evidence of their safety (Madireddy *et al.,* 2022).

Theobroma cacao L., also known as cocoa, is a tropical plant and its beans have been investigated for its enormous pharmacological properties such as anti-inflammatory, antidiabetic and cardiometabolic properties. Cocoa contains biologically active substances that may fight microbes like viruses, bacteria, and fungi (Nsor-Atindana *et al.,* 2012). Comprising about 75.52% of the fresh weight of the cocoa pod, the husk forms the outer layer and is a major by-product of cocoa cultivation (Putu *et al.,* 2023). As cocoa production rises each year, the amount of cocoa pod husk waste also increases. Due to paucity of information on the optimal utilisation of cocoa pod husk, there is a need to turn cocoa pod husk waste into value that benefits humans and cuts down on waste. Research by Akinduro *et al.* (2022) showed that cocoa by-products have significant potential as an alternative feed source. Fideles *et al.* (2023), also reported that cocoa husk is rich in fiber, supporting intestinal function, improving lipid profiles, and contributing to cardiovascular health.

Body weight, prothrombin time and Fasting blood glucose are study biomarkers necessary to provide critical insights into the physiologic, metabolic and coagulation parameters as evidence supporting the potential role of *Theobroma cacao* pod husk extract in the management of SA-induced infection. Changes in the biomarkers reflect the effects of both infection and therapeutic agents on the albino rats. This study therefore evaluates the effects of *Theobroma cacao* (cocoa) pod husk extract on the body weight, prothrombin time and fasting blood glucose (FBG) of *Staphylococcus aureus*-induced albino rats.

MATERIALS AND METHODS

Sample Collection

An isolate of *Staphylococcus aureus*, obtained from the skin infection of a primary school pupil was used for this study. The isolate was identified via VITEK-2 system and which showed resistance to Gentamycin, Ciprofloxacin and Nitrofurantoin respectively.

Ethical Issues

Ethical approvals were obtained for this study from Health Research and Ethical Committee (HREC) of Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State (OOUTH/HREC/287/2019AP).

Preparation of *Cocoa* Pod Husk Extract (CPH Extract)

Amelonado variety of cocoa was purchased from a local farmer in Ogun State and it was identified at Cocoa Research Institute (CRIN), Ibadan, by Dr Sobowale. The pods husks were separated and air-dried at room temperature. The pod husks were further divided into two portions: the first portion was burnt to ashes (CPHa) while the second portion was blended into powder (CPHb). Burning was carried out using firewood under a clean pot containing the pods. This procedure reflects the local method employed by respondents interviewed for this

study, who use cocoa pod ash in managing skin infections, while the blending was done using a clean grinding machine. The ashes were macerated in 70% ethanol (CPHaE) while the blended powder was separately macerated in 70% ethanol (CPHbE). The ethanol extraction was carried out for 72hrs and filtration process was done using Whatman filter paper. The resulting filtrate was evaporated to dryness using a rotary evaporator. The extracts were then weighed and stored in sterile containers with desiccant packs to preserve potency.

In Vivo evaluation of *cocoa* pod husk efficacy in Albino rats

A modified version of the method described by Sari *et al.,* (2023) was employed in this study. The modification involved shaving the lateral skin of the animals to remove fur, prior to topical application of the extract. Thirty-six (36) Swiss albino rats (8weeks old) of both sexes, weighing averagely 121kg were grouped into four (4) as Group A (treatment only), Group B (Infection and treatment), Group C (Infection only) and Group D (Control). Group A rats were administered only with varying concentrations (0%, 12.5%, 25%, 50% & 100%) of cocoa pod husk, group B rats were infected with *Staphylococcus aureus* and treated with varying concentrations (0%, 12.5%, 25%, 50% & 100%) of cocoa pod husk, group C rats were only infected with *Staphylococcus aureus,* while group D rats were not infected or treated with cocoa pod husk. The albino rats were acclimatized for a week under suitable condition and their weights were monitored weekly. *Staphylococcus aureus* was inoculated through skin route using standard practices. A side of the rat was shaved with sterile shaving stick and the broth culture of Staphylococcus *aureus* was rubbed on the shaved portion using a sterile cotton swab. The care, use, and procedures performed on these rats are done by professionals, which is in accordance with standard practice as compiled by European Parliament and of the Council on the protection of animals used for scientific purposes (European Parliament and of the Council, 2010). The manifestation of infection on the albino rats was examined at 48hrs interval on days 3, 5, and 7 respectively.

