



GROWTH RESPONSE AND BIOCHEMICAL CHANGES IN WISTAR RATS FED ON MIXED SPICES-SUPPLEMENTED DIET

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ABSTRACT

The research was conducted to study the growth and biochemical changes in Wistar rats fed on mixed spices-supplemented diet. *Syzygium aromaticum*, *Zingiber officinale*, *Allium sativum* and *Allium cepa* were dried, pulverized and mixed in the ratio of 1:2:2:2 respectively. Eight Wistar albino rats (81.60±17.80 g) were divided into two groups of four rats each with nearly equal average weight and randomly assigned to two experimental diets; diet A (control) was starters` mash while diet B was 1.5% mixed spices of starters` mash. The rats were maintained on their respective diets *ad libitum* for six weeks after one week of acclimatization. The feed intake, change in body weight, feed conversion ratio, fasting blood glucose, haematological and lipid profiles, and serum transaminase activities were determined using standard methods. The results showed that the feed consumed (113.4±3.39 g), feed conversion ratio (7.17±1.73) and change in body weight (22.87±6.19 g) in rats fed on diet B were not significantly different ($P>0.05$) from the control. Compared to the initial values (101.80±3.5 mg/dl), diet B did not cause significant variation ($p>0.05$) in fasting blood glucose level. The packed cell volume, hemoglobin and red blood cell were significantly increased in rats maintained on diet B. Serum concentrations of triglyceride, low density lipoprotein cholesterol, atherogenic index and serum activities of transaminases were significantly decreased ($P<0.05$) in group B compared to the control. It is therefore concluded that 1.5 % of mixed spices-supplemented diet may not affect appetite and growth, and may improve health status of an individual.

Keywords: Biochemical; Hematology; Lipid; Mixed-spices; Transaminase

INTRODUCTION

Spices are aromatic flavourings made from parts of plant that include the bark, buds, flowers, fruits, leaves, rhizomes, roots, seeds, stigmas and styles or the entire plant tops (UNIDO and FAO, 2005). The spices that are widely cultivated across the globe (Nigeria inclusive) include rhizome of *Zingiber officinale* (ginger), *Allium sativum* (garlic), *Allium cepa* (onion) and *Syzygium aromaticum* (clove). They are usually used to impart characteristic flavour (sour, sweet, salty, tangy) to food and to tenderize meat (Blaire, 2016).

In the recent years, the use of spices has been receiving serious attention from researchers due largely to their abundant phytonutrients and carminative effect both of which confer potential health benefits (Blaire, 2016). Diets containing the oils of ginger, clove or mixture of both have been reported to improve some physiological conditions in streptozotocin-induced rats (Talal and Atef, 2007). According to a review by Rajesh *et al.* (2012), (6)-gingerol in ginger and ginger oil have antioxidant effects as they were shown to respectively protect HL-60 cells from oxidative stress and to have dominative protective effects on DNA damage induced by H₂O₂. Many studies have shown ginger to have significant blood glucose lowering effect in animals

(Al-Aminet *et al.*, 2006; Li *et al.*, 2014; Saravanan *et al.*, 2014). Syrup produced from the combination of onions, garlic, tomatoes, ginger, water melon seeds, lemon and palm oil has also been shown to have significant blood glucose lowering effect in Wistar rats (Lawal *et al.*, 2016). However, little is reported about the growth and biochemical changes in normal Wistar rats maintained on mixed spices supplemented-diet, hence the importance of this study.

MATERIALS AND METHODS

The research was conducted in Dutsin-Ma LGA of Katsina State, Nigeria. Dutsin-Ma lies on latitude 12°26'N and longitude 07°29'E. The climate of Katsina State (Dutsin-Mainclusive) is the tropical wet and dry type with average annual rainfall of about 700 mm. The mean annual temperature ranges from 29°C –31°C (Abaje *et al.*, 2015).

Materials

Pelletized starter's mash, product of Vital Feed, Jos, Nigeria was purchased from dealers in Vital Feeds in Dutsin-Ma Local Government. Fresh spices (ginger, clove, garlic and onion) were bought from Dutsin-Ma central Market, Katsina State.

Experimental animals

Eight (8) male Wistar rats weighing 86.15 ± 7.31 g were purchased from Research Centre Vom, Plateau State. The rats were kept in standard wooden cages and in accordance with the rules of the National Academy of Sciences (2011). They were kept for one week to acclimatize to the experimental environment before the commencement of the experiment.

