RESEARCH ARTICLE

ISSN: 0331 - 670X

https://doi.org/10.51412/psnnjp.2023.26

The Nigerian Journal of PHARMACY

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THE OFFICIAL ORGAN OF THE PHARMACEUTICAL SOCIETY OF NIGERIA FOUNDED 1927

Nutrient, antinutrient and antimicrobial properties of Telfairia Occidentalis pod and biochemical response in experimentally compromised rats' hematology and lipid profile

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ARTICLE INFO

Article history:

Received 25 April 2023
Revised 25 May 2023
Accepted 30 June 2023
Online Published 30 September 2023

Keywords:

Monosodium glutamate,

Lipid lowering,

Blood boosting,

Dyslipidaemia,

Intoxication

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ABSTRACT

Background: Recent study indicated the prospective use of the essentially unutilized pod of *Telfairia Occidentalis* (TOP), in dietary management of monosodium glutamate, MSG, related hepatotoxicity. This study aimed to determine some minerals, vitamins, antinutrients and antimicrobial properties of TOP flour ethanol extract (TOPE) and to evaluate its hematologic and lipid profile responses against MSG intoxication in rats.

Methods: The *in vitro* determination of minerals, vitamins, anti-nutrients and antimicrobial properties of TOP by standard referenced protocols were carried out. Twenty adult male Wistar rats (120 - 175 g) randomly allotted to five groups of 4 rats each in groups A, B, C, D and E respectively received normal saline (1 mL), *via* oral gavage and daily for 14 days. The evaluation of some hematologic (red blood cell, RBC, packed cell volume, PCV and hemoglobin, HB) and lipid (triacylglycerol, TAG, low density lipoprotein cholesterol, LDL-Chol, high density lipoprotein cholesterol, HDL-Chol and total cholesterol, T-Chol) profile responses followed standard and referenced protocols.

Results: The results revealed that TOPE is composed of important bioactive minerals, vitamins and anti-nutrients mix with *in vitro* concentration-dependent antimicrobial activity. *In vivo,* TOPE compared favourably with the control and elicited a significant ($P < 0.05$) and dose-dependent mitigation of the associated dysfunctions caused by MSG intoxication in rats' hematologic and lipid profiles.

Conclusion: TOPE demonstrated dietary property and concentration-dependent antimicrobial activity. *In vivo*, TOPE improved hematologic and lipid profile functions in the rats and corrected apparent disruptions associated with MSG intoxication in rats *via* probable blood boosting and lipid lowering activities. The implications of these findings warrant follow up as they may be significant in relation to prospective application of TOPE as potential novel diet and drug for the resistance of microbial pathogens and the modulation of MSG-related disruptions in animal hematology and lipid metabolism.

1. Introduction

Dyslipidaemia and blood-related issues are major risk factors for cardiovascular diseases usually implicated with impaired hematology and lipid metabolism[1]. Human disease burden is worsened by the activities of microbial pathogens [2]. The cost implications of the management and treatment of ailments directs attention to the possible use of locally available and cheap medicinal plants [3]. *Telfairia occidentalis* (fluted Pumpkin) which belongs to the genus *Cucurbita* and family *cucubitaceae*is a common, bioactive phytochemicals-rich leafy vegetable that is widely consumed in Nigeria [4]. Fluted pumpkin leaf exerted antimicrobial, hypo-lipidemic, antianemic and improved hematological activities that were linked to its high phyto-

nutrient contents including minerals and vitamins[5]. The fruit pod of *T. occidenalis*, TOP, which contains the pumpkin seeds and constitutes about 64 % of the whole fruit weight is not utilized[6] but may be nutritionally and pharmacologically bioactive as the leaf. Recent studies emphasized on the need for detailed study on plant-based wastes *prior* to application in diets and drugs and reported the proximate properties and response of TOPE on the hepatic bioindicators and histology of MSG-compromised rats[4,,7] but none, to the knowledge of the authors reported the minerals, vitamins, antinutrients, antimicrobial, hematologic and lipid profiles of fluted pumpkin pod waste.

MSG is a popular food flavour enhancer with possibility of inadvertent consumption and abuse. It exerted varied adverse health effects in animal models which provoked plethora of studies on the possibility of natural products including *T. occidentalis* leaf extract to mitigate MSG-related effects [8]. This study aimed to determine some minerals, vitamins, anti-nutrients and antimicrobial properties of *Telfairia occidentalis* pod and the biochemical response in rats' hematology and lipid metabolism compromised by monosodium glutamate intoxication. Thus, the study objectives included the determination of minerals, vitamins anti-nutrients and antimicrobial properties of TOP and the response on the hematologic and lipid profiles of MSG burdened rats. These were acceptable indicators of nutrient, dietary, pharmacologic and metabolic status[9] needed to be assessed for a prospective food and drug or pharmafood source. Elevated serum levels of TAG, LDL- Chol but a diminished level of HDL-Chol indicated impaired lipid metabolism due to significant dyslipidaemia and inherent cardiovascular diseases[1].

