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Studies on the Effect of Processing Methods on the Antihyperglycemic activity of Herbal Teas from Leaves of *Vernonia amygdalina* Del

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ABSTRACT

The leaves of *Vernonia amygdalina* Del (Asteraceae) was processed into herbal tea using the black and green tea processing techniques. The green tea obtained was used as such while a portion of the black tea was flavored with *O. basilicum* or *O. gratissimum.* Methanol extracts of the flavored and unflavored herbal teas, obtained by cold maceration, were subjected to pharmacological studies for antihyperglycemic activity in alloxan diabetic rats and pytochemical analysis. Results showed that single oral administration of the tea extracts reduced blood glucose level of diabetic rats to varying the extents. Extract of green tea (GTE) caused a moderate reduction in blood glucose levels while black tea (BTE) evoked a mild dose-related effect. Extracts of the flavored tea caused significant (P<0.05) reductions in blood glucose levels. While the effect of extract of *O. basilicum* flavored tea (BTOBE) was dose-related, that of *O. gratissimum* flavored tea (BTOGE) was non-dose-related. A comparison of the magnitude of reduction in blood glucose level showed the order of potency: BTOBE>BTOGE>GTE>BTE. Phytochemical analysis showed relative presence of alkaloids, saponins, tannins, flavonoids, steroids and terpenoids with GTE having the highest concentration of these constituents. These findings suggest that black tea technique may be preferred for processing leaves of *V. amygdalina* into herbal tea since it preserved the antihyperglycemic activity. Green tea method may reduce the antihyperglycemic effect even though it preserves the phytochemical constituents better than black tea technique. Flavoring of black tea with *Ocimum spp* enhances the antihyperglycemic effect due to their inherent hypoglycemic activity.

Keywords: Antihyperglycemic, Black tea, Green tea, Herbal tea, Ocimum spp*, Vernonia amygdalina*

INTRODUCTION

A number of medicinal plants are used in traditional medicine in various parts of the world for treatment of diabetes (1). One of such plants is *Vernonia amygdalina* Del (Asteraceae), a shrub with characteristic bitter leaves whose morphology has been described (2). Although the leaves are very bitter in taste, the plant enjoys immense medicinal and nutritional relevance. In Nigeria, *V. amygdalina* is widely consumed in food and used in traditional medicine practice. The leaves are used as vegetable for preparing food, especially bitter-leaf soup, a delicacy popular in the southeastern parts of the country. The juice or extract is drunk as a tonic. The leaves have been shown to contain 18% protein, 8.5% fibre and microelements (3) which adds to the nutritional importance. In traditional medicine practice of southeastern Nigeria, the bitter leaf extract obtained by squeezing and washing of the leaves, is drunk

to manage blood sugar, fever and rejuvenate internal organs like the kidney. Depending on preference, the leaves may be chewed especially in the morning to achieve similar effects. A sweet after-taste on drinking water is associated with chewing the leaves. Elsewhere, the roots and leaves are used to treat fever, hiccups, kidney problem and stomach discomfort among several other uses (2, 4). Extracts of the stem, bark, roots and leaves are used as purgative, antimalarial and in the treatment of eczema (5). Pharmacologically, the hypoglycaemic, hypolipidemic (6), antiplasmodial (7) antimalarial, anthelminthic (8), antitumorigenic (9) and antihepatotoxic (10) properties have been documented. The anti-diabetic efficacy of the plant in diabetes mellitus (type 2 diabetes) has also been demonstrated (11) as well as hypoglycemic effect on postprandial blood glucose in humans (12).

Due to popular use of this plant in the management of diabetes, we explored the possibility of processing the leaves into herbal tea using the methods for producing green and black tea. Although *V. amygdalina* is drought tolerant (13), it thrives well in rainy season but produces scanty leaves during dry season which reduces availability at off seasons. Processing the leaves into herbal tea would guarantee availability on and off season and at times and in places where it is needed notwithstanding the geographical distribution. Tea is usually loosely used to describe infusions made from herbs, spices, and dried fruits (14). Herbal tea preparations do not contain actual tea leaves but are referred to as tisanes or herbal teas to avoid confusion with beverages made from the tea plant, *Camellia* spp. This Implies that herbal tea could be made from herbs with medicinal and nutritional values, and can be flavored especially when there is need to improve the palatability.

In this study, we evaluated the anti-hyperglycemic activity of the herbal tea and its flavored preparations to ascertain the effect of the processing methods on the pharmacological activity and medicinal relevance. The phytochemical constituents of the teas were also analyzed to determine the effect of the processing methods on constituents responsible for the medicinal potency of the plant.