Prior the treatment, the inoculated skin portion were subcultured on Mannitol Salt Agar (MSA) after which the treatment commenced with the topical application of cocoa pod husk and cream base formulated at different concentrations viz: 100% (i.e Cocoa Pod husk extract only), 50% (i.e Cocoa Pod husk extract and cream base 50:50), 25% (i.e Cocoa Pod husk extract and cream base 25:75), 12.5% (i.e Cocoa Pod husk extract and cream base 12.5:85.5), and 0% (i.e 100% cream base) for 5 consecutive weeks. Blood samples were obtained by pricking the rat tails to collect blood for prothrombin time test while fasting blood glucose test was assessed using a glucometer.

Data analysis

Each of the laboratory analyses was replicated in triplicate and statistical analysis were performed using Statistical Package for Social Sciences (SPSS) version 20. Multivariate analysis of variance was also used to study interactions between treatment and dose on prothrombin time and Fasting Blood Glucose and mixed model was used to determine the effect of treatment, dose on the weight of the animals over weeks. The results were expressed as Mean $\pm$ SD and values of $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Results

Table 1 compared the mean body weights between albino rats treated with various concentrations of cocoa pod husk ash extract (CPHaE) and control rats. At week 5, a significant increase in the mean body weight of the albino rats of the treatment only group were observed at 0% (131.57 ± 0.76), 12.5% (135.80 ± 0.17), 25% (139.03 ± 0.15), 50% (140.77 ± 0.23) and 100% (142.33 ± 0.15) respectively when compared with 120.10 ± 0.36 (infection only group). A statistically significant difference ($p< 0.05$) in the mean body weight of the albino rats of the infected and treatment group at 0% (124.87 ± 0.21), 12.5% (126.47 ± 0.55), 25% (127.30 ± 0.46), 50% (127.43 ± 0.50) and 100%

(128.90 ± 0.50) was observed when compared with 120.10 ± 0.36 (infection only) and 137.17 ± 0.46 (control). Furthermore, a significant increase was observed in the mean body weight of the albino rats in the control group (137.17± 0.46) as against the infection only group (120.10 ± 0.36) ($p< 0.05$). There was significant change ($p< 0.05$) in the weight of the animals over time (weeks). Also, there was significant interaction effect of dose and infection on the body weight of the animals.

Table 2 compared the mean body weights between albino rats treated with various concentrations of cocoa pod husk blend extract (CPHbE) and control rats. At the end of this study, an increase in the mean body weight of the albino rats of the treatment only group were observed at 0% (126.60 ± 0.10), 12.5% (127.77 ± 0.21), 25% (128.80 ± 0.10), 50% (129.63 ± 0.06) and 100% (132.00 ± 0.00) respectively when compared with 120.10 ± 0.36 (infection only group). Also, there was a significant difference in the mean body weight of the albino rats of the infected and treatment group at 0% (124.07 ± 0.12), 12.5% (124.93 ± 0.06), 25% (125.40 ± 0.26), 50% (126.07 ± 0.15) and 100% (136.47 ± 0.06) respectively when compared with 120.10 ± 0.36 (infection only) and 137.17 ± 0.46 (control) ($p<0.05$). The result showed that concentration of the extract has significant effect on the weight of the animals over time. Furthermore, there was significant interaction effect between treatment groups and weight of the animals over time (weeks).

Table 3 showed the comparison between the mean prothrombin time of cocoa pod husk extract–treated albino rats and control rats. A statistically significant decrease ($p <0.05$) was observed in the mean prothrombin time of albino rats in the treatment only group, when comparing CPHaE and CPHbE at varying concentrations with the infection only (91.17 ± 0.31) group. Albino rats in the infected and treatment group exhibited significant difference in the prothrombin time ($p<0.05$), when comparing CPHaE and CPHbE at varying concentrations with the control (76.60 ± 0.35) group. Furthermore, there was a significant difference between the mean prothrombin time of infected only group (91.17 ± 0.31) and control group (76.60 ± 0.35). Furthermore, there was significant interaction effect between treatment groups and concentration on Prothrombin time

