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EFFECT OF *VERNONIA AMYGDALINA* AND FISH OIL-SUPPLEMENTED BISCUITS ON LIPID PROFILE PARAMETERS AND BLOOD GLUCOSE CONCENTRATION OF DIABETIC RATS

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ABSTRACT
Biscuit, a popular snack, serve as an ideal vehicle for incorporating therapeutic agents for disease management. This study aimed to assess the effect of *Vernonia amygdalina* (VO) and fish oil (FO)-supplemented biscuits (VFSB) on the total lipid profile and blood glucose concentration of diabetic rats. Forty-nine Wistar rats were allocated into seven groups (A to G). Upon induction and confirmation of diabetes in experimental rats, fasting blood glucose (FBG) level were monitored weekly using glucometer. Twenty-four hours after the last day of treatment, rats were sacrificed and serum total cholesterol (TC), triglyceride (TG), High-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c) concentrations were assayed using assay kits. Results showed a significant ($p < 0.05$) reduction in FBG, TC, LDL-c, and TG concentrations in biscuit fed groups D, E, and F compared to group B fed VFSB. High-density lipoprotein cholesterol levels significantly increased ($p < 0.05$) in groups D, E, and F compared to Group B. In conclusion, *Vernonia amygdalina* and fish oil-supplemented biscuits exhibited a glucose-lowering effect and mitigated dyslipidemia associated with diabetes mellitus.

Keywords: Therapeutic biscuit, diabetes mellitus, *Heteroclarias*, *Vernonia*, Lipids.

INTRODUCTION

Diabetes mellitus is a metabolic disease that is characterized by hyperglycemia associated with alterations in carbohydrate, protein, and lipid metabolism. Dyslipidemia is a major risk factors for cardiovascular disease in diabetes mellitus. It is characterized by a high plasma triglyceride concentration, low high density lipoprotein (HDL) cholesterol concentration and increased concentration of low density lipoprotein (LDL) cholesterol particles. The lipid changes associated with diabetes mellitus are attributed to increased free fatty acid flux secondary to insulin resistance (Mooradian, 2009). Studies had shown that patients with type 2 diabetes mellitus (T2DM), even when in good glycemic control have abnormalities in lipid levels (Wu and Parhofer, 2014; Feingold, 2020).

The number of adults suffering from diabetes had increased from 108 million in 1980 to 422 million in 2014, and other estimates indicated more than 435 million people will be diabetic by 2030 (GBD, 2023). With the increasing prevalence of diabetes mellitus, it has become a global health concern, prompting extensive research into innovative therapeutic strategies and dietary interventions. Among the various approaches explored, the integration of natural compounds and functional foods has garnered significant attention for their potential to manage diabetes and its associated complications.

Biscuit is an important snack that can be fortified with therapeutic agents for management of specific disease. Biscuits are small, crisp and flat baked products made from wheat flour, starch, powdered sugar, glucose syrup, emulsifiers, food colour and flavours (Manley, 2003). Even though on account of its high sugar and fat contents, biscuits are sometimes viewed as unhealthy foods, there are several ways in which biscuits can be fortified (Manley 2003). Rababah *et al*. (2006) fortified biscuits using chickpea flour, broad bean flour and soy protein. Boobier *et al*. (2006) thereby converting a traditional biscuit to a functional food by adding vitamin B_{12}, folic acid, vitamin C and prebiotic fibre. Therapeutic biscuits therefore, are a type of functional food enriched with specific nutrients and bioactive compounds that provide health benefits to consumers (Lim *et al.,* 2021). These biscuits are designed to improve overall health and well-being by addressing specific health concerns such as digestive issues, heart health, and immune system function.

Vernonia amygdalina Del (Asteraceae) is a small shrub with dark green leaves and rough barks growing predominantly in tropical Africa but has been domesticated in many parts of West Africa (Igile *et al.,* 1994). It is a perennial plant with height between 1 m and 6 m (Nwosu *et al*., 2013). *V. amygdalina* Del is soft wooded and is a multipurpose and rapid regenerating shrub. Its bitter taste has made it to be fondly called "bitter leaf". *Vernonia amygdalina* has been traditionally employed in several cultures for its anti-diabetic and hypolipidemic effects. Adeoye *et al*. (2017) showed that *V. amygdalina* leaf extracts possessed hypoglycemic activity in alloxan-induced diabetic rats. Likewise, fish oil, rich in omega-3 fatty acids, has been recognized for its potential to modulate lipid metabolism and exert anti-inflammatory actions, thus offering a promising avenue for diabetic management (Calder, 2013; Devarshi *et al*., 2013).