MATERIALS AND METHODS

Drugs: Glibenclamide (Glanil™, 5 mg)
Chemicals, solvents and reagents: Alloxan monohydrate (Sigma Chemicals, USA), ethanol (Sigma Aldrich, Germany), Tween 80.
Equipment: One Touch basic glucometer (Lifescan, USA).
Animals: Adult albino rats (150-220 g) of both sexes obtained from the Department of Zoology, University of Nigeria, Nsukka were used. Animals were kept in plastic cages and maintained freely on standard pellets and water. All animal experiments were in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85 – 23, revised 1985).

Collection and preparation of plant materials

Fresh mature leaves of *V. amygdalina* were plucked from plants growing in a garden at Ekperemere Likke-Iheaka, Enugu State, Nigeria. Fresh leaves of *Ocimum gratissimum* and *O. basilicum* were purchased from the open market at Nsukka, Enugu State, Nigeria. The plant materials were identified by taxonomists in the Department of Botany, University of Nigeria, Nsukka. The leaves were destalked, sorted and cleaned. Leaves of *V. amygdalina* were spread to drain off surface moisture, and used to produce black or green tea as described below. Leaves of *O. gratissimum* and *O. basilicum* were sun dried (30-35°C, 50-55% Relative humidity) for 3days and pulverized to coarse powder using a manual mill.

Preparation and flavoring of tea

The drained *V. amygdalina* leaves (3 kg) were withered under the sun (29°C and 75% relative humidity) for 4 h and spread on wire mesh over a pot of boiling water to steam for 3 mins. The steamed leaves were cooled, ground using a manual plate mill (Corona Plate Mill) and dried in a hot air oven (Phoenix) at 80°C for 3 h (to prevent fermentation) to afford the green bitter leaf tea.

For preparation of black tea, a fresh batch of the drained leaves (6 kg) was withered under the sun (33-37°C and 57-63% relative humidity) for 5 h and ground using a manual Plate Mill (Corona Columbia) to disrupt the cells for effective fermentation. The ground leaves were spread on trays and allowed to ferment for 5 h under the sun. The fermented product was oven-dried (Phoenix hot air oven) for 3 h at 80°C, ground to coarse powder and sieved with standard sieves (Nos 30 & No 40) to afford the black bitter leaf tea. The black bitter leaf tea was flavoured by mixing it with *O. gratissimum* or *O. basilicum* at 5:1 (w/w) ratio.

Phytochemical analysis

The alkaloids, saponin, flavonoids, tannins, steroids and terpenoid content of the processed bitter leaf tea were assessed using the methods outlined by Harborne (15).

Extraction of tea

The green tea (64.49 g), black tea (70.7 g), black tea flavoured with *O. basilicum* (165 g) and black tea flavoured

with *O. gratissimum* (89.5 g) were separately extracted with methanol by cold maceration for 48 h. The mixture was agitated intermittently and filtered on the last day with repeated washing using fresh solvent. Concentration of the different filtrates in a rotary evaporator under reduced pressure afforded the green tea extract (GTE), black tea extract (BTE), black bitter leaf tea and *O. basilicum* extract (BTOBE) and black tea and *O. gratissimum* extract (BTOGE).

Assessment of pharmacological activity

The anti-hyperglycemic effect of the herbal teas and the flavored preparations was assessed by evaluating the effect of the extracts on blood glucose level of alloxan diabetic rats.

Anti-hyperglycaemic activity test

Briefly, rats for the study were fasted for 12 h but allowed free access to water. Diabetes was induced by intraperitoneal injection of alloxan monohydrate (150 mg/kg) in normal saline. Thereafter, alloxan-treated rats were allowed free access to food and water and their blood glucose levels measured every 24 h to check for successful induction of diabetes. On day 3, diabetic rats with blood glucose levels >300 mg/dl were selected for the study. The rats were fasted over night and divided into 10 groups (n = 6). Groups I and II received 200 and 400 mg/kg of GTE while groups III and IV received 200 and 400 mg/kg of BTE respectively. Groups V and VI received 200 and 400 mg/kg of BTOBE while groups VII and VIII were given 200 and 400mg/kg of BTOGE respectively. Groups IX and X served as control and received glibenclamide (5 mg/kg) or vehicle (5% v/v Tween 80). The blood glucose level (mg/dl) of the rats was measured with one touch basic glucometer before and at 1, 2, 4 and 8 h after oral administration of extracts.

Table 1: Phytochemical constituents of herbal teas

Phytochemical constituents	Relative presence		
	Control	BTE	GTE
Alkaloids	+++	++	+++
Flavonoids	++	+	-
Saponins	+	+	+++
Tannins	+	++	+++
Terpenoids	+++	++	++
Steroids	+++	+	+++

- = absent; + = Mildly present; ++ = Moderately present; +++ = abundantly present; Control = extract of dried fresh leaves of *V. amygdalina*; BTE = Black tea extract; GTE = Green tea extract.