Preparation of the spices and feed formulation

The spices (clove, ginger, garlic and onion) were air-dried and separately pulverised. Thereafter, the spices were mixed in ratio of 1:2:2:2 respectively and thoroughly mixed to achieve homogeneity. The mixed spices were mixed with the starters` mash to formulate two diets;

Diet A: Diet based on 100% starter`s mash (Control)

Diet B: Diet based on 1.5% mixed spices supplemented starter`s mash.

Each of the diets was sprinkled with water, mixed and air dried. The diets were kept in clean polyethylene bags.

Experimental design

The eight (8) experimental Wistar rats were divided into two groups of equal average weight and randomly assigned to the formulated diets. The groupings were as follow;

- A. Rats maintained on diet based on 100% starter`s mash and water (Control)
- B. Rats maintained on diet based on 1.5% inclusion level of mixed spices

Determination of feed intake and growth response

The rats were maintained on their respective experimental diets and water *ad libitum* for six weeks. To determine daily feed consumed, the quantity of feed left over was subtracted from the quantity of feed served. The average weekly feed consumptions were calculated from the daily consumptions. To determine average weekly body weight change, rats were weighed at the commencement of the experiment and thereafter on weekly basis (Idoko *et al.*, 2015).

Biochemical analysis

The weekly fasting blood glucose level was determined using glucometer. Serum lipid profiles (Total cholesterol (TC), Triglyceride and HDL-C) were determined enzymatically using lipid profile enzyme assay kits from Randox Laboratories Ltd. while LDL-C was determined using the formula $TC-TG/2.2-HDL$ (Friedewald *et al.*, 1972). Atherogenic index was computed from the determined lipid profiles;

Atherogenic index (AI) = $\log(\text{triglyceride}/\text{HDL-C})$.

Automated haematology analyser, SYSMEX KX21- JAPAN was used in estimating haematological parameters.

The activities of transaminases (aspartate aminotransaminase and alanine aminotransaminase) were determined using aspartate -aminotransferase and alanine-aminotransferase Randox assay Kits based on the method of Reitman and Frankel (1957) modified by Schmidt and Schmidt (1963).

Statistical analysis

Using SPSS (model 15) statistical package, independent sample T- test were used to compare the means. Differences between groups were considered significant at $p < 0.05$ and not significant at $p > 0.05$ confidence levels.

RESULT AND DISCUSSION

Growth performance of Wistar rats maintained on 1.5% mixed spices-supplemented diet:

The rate of feed consumption, feed conversion ratio and weight gain in rats maintained on 1.5% mixed spices-supplemented diet did not differ significantly ($p > 0.05$) from the values obtained in the rats maintained on the control diet (Table 1). The non significant variation indicates that 1.5% mixed spices supplemented diet may not have affected the physical properties of the diet. Feed consumption in animals is usually affected by components of physical properties of diets such as taste and texture of the finished feeds (Idoko *et al.*, 2016). Adequate feed intake is necessary to meet the bodies` both caloric and nutritional needs. However, Karangiya *et al.* (2016) reported birds maintained on 1% mixed spices (ginger and garlic only) to have had significantly higher feed intake. Feed conversion ratio (FCR) as a measure of feed efficiency is basically the amount of feed which is served divided by the weight increase produced in the animals to which it is delivered (Dennis, 2014), and measures the efficiency with which the bodies of animal convert animal feed into the desired output (Dan, 2013) such as increase in weight. Lower FCR is usually indicative of better quality of the diet. The non significant change in weight gain is understandable since change in weight is more or less a function of feed intake and feed conversion ratio. Therefore, long term consumption of 1.5% mixed spices-supplemented diet may not affect consumption and growth rate in individuals.