The aim of this study was to effect of *Vernonia amygdalina* del. and fish oil-supplemented biscuits on selected lipid profile parameters and blood glucose of diabetic rats. The utilization of biscuits as a delivery system for these bioactive components not only enhances palatability but also facilitates controlled and convenient consumption.

MATERIAL AND METHODS

Chemicals

Streptozotocin (STZ) (Sigma-Aldrich, St. Louis, Mo, USA) were purchased from a local research laboratory in Ilorin, Nigeria. Metformin (Eden U.K. Pharmaceutical Ltd.) was purchased from a local pharmacy store in Ilorin, Nigeria. The assay kits used for

determination of cholesterol, HDL and triglyceride were manufactured by Fortress Diagnostics Ltd., Antrim Technology Park, Antrim BT41 1 QS, United Kingdom and Nicotinamide Adenine Dinucleotide (BDH chemicals Ltd Poole England) was donated to the research group by Dr. L.A Quadri of the Department of Biochemistry, University of Ilroin. Other reagents used were of analytical grade, prepared in glass apparatus with distilled water.

Plant collection and preparation

Fresh leaves of *Vernonia amygdalina* were harvested from a garden at *Ajowa* Street, Sango road, *Ilorin*, Kwara state, Nigeria (8°31'37.12116''N 4°35'19.21848''E) in January, 2023. The identification and authentication of the plant was carried out at the herbarium unit of the Department of Plant biology, University of Ilorin, Ilorin, Kwara State. A voucher specimen no UILH/001/1324/2023 was assigned to the *Vernonia amygdalina* sample.

Vernonia amygdalina leaves collected were rinsed with tap water and air-dried to constant weight at room temperature. The dried leaves were then pulverized using an electronic blender, stored in air-tight container and kept in air-tight container prior to usage.

***Heteroclarias* viscera collection and oil extraction**

The viscera of *Heteroclarias* spp cat fish was used for this experiment. The viscera were purchased from the local market at Unity road cat fish Market, Ilorin, Kwara State, Nigeria. The fish viscera were extracted from the fish manually by the market vendors. They were bought fresh and washed to remove any blood residue. The viscera oil was extracted according to the wet (boiling) extraction method as described by Vidotti *et al.* (2011) with slight modification by skipping the centrifugation step. A weight of 1000 g chopped viscera was placed into a cooking bowl with 1000 ml of water and cooked at 100 °C for 50 minutes. The cooked viscera was left to cool down for 3 hours and the oil allowed to float on the surface of the cooked mixture. The floating oil was then collected and placed in a separating funnel in order to separate the oil from the cooked mixture. The clear oil was decanted into a reception container, sieved and heated to remove moisture in the oil.

Formulation of *Vernonia amygdalina* Del and Fish Oil-enriched Biscuits

The biscuits were formulated following the recipe of Whitley (1970) with slight modifications in the percentages of the ingredients. Four biscuits samples were formulated, namely; biscuit without *Vernonia amygdalina* and fish oil (B2), biscuit with 1 g of *Vernonia amygdalina* and fish oil (B3), biscuit with 2 g of *Vernonia amygdalina* and fish oil (B4) and biscuit with 3 g of *Vernonia amygdalina* and fish oil (B5). The control biscuit labelled B1 (a product of Yale Foods Industrial LTD, plot 1B, Block1, Oluyole Estate. P.O. Box 2033, Ring Road Ibadan, Oyo State, Nigeria) was purchased from a local vendor in Ilorin.

B2 was prepared by mixing 240 g of wheat flour with 15 g of sweetener, 1 g of salt, 4 g of baking powder, 60 g of butter and 60 g of milk, to form a dough. The dough was kneaded and rolled carefully with a rolling pin, cut into round shapes and baked in the oven for 15 minutes at 175 °C.