The level of reduction (%) in blood glucose at the various times was calculated relative to the zero hour value.

Statistical analysis

Data obtained was analysed using ANOVA and subjected to LSD post hoc test for multiple comparisons. Differences between means were accepted significant at $P<0.05$. Results were presented as Mean ± SEM.

RESULTS

Processing and extraction of herbal teas

Processing of the fresh leaves afforded black tea and green tea. Flavoring of black tea afforded *O. basilicum*- and *O. gratissimum*-flavored black tea. Extraction of the teas and flavored preparations afforded the green tea extract (GTE; 10 g, 14.62% w/w), black tea extract (BTE; 7.2 g, 16.18% w/w), black bitter leaf tea and *O. basilicum* extract (BTOBE: 9.9 g, 11.43% w/w) and black tea and *O. gratissimum* extract (BTOGE; 7.28 g, 8.13% w/w).

Phytochemical constituents of herbal teas

Phytochemical tests showed that the GTE tested positive to alkaloids, saponins, tannins, steroids and terpenoids while BTE gave positive reactions to alkaloids, saponins, tannins, flavonoids, steroids and terpenoids. Extract of unprocessed leaves used as control gave positive reactions to alkaloids, saponins, tannins, flavonoids steroids and terpenoids (Table 1).

Effect of herbal tea extracts on blood glucose level of diabetic rats

Single oral administration of the tea extracts and the flavored preparations reduced blood glucose level of diabetic rats to varying extents. The GTE caused a moderate reduction in blood glucose levels at both dose levels (Table 2) while BTE evoked a mild dose-related effect (Table 3). Extracts of the flavored preparations (BTOBE and BTOGE) significantly ($P<0.05$) reduced blood glucose at both dose levels. The effect of BTOBE was dose-related (Table 4) while that of BTOGE was non-dose-related (Table 4). A comparison of the magnitude of reduction in blood glucose level showed the order of potency: BTOBE>BTOGE>GTE>BTE (Table 5).

DISCUSSION

Processing of herbal remedies into ready-to-be used forms adds value to them and enhances their quality.

Table 2: Effect of green tea extract on blood glucose level of diabetic rats

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)				
		0 h	1 h	2 h	4 h	8 h
GTE	200	308.33 ± 22.82	244.67 ± 34.51	329.33 ± 85.62	297.67 ± 11.14	229.67 ± 23.92
	400	347.67 ± 23.89	356.67 ± 31.04	349.33 ± 26.15	287.00 ± 31.04	227.67 ± 25.88*
Glibenclamide	5	352.00 ± 51.16	178.33 ± 29.76*	138.33 ± 18.91*	91.67 ± 12.61*	123.00 ± 22.47*
Control	-	371.00 ± 33.96	246.33 ± 6.35	232.33 ± 4.26	245.00 ± 5.48	265.00 ± 17.16

n = 6;
*$P<0.05$ compared to 0 h values (*ANOVA; LSD post hoc*);
Values of blood glucose level shown are Mean ± SEM; GTE = green tea extract.

Table 3: Effect of black tea extract on blood glucose level of diabetic rats

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)				
		0 h	1 h	2 h	4 h	8 h
BTE	200	342.00 ± 36.88	212.67 ± 49.33*	256.00 ± 24.46	269.00 ± 13.15	275.00 ± 57.33
	400	367.67 ± 28.17	432.00 ± 36.12	475.00 ± 14.24	282.33 ± 15.88*	258.00 ± 36.87*
Glibenclamide	5	352.00 ± 51.16	178.33 ± 29.76*	138.33 ± 18.91*	91.67 ± 12.61*	123.00 ± 22.47*
Control	-	371.00 ± 33.96	246.33 ± 6.35	232.33 ± 4.26	245.00 ± 5.48	265.00 ± 17.16

n = 6;
*$P<0.05$ compared to 0 h values (*ANOVA; LSD post hoc*);
Values of blood glucose level shown are Mean ± SEM; BTE = Black tea extract.