The fasting blood glucose level of rats maintained on 1.5% mixed spices supplemented diet

Compared to the initial blood glucose level, 1.5% supplemented diet did not cause significant variation ($p > 0.05$) in fasting blood glucose level (Table 2). Poorly controlled hyperglycaemia can cause distressing symptoms and can severely impair an individual`s quality of life; life expectancy can be reduced, with the main causes of death being renal and cardiovascular disease (Soedamah-Muthu *et al.*, 2006). The brain depends on a continual supply of glucose and is vulnerable to hypoglycaemia. Unable to synthesize or store this primary source of energy, the brain is one of the first organs affected by lowered blood glucose levels (Zammit and Frier, 2005; Cryer *et al.*, 2003). Symptoms and signs associated with hypoglycaemia include shakiness, anxiety, nervousness, palpitations, sweating, dry mouth, pallor, and pupil dilation (Cryer, 2004). Other symptoms include diaphoresis, hunger, and paresthesias (Cryer *et al.*, 2003). It is evident from the finding in this work that though these spices are reported to have hypoglycaemic effect on induced hyperglycaemia (Eyo *et al.*, 2011; Lawal *et al.*, 2016), it may not have effect on normal rats when used as feed supplement.

Table 1: Growth performance of Wistar rats maintained on 1.5% mixed spices-supplemented diet

	A	B
Feed consumed (g)	94.98±2.55 ^a	103.44±3.38 ^a
Feed conversion ratio	6.40±1.46 ^a	7.18±1.73 ^a
Weight gained (g)	20.03±2.19 ^a	23.38±1.88 ^a

Results are means of 4 determinations ± SEM. Values along the row with the same superscript are not significantly different (P>0.05), and are significantly different if the superscripts are different.

Group A: Rats maintained on diet based on 100% starters mash (control) and water.

Group B: Rats maintained on diet based on 1.5% mixed spices- supplemented diet and water

Table 2: The fasting blood glucose level (mg/dl) of rats maintained on 1.5% mixed spices supplemented diet.

	Initial	WK1	WK2	WK3	WK4	WK5
Group A	97.40±5.46 ^a	89.80±4.97 ^a	80.00±2.43 ^a	109.20±7.59 ^a	98.80±5.95 ^a	86.80±8.44 ^a
Group B	101.80±3.54 ^a	113.00±3.27 ^a	96.60±4.98 ^a	110.80±11.68 ^a	101.60±2.23 ^a	99.20±6.52 ^a

Results are means of 4 determinations ± SEM. Values along the row with the same superscript are not significantly different (P>0.05), and are significantly different if the superscripts are different.

Group A: Rats maintained on diet based on 100% starter`s mesh (control) and water.

Group B: Rats maintained on diet based on 1.5% mixed spices- supplemented diet and water

WK= week

Haematological profile of rats maintained on 1.5% mixed spices supplemented diet

With the exception of mean corpuscular haemoglobin concentration (MCHC) and White blood cells (WBC), all determined haematological parameters were significantly higher (P<0.05) in the rats maintained on 1.5% mixed spices-supplemented diet as compared to the control. However, concentrations of MCHC and WBC did not vary significantly (Table 3). Haematology is a branch of internal medicine that deals with the physiology, pathology, aetiology, diagnosis, treatment, prognosis and prevention of blood-related disorders, and forms one of the important means of determining the deleterious effect of foreign compounds on the blood (Reddy *et al.*, 2012). The significant increase in the red blood cell indices (packed cell volume, haemoglobin and red blood cell count) could be due to improved erythropoiesis. Garlic and onion had been reported to contain compounds that improve iron metabolism by promoting its bioavailability from food grains (Enitan, 2012). Iron is needed to produce the haem component of haemoglobin. These spices are also known to contain phytochemicals that are believed to be important in

stimulation of the immune system and organs related to blood cell formation particularly the bone marrow (Jeorg and Lee, 1998). Many of the phytochemicals act as antioxidants and have good free radical scavenging properties, protecting the red blood cells against oxidative damage. Banerjee and Maulik (2000) reported that prolonged feeding of rats with garlic and ginger may lead to hypoxia thereby stimulating red blood cell production in the bone marrow. However, report by Joshua (2015) showed that aqueous extract of ginger and garlic could cause significant decrease in the studied haematological parameters.