B3 was prepared by mixing 240 g of wheat flour with 15 g of sweetener, 1 g of salt, 4 g of baking powder, 69.5 g of fish oil, 60 g of milk and 1 g of pulverized *Vernonia amygdalina* leaves, to form a dough. The dough was kneaded and rolled carefully with a rolling pin, cut into round shapes and baked in the oven for 15 minutes at 175 °C.

The above process used for the formulation of B3 was repeated for the formulation of B4 and B5 with variation in the quantity of *Vernonia amygdalina*. Two (2 g) of *Vernonia amygdalina* was used for the formulation of B4 while 3 g was used for the formulation of B4. Afterwards, the biscuits were cooled at room temperature and kept in zip lock bags.

Experimental animals

Forty nine male albino rats (*Rattus norvegicus*) with an average weight of 120g were obtained from the animal holding unit of the Department of Biochemistry, University of Ilorin, Ilorin, Kwara state. The rats were housed in well ventilated cages and allowed to acclimatize to animal house condition of; temperature 28 – 31 °C, 12 hours of natural light and 12 hours darkness and humidity 50 – 51% for 14 days. The rats were fed with growers feed (Premier feed mill, No 1, Eagle flour road, Premier Feeds Company Limited) and they were given water.

Animal handling/Ethical clearance

Ethical clearance for this study was obtained from the University of Ilorin animal use and ethical Committee with protocol identification code UERC/LSC 078.

Induction and Confirmation of Diabetes

Nicotinamide Adenine Dinucleotide (NAD) was freshly dissolved in ionized water and was administered to all experimental rats excluding group A (Control) by a single intra-peritoneal injection at a dose of 230 mg/kg. Fifteen (15) minutes later, streptozotocin was prepared by freshly dissolving in 0.1 M ice cold citrate buffer (4.5 pH) was administered to rats in group B to G (excluding group A). After 72 hours of the STZ-NA injection, blood glucose levels were determined and mice with blood glucose levels higher than 200 mg/dl were used in the following experiments.

Experimental design

Forty-nine Wistar rats were randomly divided into seven groups of 7 animals each: The grouping was done as follows:

Table 1: Animal grouping

Groups	Treatment
A(Control)	Non diabetic rats fed with biscuit without *V. amygdalina* and fish oil
B(Diabetic untreated)	Diabetic rats fed with biscuit without *V. amygdalina* and fish oil
C(reference drug control)	Diabetic rats treated with 120 mg/kg b.wt. metformin, fed with biscuit without *V. amygdalina* and fish oil
D	Diabetic rats fed with biscuit containing 1 g of *V. amygdalina* and fish oil
E	Diabetic rats fed biscuit containing 2 g of *V. amygdalina* and fish oil
F	Diabetic rats fed with biscuit containing 3 g of *V. amygdalina* and fish oil
G	Diabetic rats fed with conventional biscuit

The rats were fed daily with 65 g of the experimental biscuits for 35 days.

Animal Sacrifice and Serum Collection

The rats were sacrificed 24 hours after the last day of treatment. They were anaesthesized with dichloromethane and sacrificed by simply incising the jugular vein, Blood samples were collected into clean sample bottles and was allowed to clot at room temperature and the serum was collected using Pasteur pipette.

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Biochemical Assays

Fasting blood glucose (FBG) concentrations of all the experimental groups were determined using ACCU CHECK glucometer by withdrawing blood form the caudal vein of the rats. The FBG levels were monitored weekly throughout the experimental period. Total cholesterol, triglycerides, HDL concentrations were determined by following the manufacturer's instruction in the assay kits (Fortress Diagnostics Ltd., Antrim Technology Park, Antrim BT41 1 QS, United Kingdom). The method described by Friedewald *et al.* (1972) was employed for the determination of serum LDL concentration. This is based on a mathematical calculation using the fomular below**:**

$$\textit{Conc. of LDL-cholesterol (mmol/l)} = Total\ chol - \frac{Triglycerides}{2.2} - HDLchol \times DF$$

Where DF = dilution factor

Atherogenic Index

The atherogenic index was calculated using the formular reported by Niroumand *et al.* (2015):

$$Atherogenic\ Index = Log(\frac{Triglycerides}{High\ density\ lipoprotein\ conc})$$

Statistical analysis

Data were expressed as mean of 7 determinations ± SEM. The data were subjected to statistical analysis using the IBM® statistical package for social sciences (SPSS) software version 20. All significant differences were determined by one way analysis of variance (ANOVA). Post hoc multiple comparisons were done using Duncan's multiple range test. The level of significance was set at $p < 0.05$ (confidence level = 95%).

