EVALUATION OF THE ACTIVITY OF ZINGIBER OFFICINALE (GINGER) AQUEOUS EXTRACTS ON ALLOXAN-INDUCED DIABETIC RATS.

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Summary

In view of the varied medicinal applications of Z. officinale, the effects of the increasing dosages of its aqueous extracts on alloxan - induced diabetic Rattus novergicus for possible use in the management of diabetes mellitus was evaluated. Diabetes mellitus was induced in 54 out of a total of 63 adult Rattus novergicus using 150mg/kg of alloxan monohydrate. Increasing dosages (200, 250 and 300mg/kg) of Z. officinale aqueous extracts were given to the diabetic rats for six weeks while the control rats got either normal saline (1ml) or increasing dosages of glibenclamide ( 2.5, 3.8 and 5.0mg/kg) during the same period. Blood glucose level, total serum lipids and total serum cholesterol were assessed with routine methods. F-LSD was employed to test significant differences (P < 0.05) among treatment means. Increasing dosages of Z. officinale aqueous extracts produced a dose-dependent significant (P < 0.05) reductions in the blood glucose levels, total serum lipid and total serum cholesterol when compared with that of the control rats. The best percentage reduction in blood glucose level, total serum lipids and cholesterol were observed at 300mg/kg. It is possible to conclude from the experimental findings that Z. officinale studied exhibited promising hypoglycaemic and hypolipidaemic activity in alloxan-induced diabetic rats. It’s hypoglycaemic and hypolipidaemic effects could represent a protective mechanism against the development of hyperglycaemia and hyperlipidaemia characteristic of diabetes mellitus.

Keywords: Zingiber officinale, hypoglycaemia, hypolipidaemia, alloxan diabetic rats

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**Introduction**

*Z. officinale* is one of the most widely used members of the family Zingiberaceae and is a common condiment for various foods and beverages. *Z. officinale* has a long history of medicinal use dating back to 2,500 years in China and India for conditions such as headaches, nausea, and rheumatism and cold (1). They are characterized in traditional Chinese medicine as spicy and hot. *Z. officinale* has been reported to warm the body and treat cold extremities, improve a weak and tachy pulse, address a pale complexion and strengthen the body after blood loss (2).

The major pungent compounds in *Z. officinale*, from studies of the lipophilic rhizome extracts have yielded potentially active gingerols which can be converted to shogaols, zingerone and paradol (3). The compounds 6 - gingerol and 6 - shogaol have been shown to have a number of pharmacological activities, including antipyretic, analgesic, antitussive and hypotensive effects (4). *Z. officinale* extracts exhibit inhibition of platelet aggregation and thromboxane synthesis *in vitro* (5,6) which has led to concerns *Z. officinale* extracts may prolong bleeding, however, several European studies using *Z. officinale* orally did not find any significant anti coagulant effects *in vivo* (7). There is scanty of research work on the hypoglycaemic and hypolipidaemic effects of *Z. officinale* and hence the need to embark on this study became necessary.

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia (high blood sugar) with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (8). In 2006, according to the World Health Organization, at least 171 million people world wide suffer from diabetes (9). The incidence is increasing rapidly and it is estimated that by the year 2030, this number will double (9). Diabetes is a common and very prevalent disease affecting the citizens of both developed and developing countries (10). The greatest increase in prevalence is however expected to occur in Asia and Africa, where more patients will likely be found by 2030. The national diabetes information clearing house estimates that the management of diabetes mellitus costs $132 billion in the United States alone every year. (9) Pointed out that 1 in 3 Americans born after year 2000 may develop diabetes in their lifetime. Statistical projections from India suggested that the number of diabetes will rise from 15 million in 1995 to 57 million in the year 2025, thus making India the country with the highest number of diabetics in the world (11, 12). The use of herbal products for medicinal benefits has played an important role in nearly every culture on earth and for many years, the search for anti-diabetic products will continue to focus on plants and other natural resources (13). The cost of administrating modern antidiabetic drugs is beyond the reach of most people in the low income group and those living in the rural areas, hence the use of plants for the treatment of common diseases such as diabetes are very common. In line with the (14) expert committee on diabetes which recommends that traditional methods of management of diabetes should be further investigated. Also considering the economic resource constraints and cheapness of these herbal products, this present study was designed to determine the effects of increasing dosages of *Z. officinale* (Ginger) on alloxan induced diabetic *R. novergicus* and its possible mechanisms of action, for possible use in the control of hyperglycaemia and hyperlipidaemia characteristic of diabetes mellitus.
Materials and Methods