Lipid profiles of rats maintained on 1.5% mixed spices supplemented diet

Rats maintained on 1.5% mixed spices-supplemented diet had significantly (P<0.05) lower serum concentrations of triglyceride, LDL-C and atherogenic index but significantly (P<0.05) higher HDL-C. There was no significant variation in the total cholesterol concentration (Table 4). The significantly higher HDL-C, and lower LDL-C, triglyceride and atherogenic index in rats maintained on 1.5% mixed spices supplemented diet could be due to improved lipid

metabolism in the rats. Spices contain bioactive compounds that are of health benefits. Allicin, for instance which is one of the most bioactive compounds in garlic is thought to have hypolipidemic effect (Lawson, 1998) due to its inhibitory effect on cholesterol synthesis (Katherine and Karyn, 2004). The LDL-C lowering effect of spices is instructive since LDL-C is the major atherogenic lipoprotein. Nwachukwu and Iweala (2013) had reported hypolipidemic effect of mixed spices of ginger, garlic and black pepper in guinea

pigs. Takasaki (2005) and Dobiášová (2004) reported atherogenic indices to be powerful indicators of the risk of atherosclerosis with increase in their values indicating increased risk of developing cardiovascular diseases. Diets supplemented with mixed spices may therefore reduce the risk of atherosclerosis and other cardiovascular diseases caused by imbalance in lipid metabolism

Table 3: Haematological profiles of rats maintained on 1.5% mixed spices supplemented diet

	Group A	Group B
PVC (%)	35.33±0.88 ^a	47.00±0.00 ^b
HB(g/dL)	11.43±0.13 ^a	15.67±0.00 ^b
MCHC(g/dL)	33.39±0.04 ^a	33.28±0.02 ^a
RBC (10⁶/uL)	1.61±0.05 ^a	2.00±0.10 ^b
WBC(10³/uL)	2.10±0.12 ^a	2.33±0.12 ^a
PLATLET(10³/uL)	485.00±1.00 ^a	475.00±6.35 ^a

Results are means of 3 determinations ± SEM .Values along the row with the same superscript are not significantly different (P>0.05), and are significantly different if the superscripts are different.

Group A: Rats maintained on diet based on 100% starters mash (control) and water.

Group B: Rats maintained on diet based on 1.5% mixed spices- supplemented diet and water

MCHC: Mean corpuscular haemoglobin concentration

RBC: Red blood cells

HB: Haemoglobin

PCV: Packed cell volume

WBC: White blood cells

Table 4: Lipid profiles of rats maintained on mixed spices supplemented-diets

Parameter	Group A	Group B
Total Cholesterol(mmol/l)	0.23±0.01 ^a	0.21±0.02 ^a
Triglyceride(mmol/l)	0.41±0.03 ^a	0.37±0.01 ^b
HDL-C(mmol/l)	2.86±0.04 ^a	2.91±0.06 ^b
LDL-C	0.26±0.01 ^a	0.23±0.03 ^b
AI	-0.85±0.02 ^a	-0.89±0.01 ^b

Results are means of 3 determinations ± SEM .Values along the row with the same superscript are not significantly different (P>0.05), and are significantly different if the superscripts are different.

Group A: Rats maintained on diet based on 100% starters mash (control) and water.

Group B: Rats maintained on diet based on 1.5% mixed spices- supplemented diet and water

HDL-C- High density lipoprotein-cholesterol

LDL-C- Low density lipoprotein-cholesterol

AI- Atherogenic Index

Table 5: Serum transaminase activities in rats maintained on mixed spices-supplemented diet

Parameter	Group A	Group B
ALT(U/I)	54.67 ± 2.73 ^a	39.00 ± 2.08 ^b
AST(U/I)	49.00 ± 1.53 ^a	27.00 ± 2.52 ^b

Values are expressed as mean of 4 determinations ± S.E.M. Values along the row with the same superscript are not significantly different ($P > 0.05$), and are significantly different if the superscripts are different.

Group A: Rats maintained on diet based on 100% starters mesh (control) and water.

Group B: Rats maintained on diet based on 1.5% mixed spices- supplemented diet and water

Serum transaminase activities in rats maintained on 1.5% mixed spices supplemented diet

The serum activities of alanine-aminotransferase (ALT) and aspartate transaminase (AST) of rats fed on 1.5 % mixed spices supplemented diet were significantly decreased ($P < 0.05$) compared with rats fed on the control diet (Table 5). The observed significant decrease in activities of serum ALT and AST could mean that 1.5 % mixed spices might have decreased the liver membrane permeability. When damaged, membranes of liver cells can become permeable, allowing for escape of aspartate transaminase and alanine-aminotransferase into the bloodstream (Green and Flamm, 2002). They are therefore used as biomarkers to predict possible toxicity in some organs such as liver cytolysis (Shahjahan *et al.*, 2004). This finding is in agreement with Joshua (2015) who reported that the combination of garlic and ginger led to a significant reduction in serum activities of the liver enzymes and preserved structural integrity of liver tissues. Alef *et al.* (2013) reported that the administration of whole ginger markedly attenuated lead induced hepatotoxicity in rats as indicated by significant decrease in AST activity of lead exposed rats after ginger treatment. The observed decrease in serum activities of these enzymes may therefore be due to improved structural integrity and not due to decreased synthesis. Long term consumption of 1.5% mixed spices-supplemented diet may therefore have curative effect on the liver.