2. Materials and Methods

2.1 Materials, Sample Collection, Preparation and Extraction

MSG (a product of Ajinomoto® marketed by West African Seasoning Company Limited, Nigeria) was procured while *T. Occidentalis* pod sample was collected in the month of April, 2016 from Ndioro district in Ikwuano, Abia State, Nigeria, prepared and extracted with 98 % analytical ethanol (Sigma-Aldrich, USA) as reported recently[7]. The choice of ethanol was due to its provision for wider zone of inhibition [10]. Microbial pathogens comprising six bacteria (*N. gonorrhea, E. coli, S. aureus, S. typhi, P. aeroginosa* and *S. Pneumonea*) and five fungi (*A. niger, H. capsulatum, C. albicans, P. notatum and F. spp.*) were clinical isolates obtained from the Department of Pharmaceutical Microbiology Laboratory, College of Health Sciences, University of Nigeria Teaching Hospital (UNTH) Enugu, Nigeria.

2.2 Ethical adherence and Experimental Animals and Design

The study adhered strictly to the ethical guidelines on animal use as stipulated by the National Research Council, NRC, USA. [11]. The animal study design involved random allotment (after acclimatization) of twenty adult male Wistar rats (120 - 175 g) to five groups of 4 rats. Rats in each groups A, B, C, D and E respectively received normal saline (1 mL), MSG (8000 mg/kg), TOPE (200 mg/kg), MSG (8000 mg/kg) + TOPE (200 mg/kg) and MSG (8000 mg/kg) + TOPE (400 mg/kg) *via* oral gavage and daily for 14 days. Following overnight fasting, the rats were sacrificed by cervical dislocation, and blood samples were collected by cardiac puncture into plain bottles. The blood thus collected was allowed to clot after standing for 10 minutes at ambient temperature. Thereafter, the respective serum was separated by centrifuging the coagulated blood samples at 3000 × g for 15 minutes. T-Chol, TAG, HDL-Chol, LDL-Chol, RBC, HB and PCV were determined in the serum.

2.2 Determination of minerals, vitamins and antinutrients compositions of TOPE

The content of the minerals (Zinc, Zn, sodium, Na and iron, Fe) was determined using the respective method by Association of Official Analytical Chemists[12] without any modification. The concentration of vitamins (A, C and E) was determined by the method of Pearson [13] as described recently [14] which involved soaking in appropriate medium, centrifugation and taking the absorbance against the reagent blank. Phytate was determined by measuring the absorbance of developed colour at 519 nm against the reagent blank as described by Russel [15]. Trypsin inhibitor content was determined based on the method of Prokopet and Unlenbruck[16]which involved reading the absorbance of the developed colour against the blank at 410 nm. Tannin content was determined based on Folin-Dennis colorimetric method and measuring the absorbance of developed colour at 780 nm against the regent blank set at zero as described by Pearson [13].

2.3 Determination of antimicrobial activities of TOPE

The determination of the antimicrobial activity of the eleven microbial pathogens was as reported earlier [16-20]. This was based on the disk diffusion technique using 10 mm

discs obtained as 50 mg of Ketoconazole (antifungal) and Ciprofloxacin (antibacterial) standard strains.

2.4 Determination of response of TOPE on some hematologic and lipid profiles in MSG-compromised rats' serum

The determination of RBC, HB and PCV followed the respective method by Ochei and Kolhatker[21]. Total cholesterol concentration was determined using the method of Allain *et al*. [22]. The concentration of triacylglycerol and HDL-cholesterol respectively was determined using the method of Albers *et al*. [23].

2.5 Statistical analysis

The descriptive statistics and test for significance in mean of the data were by one-way analysis of variance (ANOVA) with the statistical package for social sciences (SPSS) version 16. The Duncan's multiple range tests were used to identify the means that differ significantly at $P < 0.05$. Results were expressed as mean ± standard error of mean, SEM.

3. Results

The composition (%) in TOPE of the determined mineral was highest for sodium (406.24±0.58) followed by zinc (198.19±24.28) and iron (91.87±5.12) while that of the antioxidant vitamin was least for E (20.16±1.05) followed by A (141.37±3.51) and highest for C (343.75±3.73).