Plant Material
The *Z. officinale* used for the experiment was bought from the Ogige Market, Nsukka, Nigeria. The plants were identified to species level at the Herbarium unit, Department of Botany, University of Nigeria, Nsukka where voucher specimen were kept.

Animal Model
Sixty three (63) adult white wistar strain albino rats (*R. norvegicus*) weighing 200 to 250g, bred in the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. They were fed *ad libitum* with 30% crude protein (Guinea feed) commercial feed. They were allowed to acclimatize under standard photoperiodic condition in a clean rat cage in the Physiology Research Laboratory, Department of Zoology, University of Nigeria, Nsukka. All animals were maintained under the standard laboratory condition for temperature (26 ± 2°C) and light (12 hours day length) and were allowed free access to food and water.

Preparation of Plant Extracts
The methods of (16) and (17) were used. Fresh health plant of *Z. officinale* (2000 g) were washed, cut into small pieces and homogenized in a waring blender. The resulting mixture was soaked in 2L of distilled water. The mixture was allowed to stand for twenty four hours with intermittent shaking. Following filtration, the filtrates were heated to dryness in a water bath and the weight of the crude extract determined. The extract was kept in refrigerator (4°C) thereafter. The extract was later reconstituted in normal saline (0.85% NaCl) at a concentration of 1g/ml before administration.

Induction Of Diabetes Mellitus
The methods of Osinubi *et al.* (13) and (18) were used to induce diabetes in the rats. 150 mg of alloxan per kg body weight of rat was administered intraperitoneally after overnight fast (access to only water) of twelve hours to make them more susceptible to developing diabetes. Only rats with serum glucose levels between (250 – 400 mg/dl) after two weeks were considered diabetic and used for the experiment.

Experimental Design
The study was carried out on alloxan- induced diabetic rats for six weeks. The animals were fasted for sixteen hours before each experiment and blood sample collected from the eye of the rats. All parameters assessed were determined before the extract treatments of the animals (initials) and subsequently evaluated weekly for six weeks. The experimental design was the three by three Latin square design using 63 rats divided into two major groups:

- **Group I**: nine non diabetic rats (non diabetic control).
- **Group II**: fifty four alloxan induced diabetic rats.

**Group I** rats were divided into 3 subgroups (Ia, Ib, Ic) of 3 rats each in different cages and receives 1.0ml of normal saline intraperitoneally daily.

**Group II** (fifty - four alloxan induced diabetic rats) were divided into 2 subgroups (IIa, IIb). Subgroups IIa, (twenty seven rats) were divided into 3 replicates (IIa1, IIa2, IIa3)
respectively, each replicate had three rats and received 200 mg/kg, 250 mg/kg or 300 mg/kg of *Z. officinale* aqueous extracts intraperitoneally daily.

The subgroups IIb was the diabetic control (twenty-seven rats) and were divided into 3 replicates (IId1, IId2 and IId3) each replicate had three rats and were administered 2.5mg/kg, 3.8mg/kg and 5.0mg/kg of standard antidiabetic drug (glibenclamide) daily for six weeks.

**Blood Glucose Level Determination**

The glucose in a protein-free supernatant prepared from whole blood, serum or plasma was heated with a solution of a primary aromatic amine, O-toluidine, in glacial acetic acid. A green colour produced, probably a glycosylamine, the absorbance of which was measured using a spectrophotometer at 630 nm wavelength. (19).