CONCLUSION

This study having examined growth response and biochemical changes in Wistar rats fed on mixed spices (cloves, ginger, garlic and onion) supplemented diet, it is concluded that use of mixed spices-supplemented diet may not influence weight gain and may not alter fasting blood glucose level of an individual. Consumption of mixed spices-supplemented diet may also have a curative effect on diseased or damaged liver and reduce the risk of atherogenic complications. However, further investigation on the cause of decreased serum ALT and AST rats fed on 1.5 % mixed spices supplemented diet is necessary since it could also be due to decreased synthesis of the enzymes in the organs.

REFERENCES

- Abaje, I.B., Ogoh, A.O., Amos, B.B and Abashiya, M. (2015). Climate Change, Flood Disaster Assessment and Human Security in Katsina State. *Nigeria American Journal of Human Ecology*, 4 (4):47-56
- Al-Amin, Z.M., Thomson, M., Al-Qattan, K.K., Peltonen-Shalaby, R., and Ali, M. (2006). Anti-diabetic and hypolipidaemic properties of ginger (*Zingiber officinale*) in streptozotocin-induced diabetic rats, *British Journal of Nutrition* 96(4):660-666
- Banerjee, S.K. and Maulik, S.K. (2000). Effect of garlic on cardiovascular disorders: A review. *Nutrition Journal*, 1(1):1-4
- Blaire, M. (2016). Adding Health Benefits and Flavor to Your Food With Herbs and Spices. <https://vanderbilt.edu/olli/class-materials/2016SummerWellnessSpices.pdf>. Accessed 25/10/2017
- Katherine, B.C and Karyn, M.S. (2004). The ABC clinical guide to herbs. *Journal of Natural Products* 67(6):1074-1075
- National Academy of Sciences. (2011). Care and guide for the use of laboratory animals... <https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf>
- Cryer, P.E., Davis, S.N. and Shamoan, H. (2003): Hypoglycemia in diabetes. *Diabetes Care*, 26:1902–1912
- Cryer, P.E. (2004). Current concepts: Diverse causes of hypoglycemia-associated autonomic failure in diabetes. *The New England Journal of Medicine* 350:2272–2279
- Dan, S. (2013). Beef Cattle Feed Efficiency University of Illinois lib.dr.iastate.edu/cgi/viewcontent.cgi?article=1027&context=driftlessconference. Accessed 05/11/2017