Table 1: Some minerals, vitamins and antinutrients composition of TOPE

Minerals	Zinc (198.19±24.28 %)	Iron (91.87±5.12 %)	Sodium (406.24±0.58 %)
Vitamins	Vitamin A (141.37±3.51 %)	Vitamin C (343.75±3.73 %)	Vitamin E (20.16±1.05 %)
Antinutrients	Tannin (54.92±0.54 mg/100g)	Trypsin inhibitor (4.33+0.01 µ1/mg)	Phytate (0.74+0.01 mg/100g)

Results are mean ± standard error of mean, SEM (n = triplicate determinations)

Phytate (0.74+0.01 mg/100g) was the least followed by trypsin inhibitor (4.33+0.01 µL/mg) while tannin (54.92±0.54 mg/100g) was the highest of the determined antinutrients (Table 1). The antifungal and antibacterial activity (mean diameter of inhibition zone, mm) of TOPE is depicted on Tables 2 and 3. Generally, TOPE elicited concentration dependent antimicrobial activity against the tested pathogens that, at the highest tested concentration, mostly compared favourably with the standard drugs, Ketoconazole and Ciprofloxacin.

Table 2: Antifungal property of TOPE

Pathogens	2000 mg/mL	1000 mg/mL	500 mg/mL	250 mg/mL	125 mg/mL	Ketoconazole
A.niger	21.00±0.58	11.00±0.58	6.00±0.58	2.00±0.58	0.33±0.33	34.00±0.58
H.capsulatum	16.33±0.33	7.00±0.58	3.00±0.58	0.00±0.00	0.00±0.00	26.67±0.88
C.albicans	19.00±0.58	9.00±0.58	5.33±0.33	1.33±0.33	0.00±0.00	50.00±1.15
P.notatum	11.33±0.88	5.00±0.58	1.67±0.33	0.00±0.00	0.00±0.00	28.00±1.15
Fusarium Spp	13.00±0.58	6.00±0.58	2.00±0.58	0.00±0.00	0.00±0.00	21.67±0.88

Results are mean ± standard error of mean, SEM (n = triplicate determinations)

At the highest tested concentration, TOPE caused the highest antifungal activity against *A. niger* (21.00±0.58) followed by *C. albicans* (19.00±0.58), *H. capsulatum* (16.33±0.33), *P. notatum* (11.33±0.88) and, the least, *Fusarium Spp* (13.00±0.58). TOPE elicited antifungal activity against A. niger (0.33±0.33) at the least tested concentration 125 mg/mL but against *A. niger* (2.00±0.58) and *C. albicans* (1.33±0.33) at 250 mg.mL. Similarly, at the highest tested concentration, TOPE caused the highest bacterial activity against *E. coli* (37.00±0.58) followed by *S. aureus* (31.67±0.88), *N. gonorrhea* (20.67±0.58), *S. pneumonia* (20.33±0.88), *S. typhi* (16.00±0.58) and, the least, *P. aeroginosa* (12.33±0.88). TOPE elicited antibacterial activity against E. coli (0.67±0.33) and *S. aureus* (0.33±0.33) at the least tested concentration of 125 mg/mL and showed activity against the tested bacteria except *P. aeroginosa* (0.00±0.00) at 250 mg/mL

Table 3: Antibacterial property of TOPE

Pathogens	2000 mg/mL	1000 mg/mL	500 mg/mL	250 mg/mL	125 mg/mL	Ciprofloxacin
E. coli	37.00±0.58	16.67±0.88	7.67±0.88	3.00±0.58	0.67±0.33	68.00±1.15
N. gonorrhea	20.67±0.58	10.33±0.58	4.67±0.33	1.33±0.33	0.00±0.00	64.67±0.88
S. aureus	31.67±0.88	15.00±0.58	7.00±0.58	3.00±0.58	0.33±0.33	54.00±1.15
P. a uroginosa	12.33±0.88	5.00±0.58	2.00±0.58	0.00±0.00	0.00±0.00	37.33±0.88
S. typhi	16.00±0.58	8.00±0.58	4.00±0.58	1.33±0.33	0.00±0.00	72.00±1.15
S. pneumonia	20.33±0.88	10.00±0.58	5.00±0.58	1.67±0.33	0.00±0.00	67.67±0.88

Results are mean ± standard error of mean, SEM (n = triplicate determinations)

Table 4: Response of *T. occidentalis* pod flour extract, TOPE, on some serum hematologic indicators in MSG-compromised rats