**Determinations of Total Serum Cholesterol**

The cholesterol of the serum was oxidised to a tetraene derivative by ferric ions derived from ferric perchlorate using four test tubes marked test, control, standard and blank. The absorbance was measured using spectrophotometer at 590nm wavelength and compared with that of a pure solution of cholesterol (19).

**Determination of Total Lipids in Serum**

Serum 0.05ml, was pipetted into a test tube (15ml), containing 2.00ml of concentration Sulphuric acid (d= 1.84). The tube was swirled carefully, closed with a glass ball and placed in a bath of boiling water for 10 minutes. After cooling in cold water, 0.1ml was transferred into another test tube (15ml), containing 2.5ml of phosphoric acid-vanillin reagent acid the solution was mixed carefully. The intensity of the pink colour that develops reaches its maximum after 30 minutes; it begins to fade after about 50minutes. The absorbance of the sample was measured at 546nm against the blank. The amount of lipid was read off an analytical care, which is obtained by analyzing four different amounts of total lipid of serum. Instead of total lipids, triolein was used as reference material. In this case, the values must be multiplied by a factor of 0.76. A standard solution of triolein (10g/L) was used (19).

**Data Analysis**

The data collected were pooled and analyzed for their central tendencies using descriptive statistic, values were expressed as mean ± standard deviation of the observations. F-LSD was employed to test the significant differences (P < 0.05) among treatment means. All analyses were performed using (20) for windows.

**Results**

**Blood glucose levels**

The increasing dosages (200, 250 and 300mg/k) of *Z. officinale* aqueous extracts produced dose-dependent significant (P < 0.05) reductions in the blood glucose levels of diabetic rats after 6 weeks of treatment when compared with that of the control rats (Table 1). *Z. officinale* at 200mg/kg reduce fasting blood glucose levels by 51.4% (303.3±35.8 to 147.2±2.3), at 250mg/kg it reduced it by 56.9% (319.0±58.0 to 137.3±2.2). while at 300mg/kg it reduced it by 56.7% (297.2±32.8 to 128.6±2.1).
Glibenclamide at 2.5mg/kg reduce fasting blood glucose levels by 76.4% (313.0±40.3 to 73.8±4.6), at 3.8mg/kg it reduced it by 80.1 % (319.4±54.0 to 63.6±2.2) while at 5.0mg/kg it reduced it by 81 % (310.7±35.0 to 59.0±1.6). The most effective percentage reduction in blood glucose level was observed at 300mg/kg. Normal saline at 1ml/kg had no effect on fasting blood glucose level.

**Total Serum Lipids**
The increasing dosages (200, 250 and 300mg/kg) of *Z. officinale* aqueous extracts produced a dose-dependent, significant (P < 0.05) reductions in the total serum lipids of diabetic rats after 6 weeks of treatment when compared with that of the control rats (Table 2). *Z. officinale* at 200mg/kg reduce total serum lipids by 15.6% (183.3±8.1 to 154.9±6.4), at 250mg/kg it reduced it by 18.0% (184.1±8.8 to 150.9±7.9) while at 300mg/kg it reduced it by 19.8 % (183.0±8.3 to 146.7±6.9). Glibenclamide at 2.5mg/kg reduce total serum lipids by 22.9% (183.7±7.4 to 141.6±4.9), at 3.8mg/kg it reduced it by 27.1 % (183.3±7.7 to 133.7±3.7) while at 5.0mg/kg it reduced it by 33.1 % (182.9±8.3 to 122.4±4.4). The most effective percentage reduction in total serum lipids was observed at 300mg/kg. Normal saline at 1ml/kg had no effect on total serum lipids.