- Dennis, D. (2014). Feed conversion ratio: critically important but often misused. https://web.archive.org/web/20140503042415/http://www.pig333.com/swin_markets_commentaries/feed-conversion-ratio-critically-important-but-often-misused_8570/. Accessed 10/11/2017
- Dobiášová, M. (2004). Atherogenic Index of Plasma [log(triglyceride/HDLCholesterol)]: Theoretical and Practical Implications. *Clinical Chemistry*, 50 (7): 1113-1115.
- Enitan, S. S., Ajeigbe, K.O., Josiah, S. J. and Ehiaghe, F. A. (2012). Haematological and hepatotoxic potential of Onion (*allium cepa*) and garlic (*allium sativum*) extracts in rats. *European Journal of Medicinal Plants* 2(4): 290-307
- Eyo, J.E., Ozougwu, J.C. and Echi, P.C. (2011). Hypoglycaemic effects of *alliumcepa*, *alliumsativum* and *zingiberofficinale* aqueous extracts on alloxan-induced diabetic *rattusnovergicus*. *Medical journal of Islamic world academy of sciences* 19 (3): 121-126
- Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. (1972). Estimation of low-density lipoprotein cholesterol without the use of the preparative ultracentrifuge. *Clinical Chemistry*, 18 (6): 499-502
- Green, R.M. and Flamm, S. (2002). AGA Technical review on the evaluation of liver chemistry tests. *Gastroenterology*, 123 (4):1367-1384.
- Idoko, A. S., Lawal, N., and Ilouno, L. E. (2015). Studies on the Oxidative Balance in Rats Maintained on Ripe Musa paradisiaca Peel. *International Journal of Biochemistry Research & Review* 10(3): 1-7
- Idoko, A.S; Oladiji, A.T. and Ilouno, L. E. (2016). Growth Performance of Rats Maintained On Citrullus colocynthis Seed Coat-based Diet. *IOSR Journal of Biotechnology and Biochemistry Volume* 1(4): 09-14
- Jeorg, H.G. and Lee, Y.W. (1998). Protective effect of daily sulphide on Nnitrosodimethylamine-induced immunosuppression in mice. *Cancer Letters*, 11, 73-79
- Karangiya, V.K., Savsani, H.H., Shrikant, S.P., Garg, D.D., Murthy, K.S., Ribadiya, N.K. and Ekariya, S.J. (2016). Effect of dietary supplementation of garlic, ginger and their combination on feed intake, growth performance and economics in commercial broilers. *Veterinary World*, 9(3): 245-250.
- Lawal, N., Idoko, A. S. and Sullubawa, A. Z. (2016). Serum Glucose, Lipid Profile and Oxidative Stress Markers of Salt-induced Metabolic Syndrome Rats Administered Antioxidant Rich Nutraceutical. *International Journal of Biochemistry Research & Review* 10(3): 1-7
- Lawson, L.D. (1998): *Phytomedicines of Europe: Chemistry and Biological Activity*. Washington DC: ACS Symposium Series 91, American Chemical Society, 176-209
- Li, Y., Tran, V.H., Kota, B.P., Nammi, S., Duke, C.C. and Roufogalis, B.D. (2014). Preventative effect of *Zingiber officinale* on insulin resistance in a high-fat high-carbohydrate diet-fed rat model and its mechanism of action. *Basic & Clinical Pharmacology & Toxicology*, 115:209-215.
- Nwachukwu, N. and Iweala, E. E. J. (2013). The influence of mixed spiced diet on haematological properties and lipid profile of guinea pigs. *Journal of Medicinal Plants Research* 7(1): 1-6
- Rajesh, K. M., Anil, K. and Ashok, K. (2012). Pharmacological Activity of *Zingiber officinale*. *International journal of pharmaceutical and chemical sciences* 1(3): 1422-1427
- Reddy, G.D., Reddy, A.G., Rao, G.S. and Kumar, M.V. (2012). Pharmacokinetic interaction of garlic and atorvastatin in dyslipidemic rats. *Indian Journal of Pharmacology*, 44: 246- 252.
- Reitman, S. and Frankel, S. (1957). A Colorimetric Method for the Determination of Serum GOT and GPT. *American Journal of Clinical Pathology* 28:56-63
- Saravanan, G., Ponmurugan, P., Deepa, M.A. and Senthilkumar, B. (2014). Anti-obesity action of gingerol: effect on lipid profile, insulin, leptin, amylase and lipase in male obese rats induced by a high-fat diet. *The Journal of the Science of Food and Agriculture* 94 (14). <https://doi.org/10.1002/jsfa.6642>
- Schmidt, E., and Schmidt, F.W. (1963). Determination of serum GOT and GPT. *Enzy. Biol. Chem.* 3.1
- Soedamah-Muthu, S.S., Fuller, J.H. and Mulnier, H.E. (2006) All-cause mortality rates in patients with type 1 diabetes mellitus compared with a non-diabetic population from the UK general research database, 1992-1999. *Diabetologia* 49:660-6
- Takasaki, Y. (2005). Serum Lipid Levels and Factors Affecting atherogenic Index in Japanese Children. *Journal of Physiological Anthropology and Applied Human Science*, 24 (4): 511-515.
- Talal, A. Z. and Atef, M.A. (2007). Effects of ginger and clove oils on some physiological parameters in streptozotocin-diabetic and non-diabetic rats. *Journal of Medical Sciences*, 7(2):267-275

United Nations Industrial Development organization (UNIDO) and Food and Agriculture Organization of the United Nations (FAO) (2005). Herbs, spices and essential oils post-harvest operations in developing countries.

Zammit, N.N. and Frier, B.M. (2005). Hypoglycemia in type 2 diabetes. *Diabetes Care* 28:2948–2961