RESULTS

Fasting blood glucose concentration of diabetic rats fed *Vernonia amygdalina* and fish oil-supplemented biscuits

Table 2 shows the result of fasting blood concentration of diabetic rats fed with *Vernonia amygdalina* and fish oil-supplemented biscuits. There was a significant increase ($p < 0.05$) in the FBG level of all experimental rats following the induction of diabetes in the rats. The blood glucose of group B remained consistently high throughout the experimental period. Groups D, E and F had a significantly decreased ($p < 0.05$) blood glucose level compared to groups B and C from week 2 till the end of the experiment. The result group G was not significantly different to group B. None of the FBG result of the experimental rats compared favourably to the control (group A).

Total cholesterol and triglyceride concentrations of diabetic rats fed with *Vernonia amygdalina* and fish oil-supplemented biscuits

The results of total cholesterol triglycerides concentrations are shown in Table 3. There was a significant increase ($p < 0.05$) in the concentrations of serum cholesterol and triglyceride levels of group B compared to control group (A). This increase was significantly reduced in rats in groups C to F, but group G was not significantly different to group B.

Table 2: Fasting blood glucose concentration of diabetic rats fed Vernonia amygdalina and fish oil-supplemented biscuits

	Fasting Blood Glucose (mg/dL)					
Group	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
A	81.57 ± 5.79^{a}	82.86 ± 6.27^{a}	83.83 ± 6.78^{a}	75.83 ± 6.04^{a}	76.83 ± 4.30^{a}	70.17 ± 3.23^{a}
B	77.43± 4.04^{a}	258.57 ± 4.49^{b}	266.71 ± 3.97^{b}	272.29± 8.82^{b}	295.71± 4.70^{b}	304.00± 3.75^{b}
C	82.43± 4.46^{a}	239.00 ± 8.07^{c}	237.00 ± 3.87^{c}	206.00 ± 4.04^{c}	186.67± 1.17^{c}	150.33± 0.33^{c}
D	81.57± 2.93^{a}	258.33 ± 6.56^{b}	208.50 ± 4.99^{d}	206.40 ± 5.55^{c}	177.60± 3.94^{d}	146.00± 1.94^{d}
E	83.86± 2.94^{a}	250.43 ± 6.68^{b}	222.50± 5.39^{cd}	201.17 ± 7.95^{c}	180.25± 2.76^{d}	134.40 ± 1.88^{e}
F	85.14± 6.23^{a}	252.57 ± 6.67^{b}	232.00 ± 5.62^{c}	202.20 ± 4.94^{c}	175.80± 3.68^{d}	124.40± 1.88^{f}
G	85.71± 1.66^{a}	258.29 ± 6.97^{b}	259.20± 8.12^{c}	249.67 ± 4.88^{c}	260.33± 4.67^{e}	229.50 ± 3.50^{g}

Values are mean of 7 replicates **±** SEM. Values carrying the different alphabet superscripts down the column are statistically different ($p < 0.05$); A - Non-diabetic rats fed with biscuit without *Vernonia amygdalina* and fish oil; B - Diabetic untreated rats fed with biscuit without *Vernonia amygdalina* and fish oil; C - Diabetic rats treated with 120 mg/kg b.wt. metformin and fed biscuit without *Vernonia amygdalina* and fish oil; D - Diabetic rats fed with biscuits containing 1 g of *Vernonia amygdalina* and fish oil; E - Diabetic rats fed with biscuits containing 2 g of *Vernonia amygdalina* and fish oil; F - Diabetic rats fed with biscuits containing 3 g of *Vernonia amygdalina* and fish oil; G - Diabetic rats fed with conventional biscuits purchased commercially

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Table 3: Total cholesterol and triglyceride concentrations of diabetic rats fed with Vernonia amygdalina and fish oil-supplemented biscuits

Groups	TC (mmol/L)	TG(mmol/L)
A	2.36± 0.10^{a}	1.12 ± 0.02^{a}
B	2.89± 0.12^{b}	1.29 ± 0.04^{b}
C	2.29± 0.36^{b}	1.15 ± 0.03^{a}
D	2.29± 0.12^{a}	1.14 ± 0.02^{a}
E	2.24± 0.08^{a}	1.12 ± 0.06^{a}
F	2.22± 0.13^{a}	1.16 ± 0.02^{a}
G	2.89 ± 0.10^{b}	1.28 ± 0.08^{b}