Groups	PCV (%)	HB (g/dl)	RBC ($\times10^{12}$/L)
Control	39.25 ± 0.48^{c}	18.10 ± 0.10^{d}	24.75 ± 4.79^{b}
MSG (8000 mg/kg)	26.25 ± 0.63^{a}	13.18 ± 0.20^{a}	24.50 ± 2.89^{a}
TOPE (200 mg/kg)	58.75 ± 0.48^{d}	22.40 ± 0.75^{e}	27.25 ± 4.79^{e}
MSG (8000 mg/kg) + TOPE (200 mg/kg)	32.00 ± 1.08^{b}	15.00 ± 1.51^{b}	25.25 ± 4.79^{c}
MSG (8000 mg/kg) + TOPE (400 mg/kg)	32.25 ± 0.48^{b}	17.20 ± 0.11^{c}	25.50 ± 2.89^{d}

Results represent mean± S.E.M of group serum results obtained (n = 4). Mean values in the same column having different letters of the alphabet, are statistically significant at $P < 0.05$. Control group (A), MSG group (B), TOPE (200 mg/kg) group (C), MSG + TOPE (200 mg/kg) group (D) and MSG + TOPE (400 mg/kg) group (E)

In vivo, the PCV (%), HB (g/dl) and RBC ($\times10^{12}$/L) concentration, respectively in the MSG-compromised rats (26.25±0.63, 13.18±0.20 and 245.00±2.89) decreased ($P < 0.05$) below those of the rats in the control and the other groups. These hematologic parameters in TOPE-treated rats (58.75±0.48, 22.40±0.75 and 272.50±4.79) increased ($P < 0.05$) above that of the control and others but increased ($P < 0.05$) in MSG plus TOPE rat groups compared to MSG-treated group (Table 4).

Table 5: Response of *T. occidentalis* pod flour extract, TOPE, on some serum lipid profile in MSG-compromised rats

Groups	T. Chol (mg/100mL)	TAG (mg/100mL)	HDL-Chol (mg/100mL)	LDL-Chol (mg/100mL)
Control	3.40±0.07^{e}	1.46±0.04^{d}	1.64±0.07^{e}	0.85±0.05^{a}
MSG (8000 mg/kg)	3.74±0.06^{d}	1.56±0.04^{e}	0.80±0.05^{a}	2.99±0.06^{e}
TOPE (200 mg/kg)	3.18±0.09^{a}	1.40±0.04^{b}	0.97±0.06^{d}	2.44±0.06^{d}
MSG (8000 mg/kg) + TOPE (200 mg/kg)	3.28±0.09^{c}	1.43±0.03^{c}	0.91±0.04^{c}	2.16±0.07^{b}
MSG (8000 mg/kg) + TOPE (400 mg/kg)	3.23±0.08^{b}	1.38±0.05^{a}	0.83±0.07^{b}	2.30±0.09^{c}

Results represent mean± S.E.M of group serum results obtained (n = 4). Mean values in the same column having different letters of the alphabet, are statistically significant at $P < 0.05$. Control group (A), MSG group (B), TOPE (200 mg/kg) group (C), MSG + TOPE (200 mg/kg) group (D) and MSG + TOPE (400 mg/kg) group (E)

Similarly, MSG-compromised rats had an increased ($P < 0.05$) concentration (mg/100mL) of T-Chol (3.74±0.06), TAG (1.56±0.04) and LDL-Chol (2.99±0.06) but a decreased ($P < 0.05$) HDL-Chol (0.80±0.05) compared to the other groups. The concentration of T-Chol and TAG in rat groups exposed to only TOPE or MSG plus varying doses of TOPE decreased ($P < 0.05$) below those of the control and MSG-treated rats. However, the concentration of LDL-Chol and HDL-Chol in rat groups exposed to only TOPE or MSG plus varying doses of TOPE decreased ($P < 0.05$) and increased ($P < 0.05$) respectively as compared to the MSG-treated rats (Table 5).

4. Discussion

The activities of microbial pathogens, impaired hematology and lipid metabolism are important human health risk factors with high disease burden, management cost and need for cheaper plant wastes-based pharmafood alternatives against, for instance MSG-induced metabolic dysfunctions[1-3,7]. Thus, the study determined some minerals, vitamins, anti-nutrients and antimicrobial properties of *Telfairia occidentalis* pod and the biochemical response in rats' hematology and lipid metabolism compromised by monosodium glutamate intoxication which served as acceptable indicators of nutrient, dietary, pharmacologic and metabolic status [9]. The dietary component vitamin A is essential for sight, while the antioxidant vitamins C and E and minerals zinc and iron are involved in antioxidation metabolism[24]. *T. occidentalis* pod flour extract (TOPE) is rich in these important bioactive minerals and vitamins suggesting its prospective nutraceutical relevance as a novel pharmafood source. Comparatively lower vitamin A and vitamin C contents were recorded for full fat and defatted *T. occidentalis* seed flour [25]. Generally, anti-nutrients could complex with either minerals including iron and zinc to reduce their bioavailability or protein to inhibit protein digestion [26]. Aside this shortcoming, antinutrients when consumed at low concentration could offer significant health benefits in animals, including antioxidative, and hypolipidemic effects[27,28].