Table 4 presents the comparison of mean fasting blood glucose (FBG) between cocoa pod husk extract–treated albino rats and control rats. There was a significant decrease in the mean FBG of albino rats at varying concentrations of CPHaE and CPHbE in the treated only group when compared with the infected only (91.67 ± 0.12) group. A significant difference was observed in the infected and treatment group at varying treatment concentrations of CPHaE and CPHbE when compared to the control (85.03 ± 0.55). However, there was a statistically significant difference between the mean FBG of infected only group (91.67 ± 0.12) and control group (85.03 ± 0.55). Also, there was significant interaction effect between treatment groups and concentrations on Fasting blood glucose.

Table 1. Comparison of Mean Body Weights Between Albino Rats Treated with Cocoa Pod Ash Husk Extract (CPHaE) and Control Rats.

Groups	Treatment Concentration (%)	Weight (kg) Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
Treatment Only	0	120.77±0.15^{ab}	121.80±0.26^{ab}	123.40±0.60^{b}	125.60 ± 0.20^{ef}	128.43 ± 0.47^{f}	131.57 ± 0.76^{f}
	12.5	121.23±0.6^{abc}	122.67±0.25^{bcd}	125.00±0.10^{c}	127.87±0.46^{g}	131.47±0.66^{g}	135.80±0.17^{g}
	25	121.47±0.49^{bcd}	123.43±0.59^{de}	126.17±0.29^{d}	129.57±0.58^{h}	134.00±0.26^{h}	139.03±0.15^{i}
	50	122.43±0.40^{ef}	124.57±0.58^{fg}	127.27±0.35^{e}	130.87±0.65^{i}	135.63±0.67^{i}	140.77±0.23^{j}
	100	122.77±0.15^{f}	125.33±0.70^{g}	128.07±0.15^{f}	131.87±0.15^{j}	136.77±0.81^{j}	142.33±0.15^{k}
Infected And Treated	0	120.40±0.52^{a}	121.43±0.46^{a}	122.53±0.45^{a}	123.47±0.47^{b}	124.23±0.25^{b}	124.87±0.21^{b}
	12.5	120.60±0.90^{ab}	121.60±0.36^{a}	122.67±0.23^{a}	123.90±0.40^{bc}	125.20±0.30^{c}	126.47±0.55^{c}
	25	121.30±0.26^{abc}	122.30±0.44^{abc}	123.43±0.70^{b}	124.57±0.49^{cd}	125.90±0.44^{cd}	127.30±0.46^{d}
	50	121.73±0.25^{cdc}	122.67±0.40^{bcd}	123.80±0.26^{b}	125.03±0.76^{de}	126.30±0.66^{d}	127.43±0.50^{d}
	100	122.47±0.49^{ef}	123.53±0.55^{de}	124.70±0.26^{c}	126.03±0.15^{f}	127.37±0.45^{e}	128.90±0.50^{e}
Infection Only		121.10±0.17^{abc}	123.10±0.85^{cd}	122.50±0.36^{a}	121.90±0.20^{a}	121.00±0.30^{a}	120.10±0.36^{a}
Control		122.23±0.57^{def}	124.17±0.42^{ef}	128.47±0.49^{f}	131.00±0.56^{i}	133.90±0.10^{h}	137.17±0.46^{h}

Columns with values bearing different superscript are significantly different $p<0.05$

Table 2. Comparison of Mean Body Weights between Albino Rats Treated with Cocoa Pod Husk Blend Extract (CPHbE) and Control Rats.