Values are mean of 7 replicates ± SEM. Values carrying the different alphabet superscripts down the column are statistically different ($p < 0.05$); A - Non-diabetic rats fed with biscuit without *Vernonia amygdalina* and fish oil; B - Diabetic untreated rats fed with biscuit without *Vernonia amygdalina* and fish oil; C - Diabetic rats treated with 120 mg/kg b.wt. metformin and fed biscuit without *Vernonia amygdalina* and fish oil; D - Diabetic rats fed with biscuits containing 1 g of *Vernonia amygdalina* and fish oil; E - Diabetic rats fed with biscuits containing 2 g of *Vernonia amygdalina* and fish oil; F - Diabetic rats fed with biscuits containing 3 g of *Vernonia amygdalina* and fish oil; G - Diabetic rats fed with conventional biscuits purchased commercially; TC – Total cholesterol; TG – Triglyceride; mmol – millimol; L - litre

High Density Lipoprotein cholesterol, Low Density Lipoprotein cholesterol and Atherogenic Index of diabetic rats fed *Vernonia amygdalina* and fish oil-supplemented biscuits

Table 4 shows the result of high density lipoprotein cholesterol, low density lipoprotein cholesterol and atherogenic index of diabetic rats fed *Vernonia amygdalina* and fish oil-supplemented biscuits. There was a significant increase ($p < 0.05$) in HDL concentration of groups E and F compared to B, while group G was not significantly different ($p > 0.05$) to A and B. LDL-c decreased significantly ($p < 0.05$) in groups D, E, F compared to B and G. The result for atherogenic index followed the same pattern.

Table 4: High Density Lipoprotein, Low Density Lipoprotein and Atherogenic Index of diabetic rats fed Vernonia amygdalina and fish oil-supplemented biscuits

Group	HDL-c (mmol/L)	LDL-c (mmol/L)	AI
A	0.77 ± 0.01^{a}	1.08 ± 0.12^{a}	0.16 ± 0.01^{a}
B	0.71 ± 0.01^{a}	1.71 ± 0.34^{b}	0.21 ± 0.01^{b}
C	0.82 ± 0.03^{b}	1.42 ± 0.14^{b}	0.20 ± 0.03^{b}
D	0.77 ± 0.04^{ab}	1.01 ± 0.08^{a}	0.16 ± 0.02^{a}
E	0.81 ± 0.02^{bc}	1.02 ± 0.15^{a}	0.15 ± 0.02^{a}
F	0.90 ± 0.03^{c}	1.08 ± 0.14^{a}	0.15 ± 0.04^{a}
G	0.74 ± 0.03^{ab}	1.41 ± 0.09^{b}	0.20 ± 0.02^{b}

Values are mean of 7 replicates ± SEM. Values carrying the different alphabet superscripts down the column are statistically different ($p < 0.05$); A - Non-diabetic rats fed with biscuit without *Vernonia amygdalina* and fish oil; B - Diabetic untreated rats fed with biscuit without *Vernonia amygdalina* and fish oil; C - Diabetic rats treated with 120 mg.kg. b.wt. metformin and fed biscuit without *Vernonia amygdalina* and fish oil; D - Diabetic rats fed with biscuits containing 1 g of *Vernonia amygdalina* and fish oil; E - Diabetic rats fed with biscuits containing 2 g of *Vernonia amygdalina* and fish oil; F - Diabetic rats fed with biscuits containing 3 g of *Vernonia amygdalina* and fish oil; G - Diabetic rats fed with conventional biscuits purchased commercially; HDL-c – High density lipoprotein cholesterol; LDL-c – Low density lipoprotein cholesterol; AI – Atherogenic Index