Comparatively higher phytate but lower tannin contents were recorded for full fat and defatted *T. occidentalis* seed flour [25] which suggests appropriate antinutrients mix in the TOP that could not undermine the nutrient value of TOP. For instance, the phytate content reported herein (0.74+0.007) is particularly lower than that reported for cowpea (2.9), pigeon pea (2.4) and African yam beans (2.4) [29]. The content of the determined antinutrients in the TOP could be easily lowered further to negligible and non-toxic level by simple processing to offer health benefits in animals[30,31]. This implies that the determined mineral, vitamin and antinutrient mix in TOP could be bioavailable and safe for animal consumption. TOPE elicited concentration dependent antimicrobial activity against the tested pathogens that, for a crude extract, at the highest tested concentration mostly compared favorably with the standard drugs. Activity

against *E. coli* and *S. aureus* at the highest tested concentration was markedly higher while the activity against *S. typhi* and *C. albicans* compared favourably with that reported for ethanol extract of *T. occidentalis* leaves [10]. Activity against *A. niger* and *P. aeroginosa* reported herein compared with that (8.50±0.57 and 7.75±0.50, respectively) reported for methanolic extract of *T. occidentalis* leaves [32]. Thus, TOPE portends promising dietary and antimicrobial potentials required for a prospective pharmafood source.

The determined indicators of hematological function, PCV (%), HB (g/dl) and RBC ($\times 10^{12}$/L) were lowered in the MSG-compromised rats compared to control and the other rat groups. The observed reduction in these parameters was significant ($P < 0.05$) and could be reflecting impairment in the hematological function of the rats exposed to intoxicating dose of MSG. In contrast, these indicators of hematological functions increased markedly and significantly in rats exposed to TOPE compared to others, indicating that TOPE improved these indicators of hematological function in the rats and mitigated the MSG-induced hematologic effect irrespective of dose. The result compared with the significant improvement in rats' hematology following the incorporation of *T. occidentalis* leaves diet in fish [33]. The concentration of PCV, HB and RBC reported herein was higher than those reported earlier for *T. occidentalis* leaves[32,35]. The present outcome suggests that TOPE could cause superior influence on the hematological function that could lead to higher blood boosting potential in the rats. The results further suggest that TOPE could mitigate the hematologic dysfunctions associated with the reduction of these indicators following MSG intoxication in the rats.

The results herein showed that rats that received intoxicating dose of MSG had significant changes in the determined indicators of lipid metabolism, suggesting impaired lipid metabolism, significant dyslipidaemia and inherent cardiovascular diseases in the rats exposed to high dose of MSG. Elevated serum levels of triacylglycerol (TAG), low density lipoprotein (LDL)- cholesterol, but a diminished level of high density lipoprotein (HDL)-cholesterol as observed in this study had indicated impaired lipid metabolism suggestive of significant dyslipidaemia and inherent cardiovascular diseases [1,36]. Exposure of TOPE to rats elicited similar responses on these indicators of lipid metabolism compared with the control but opposite responses on these tested lipid profiles indicators compared to rats in MSG group and other rat groups. This outcome demonstrates that TOPE could mitigate the MSG-induced effect on the tested indicators of lipid metabolism irrespective of dose. The significant reduction in the tested lipid profiles by TOPE is consistent with earlier report [37] and the reviewed pharmacotherapeutic benefits of *T. occidentalis*[5]. Thus, TOPE improved hematologic and lipid profile functions in the rats and mitigated associated dysfunctions caused by MSG intoxication in rats *via* probable blood boosting and lipid lowering activities. The results herein suggest that TOPE could offer therapeutic benefits in rats' hematology and lipid metabolism compromised by MSG intoxication warranting follow up.

2. Conclusion

Thus, TOPE demonstrated dietary property and concentration-dependent antimicrobial activity. *In vivo*, TOPE improved hematologic and lipid profile functions in the rats and corrected apparent disruptions associated with MSG intoxication in rats *via* probable blood boosting and lipid lowering activities. The implications of these findings warrant follow up as they may be significant in relation to prospective application of TOPE as potential novel diet and drug for the resistance of microbial pathogens and the modulation of MSG-related disruptions in animal hematology and lipid metabolism.

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