Groups	Treatment Concentration (%)	Weight (kg)					
		Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
Treatment Only	0	119.50±0.26^{a}	120.27±0.06^{a}	121.27±0.32^{a}	122.57±0.29^{bc}	124.37±0.40^{c}	126.60 ± 0.10^{f}
	12.5	120.03±0.06^{b}	121.00±0.00^{bc}	122.23±0.06^{b}	122.57±0.15^{bc}	125.40±0.26^{d}	127.77 ± 0.21^{g}
	25	120.30±0.10^{bc}	121.50±0.26^{cd}	122.93±0.15^{cd}	124.53±0.06^{e}	126.37±0.57^{e}	128.80 ± 0.10^{h}
	50	120.79±0.17^{de}	122.10±0.10^{e}	123.63±0.15^{e}	125.23±0.06^{f}	127.10±0.20^{f}	129.63 ± 0.06^{i}
	100	121.23±0.11^{f}	122.67±0.25^{fg}	124.57±0.58^{f}	126.87±0.06^{g}	129.40±0.44^{g}	132.00 ± 0.00^{j}
Infected And Treated	0	120.03±0.06^{b}	120.67±0.12^{ab}	121.50±0.10^{a}	122.30±0.52^{ab}	123.23±0.31^{b}	124.07 ± 0.12^{b}
	12.5	120.57±0.06^{cd}	121.30±0.20^{cd}	122.10±0.10^{b}	123.00±0.00^{c}	123.87±0.21^{c}	124.93 ± 0.06^{c}
	25	121.00±0.00^{ef}	121.77±0.15^{de}	122.60±0.10^{bc}	123.53±0.55^{d}	124.40±0.23^{c}	125.40 ± 0.26^{d}
	50	121.30±0.17^{f}	122.23±0.06^{ef}	123.10±0.10^{d}	124.07±0.23^{e}	125.07±0.21^{d}	126.07 ± 0.15^{e}
	100	121.90±0.26^{g}	122.87±0.06^{g}	132.87±0.06^{h}	134.00±0.10^{i}	135.37±0.42^{i}	136.47 ± 0.06^{k}
Infection Only		121.10±0.17^{ef}	123.10±0.85^{g}	122.50±0.36^{bc}	121.90±0.20^{a}	121.00 ± 0.30^{a}	120.10 ± 0.36^{a}
Control		122.23±0.57^{g}	124.17±0.42^{f}	128.47±0.49^{g}	131.00±0.56^{h}	133.90 ± 0.10^{h}	137.17 ± 0.46^{l}

Columns with values bearing different superscript are significantly different $p<0.05$

Table 3. Comparison of Mean Prothrombin Time between Cocoa Pod Husk Extract–Treated Albino Rats and Control Rats.

Groups	Cocoa pod extract concentrations (%)	Prothrombin time (secs) Mean ± SEM CPHaE	CPHbE
Treatment Only	0	77.40 ± 0.44^{e}	77.90 ± 0.20^{e}
	12.5	76.10 ± 0.20^{d}	76.80 ± 0.10^{d}
	25	72.77 ± 0.23^{c}	74.87 ± 0.31^{c}
	50	68.30 ± 0.36^{b}	71.50 ± 0.17^{b}
	100	63.40 ± 0.56^{a}	67.33 ± 0.21^{a}
Infected and treated	0	89.37 ± 0.42^{j}	89.67 ± 0.15^{i}
	12.5	88.57 ± 0.35^{i}	89.40 ± 0.17^{i}
	25	87.47 ± 0.90^{h}	88.87 ± 0.15^{h}
	50	86.07 ± 0.25^{g}	88.30 ± 0.26^{g}
	100	84.17 ± 0.21^{f}	87.40 ± 0.60^{f}
Infection only		91.17 ± 0.31^{jk}	
Control		76.60 ± 0.35^{d}	

Columns with values bearing different superscript are significantly different $p<0.05$

Table 4. Comparison of Mean Fasting Blood Glucose (FBG) Between Cocoa Pod Husk Extract– Treated Albino Rats and Control Rats.

Groups	**Cocoa pod extract concentrations (%)**	**FBG (mg/dl) Mean ± SEM CPHaE**	**CPHbE**
Treatment Only	0	80.67 ± 0.06^{e}	80.47 ± 0.06^{e}
	12.5	79.20 ± 0.26^{d}	79.33 ± 0.06^{d}
	25	77.47 ± 0.49^{c}	78.10 ± 0.10^{c}
	50	75.70 ± 0.10^{b}	76.77 ± 0.15^{b}
	100	73.57 ± 0.12^{a}	75.33 ± 0.38^{a}
Infected And Treated	0	91.12 ± 0.25^{j}	91.37 ± 0.25^{k}
	12.5	89.77 ± 0.06^{i}	90.63 ± 0.06^{j}
	25	88.43 ± 0.31^{h}	89.80 ± 0.00^{i}
	50	86.87 ± 0.12^{g}	88.80 ± 0.10^{h}
	100	85.33 ± 0.06^{f}	87.60 ± 0.30^{g}
Infection only		91.67 ± 0.12^{k}	
Control		85.03 ± 0.55^{f}	