DISCUSSION

Biscuit is an important snack that can be fortified with therapeutic agents for management of specific disease. The significant reduction in FBG concentration of rats fed with the different inclusion levels of *Vernonia amygdalina* and fish oil-supplemented biscuits (groups D, E and F) showed that the test biscuits elicited glucose-lowering effect in STZ-Nicotinamide induced diabetic rats. The test agents, *Vernonia amygdalina* leaves had been reported to possess bioactives with demonstrated antihyperglycemic effects (Atangwho *et al*., 2014; Asante *et al*., 2016; Alara *et al*., 2017). Likewise, fish oil, rich in omega-3 fatty acids, has been recognized for its potential to modulate lipid metabolism and exert anti-inflammatory actions, thus offering a promising avenue for diabetic management (Calder, 2013; Devarshi *et al*., 2013). Fish oil is a rich source of n-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Yamazaki *et al*., 2011). There is evidence for the role of n-3 fatty acids in insulin resistance and diabetes, as other studies have shown similar effects of fish oil on insulin resistance in obese rats (Yamazaki *et al*., 2011) as well as in humans (Ramel *et al*., 2008).

Over the years, various researchers had demonstrated that sugar and fat can be replaced in biscuits to benefit diabetic patients. Gallagher *et al.* (2003) reported that sugar in biscuits can be reduced by 20-30% with the incorporation of the oligofructose

Raftilose. Dietary fibres with proven hypolipidaemic or antidiabetic properties had been included in the recipe. Ahmed *et al.* (2022) developed a fortified biscuit with antidiabetic potential using indigenous medicinal plants and herbs such as *Moringa oleifera* leaves, *Aloe vera*, *Momordica charantia*, *Mentha spicata*, *Hydrocotyle asiatica* and *Costus igneus* plants. Similar to our findings in this study, fortification of biscuits with medicinal plants improved the therapeutic properties of the biscuits with increased antidiabetic potency.

Lipid abnormalities in patients with diabetes, often termed "diabetic dyslipidemia, are typically characterized by high total cholesterol (T-Chol), high triglycerides (TG), low high density lipoprotein cholesterol (HDL-C) and increased levels of small dense LDL particles. The significant reduction in the total cholesterol and triglyceride concentrations of rats fed with the *Vernonia amygdalina* and fish oil-supplemented biscuits in this study suggests that the formulated biscuit was able to ameliorate the dyslipidaemia associated with diabetes mellitus. This observation is consistent with the findings of Nwanjo (2005) in which VA significantly attenuated the hepatic triglyceride and LDL cholesterol levels of diabetic rats. The crucial risk factor for cardiovascular diseases (CVD) includes a low level of HDL-cholesterol. The association between a low level of HDL-cholesterol and an increased risk of CVD has been well established through epidemiological and clinical studies (Assmann and Gotto 2004). Since low level of HDL-cholesterol plays a direct role in the atherogenic process, therapeutic intervention to raise HDL-cholesterol together with other risk factors is widely encouraged. In this study, treatment with VA and fish oil-supplemented biscuits led to significant elevation of plasma HDL-cholesterol, indicating its promising protective role against CVD.

LDL cholesterol is another primary target of CVD risk-reduction therapy (Kwiterovich 1997). In this study, a significant reduction in the LDL-c levels of diabetic rats fed with *Vernonia amygdalina* and fish oil-supplemented biscuits was recorded. Excess LDL can be deposited in the blood vessel walls and becomes a major component of atherosclerotic plaque lesions. Therefore, plasma LDL cholesterol level may be used for monitoring the treatment of patients with elevated blood cholesterol levels. Similarly, significant reduction was recorded for the atherogenic index of rats fed with *Vernonia amygdalina* and fish oil-supplemented biscuits in this study. Atherogenic index is an index composed of triglycerides and high-density lipoprotein cholesterol (Dobiasova and Frohlich, 2001). It has been used to quantify blood lipid levels and commonly used as optimal indicator of dyslipidemia and associated diseases (e.g., cardiovascular diseases) (Bora *et al*., 2017; Cai *et al*., 2017; Yang *et al*., 2017).

CONCLUSION

In conclusion, *Vernonia amygdalina* and Fish oil-supplemented biscuits elicited glucose-lowering effect and ameliorated dyslipidaemia associated with diabetes mellitus. Therefore, the formulated biscuit is a promising therapeutic biscuit whose consumption will benefit diabetic patients and dyslipidaemia. Delivering therapeutic agents through biscuits as a vehicle is an important emerging technology which offers a promising outcome for disease management if properly designed to still retain it therapeutic activity after incorporating into food products.

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