Table 4: Effect of extracts of flavored black tea on blood glucose level of diabetic rats

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)				
		0 h	1 h	2 h	4 h	8 h
BTOBE	200	381.00 ± 12.05	358.00 ± 24.09	318.00 ± 9.13*	248.00 ± 15.34*	173.67 ± 29.76*
	400	401.00 ± 39.99	195.00 ± 1.67*	127.33 ± 21.24*	75.67 ± 2.38*	65.67 ± 2.01*
BTOGE	200	363.67 ± 16.09	328.00 ± 20.69*	310.33 ± 7.49*	209.33 ± 35.16*	236.33 ± 70.62*
	400	298.67 ± 40.27	177.33 ± 11.05	216.00 ± 26.49	128.00 ± 7.86*	84.33 ± 3.11*
Glibenclamide	5	352.00 ± 51.16	178.33 ± 29.76*	138.33 ± 18.91*	91.67 ± 12.61*	123.00 ± 22.47*
Control	-	371.00 ± 33.96	246.33 ± 6.35	232.33 ± 4.26	245.00 ± 5.48	265.00 ± 17.16

n = 6;
*$P<0.05$ compared to 0 h values (*ANOVA; LSD post hoc*);
Values of blood glucose level shown are Mean ± SEM; BTOBE = Extract of black tea flavored with *O. basilicum*; BTOGE = Extract of black tea flavored with *O. gratissimum*

It also ensures their availability especially in places where the plants are not endemic and encourages use due to ease of administration. Leaves of *V. amygdalina* have proven and documented anti-hyperglycemic (6) and anti-diabetic (11, 12) activities which prompted our interest to process the leaves into teas. Evaluation of the antihyperglycemic effect of the teas in alloxan diabetic rats showed that the black tea at both dose levels lowered glucose level better than green tea which caused an inconsistent effect and was almost devoid of activity. No matter the type of processing or method employed and the quality of the finished product, it is important that the pharmacological activity and hence medicinal relevance of the plant material is retained if not enhanced. The results of this study have shown that processing of leaves of *V. amygdalina* into herbal tea by green tea method reduces the anti-hyperglycemic potency and hence its usefulness as anti-diabetic remedy.

It is equally important that the processing methods do not alter the phytochemical constituents of the plant responsible for the pharmacological activity. Phytochemical studies of the herbal teas showed the presence of alkaloids, saponins, tannins, flavonoids,

Table 5: Percentage reduction in blood glucose in hyperglycemic rats

Extract	Dose (mg/kg)	Reduction in blood glucose (%)			
		1 h	2 h	4 h	8 h
GTE	200	20.65	-6.81	3.46	25.51
	400	-2.59	-0.48	17.45	34.52
BTE	200	37.82	25.15	21.35	19.59
	400	-17.53	-29.19	23.21	29.83
BTOBE	200	6.04	16.54	34.91	54.42
	400	51.37	68.25	81.13	83.62
BTOGE	200	9.81	14.67	42.44	35.02
	400	40.63	27.68	57.14	71.76
Glibenclamide	5	49.34	60.70	73.96	65.06
Control	-	33.60	37.38	33.96	28.57

n = 6; Reduction (%) in blood glucose was calculated relative to 0 h values; GTE = Green tea extract; BTE= Black tea extract; BTOBE = Extract of black tea flavored with *O. basilicum*; BTOGE = Extract of green tea flavored with *O. gratissimum*.

steroids and terpenoids to varying extents. Green tea showed higher level of the evaluated phytochemical constituents than black tea. This implies that the green tea method preserved the constituents better than the black tea process. This is, however, not consistent with the higher anti-hyperglycemic effect of black tea and suggest that black tea method may not adversely affect the phytochemical constituents responsible for the anti-hyperglycemic effect of this plant. Thus, although preferred over black tea method, the green tea method may not preserve the intactness of the phytochemical constituents.

Flavoring enhances the palatability of preparations meant for oral administration. The *Ocimum spp* used as flavoring agents in this study are widely employed as condiments or spice and flavors in food. They are not sweeteners and their use was primarily intended to relatively mask the bitterness of the herbal black tea. Although the effect of the flavors on the organoleptic properties of the herbal tea was not evaluated in this study, flavoring with the *Ocimum spp.* enhanced the anti-hyperglycemic effect. Extract of *O. basilicum*-flavored black tea caused the greatest reduction in blood glucose level of diabetic rats. The effects of these *Ocimum* spp may not be unconnected with their individual antihyperglycemic properties. The hypoglycemic activity of *O. gratissimum* (16) and *O. basilicum* (17) has been documented. Thus, these *Ocimum spp* may contribute to the anti-hyperglycemic effect of herbal tea from this plant in addition to serving as possible flavoring agents.

CONCLUSION

The results of this study showed that processing of leaves of *V. amygdalina* by green tea method reduced its antihyperglycemic activity despite preserving the phytochemical constituents better than the black tea method. Flavoring with *O. basilicum* and *Ocimum gratissimum* not only served as flavoring agents but also greatly enhanced the anti-hyperglycaemic activity of the flavored black tea. Thus, flavoring of black tea with *O. basilicum* or *O. gratissimum* may augment its anti-hyperglycemic activity.

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