Columns with values bearing different superscript are significantly different $p<0.05$

Discussion

This study investigated the effects of *Theobroma cacao* pod husk extract on body weight, prothrombin time, and fasting blood glucose (FBG) in *Staphylococcus aureus*–induced albino rats. A significant change in the weight of the animals in the respective groups, over the weeks was observed. A shorter prothrombin time was observed in infected and treated group when compared with infection only. However, the FBG levels reduced significantly in treatment only group when compared with control while the FBG levels increased in infected and treated group.

The Albino rats treated with varying concentrations of cocoa pod husk extract showed dose-treatment variations in total body weight when compared with the infected untreated and control groups respectively. This disagrees with Madireddy *et al.,* (2022), who reported no treatment-related changes in the body weight of LN18178-administered rats. The disparity might be subject to combined composition of the extracts at 3.5% *Punica granatum* fruit rind and 0.5% *Theobroma cacao* seed. LN18178 (Tesnor®) is a standardized, proprietary blend of aqueous ethanol extracts derived from *Punica granatum* fruit rind and *Theobroma cacao* seeds.

Moreover, the increase in the body weight observed in the albino rats infected with *Staphylococcus aureus* and treated with varying concentrations of cocoa pod husk, suggests that the extract may help mitigate some effects of infection on body weight. The albino rats treated with Amelonado ash extract exhibited more significant changes in body weight compared to those treated with Amelonado blend extract. This might possibly due to the presence of oxides formed during the burning process. Furthermore, this result is in consonance with the study of Sukanto *et al.,* (2024) who reported that the ethanol extract gel of cocoa pod husk enhanced the number of gingival fibroblasts in *P. gingivalis*-induced rats.

Cocoa pod husk contains bioactive constituents such as flavonoids and phenolic compounds (Rahayu *et al*., 2023) which have been reported to influence endothelial function, platelet aggregation as well as the coagulation factor activity. This study reported a significant dose-dependent reduction in the prothrombin time of the albino rats treated only with varying concentrations of cocoa pod husk extract. A shorter prothrombin time observed at higher concentrations of the extract, suggests that the extract may influence the blood coagulation process. However, further studies are needed to confirm any effect on inflammation-mediated coagulation abnormalities.

Inflammation may develop quickly and is subsequently followed by tissue regeneration, a process that involves the proliferation of fibroblast (Cialdai *et al.,* 2022). According to Sukanto *et al.,* (2024), cocoa pod skin is rich in flavonoids, saponins, tannins, and alkaloids, compounds that may enhance the quality of connective tissue and epithelium during wound healing. In the course of wound healing, fibroblast proliferation is triggered, leading to increased collagen production. Based on the report of Diller and Tabor (2022), fibroblasts play a key role in collagen synthesis. As the main component of the extracellular matrix, collagen imparts strength to scar tissue during wound repair. In addition, tannins in cocoa pod skin contribute to hemostasis and the healing of mucous membranes. Akinrotohun *et al.,* (2021) also reported that selected formulations of cocoa pod husk ash extract (B and C) exhibited inhibitive activities against proliferation of the skin commensal, *S. aureus*. The formulation was used as topical application for the control of shaving bump.

From this study, a significant reduction in the FBG levels was observed in the albino rats. The reduction displayed a possible role of *Theobroma cacao* pod husk extract in improving the glucose regulation under inflammatory conditions. According to Putu *et al.*, (2023), cocoa pod shell contains saponins which can reduce blood glucose levels by inhibiting enzymes that break down disaccharides into monosaccharides. Hence, administration of *Theobroma cacao* pod husk extract was associated with a significant decrease in FBG levels in the treated albino rats.

CONCLUSION

This study showed that *Theobroma cacao* pod husk extract exerted its effects on the body weight, prothrombin time and Fasting Blood Glucose (FBG) parameters of *Staphylococcus aureus*-induced albino rats. The findings from this study are in consonance with the fact that the underutilized agricultural by-product, cocoa pod husk, possesses biologically active compounds that may influence metabolic and hematological functions.

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