**Total Serum Cholesterol.**
The increasing dosage (200, 250 and 300mg/kg) of *A. sativum* aqueous extracts produced dose-dependent significant (P < 0.05) reductions in the total serum cholesterol of diabetic rats after 6 weeks of treatment when compared with that of the control rats (Table 3). *Z. officinale* at 200mg/kg reduce total serum cholesterol by 7.5% (129.1±6.0 to 119.4±2.9), at 250mg/kg it reduced it by 12.9% (129.4±5.6 to 112.7±3.9) while at 300mg/kg reduced it by 16.1 % (130.7±4.4 to 109.7±7.5). Glibenclamide at 2.5mg/kg reduced total serum cholesterol by 22.9% (129.4±4.4 to 99.7±3.2), at 3.8mg/kg it reduced it by 29.5% (129.1±4.3 to 91.0±3.7) while at 5.0mg/kg it reduced it by 32.9 % (129.4±3.7 to 86.8±3.1) after 6 weeks of treatment. The most effective percentage reduction in total serum cholesterol was observed at 300mg/kg. Normal saline at 1ml/kg had no effects on total serum cholesterol.
Table 1: Effects of the increasing dosage of *Z. officinale* extracts on blood glucose level of alloxan diabetic *Rattus novergicus*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dosage</th>
<th>WK0</th>
<th>WK1</th>
<th>WK2</th>
<th>WK3</th>
<th>WK4</th>
<th>WK5</th>
<th>WK6</th>
<th>% reduction after 6 weeks</th>
</tr>
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<tbody>
<tr>
<td>NS</td>
<td>1.0ml</td>
<td>55.4±5.6</td>
<td>55.3±5.0</td>
<td>55.4±5.0</td>
<td>55.7±8.1</td>
<td>55.4±8.6</td>
<td>55.7±5.8</td>
<td>55.3±4.1</td>
<td>0.01</td>
</tr>
<tr>
<td>ZO</td>
<td>200mg</td>
<td>303.3±35.8</td>
<td>239.0±26.4</td>
<td>193.8±7.9</td>
<td>172.6±11.9</td>
<td>160.8±8.4</td>
<td>152.8±10.0</td>
<td>147.2±2.3</td>
<td>51.4</td>
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<tr>
<td>ZO</td>
<td>250mg</td>
<td>319.0±8.0</td>
<td>237.1±9.0</td>
<td>188.2±3.7</td>
<td>177.0±4.9</td>
<td>157.9±2.6</td>
<td>150.0±4.8</td>
<td>137.3±2.2</td>
<td>56.9</td>
</tr>
<tr>
<td>ZO</td>
<td>300mg</td>
<td>297.2±5.8</td>
<td>204.9±14.1</td>
<td>179.3±5.2</td>
<td>164.0±3.1</td>
<td>151.0±1.6</td>
<td>139.0±2.4</td>
<td>128.6±2.1</td>
<td>56.7</td>
</tr>
<tr>
<td>GL</td>
<td>2.5mg</td>
<td>313.0±4.0</td>
<td>152.6±20.6</td>
<td>118.0±5.0</td>
<td>108.7±2.1</td>
<td>102.8±1.6</td>
<td>98.8±3.8</td>
<td>73.8±2.6</td>
<td>76.4</td>
</tr>
<tr>
<td>GL</td>
<td>3.8mg</td>
<td>319.4±4.0</td>
<td>126.1±22.4</td>
<td>94.1±4.8</td>
<td>82.7±2.9</td>
<td>75.2±5.5</td>
<td>68.0±2.3</td>
<td>63.6±2.2</td>
<td>80.1</td>
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<tr>
<td>GL</td>
<td>5.0mg</td>
<td>310.7±35.0</td>
<td>108.4±11.4</td>
<td>85.0±2.7</td>
<td>77.8±2.2</td>
<td>72.6±1.8</td>
<td>65.3±2.0</td>
<td>59.0±1.6</td>
<td>81.0</td>
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</tbody>
</table>

Values given represent the Mean±SD of 9 observations. NS = Normal saline represents Non Diabetic Control, ZO = *Zingiber officinale* and GL = glibenclamide represents Diabetic control. P < 0.05, FLSD = 15.317

Table 2: Effects of the increasing dosage of *Z. officinale* extracts on total serum lipids of alloxan Diabetic *Rattus novergicus*. 

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<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dosage</th>
<th>WK0</th>
<th>WK1</th>
<th>WK2</th>
<th>WK3</th>
<th>WK4</th>
<th>WK5</th>
<th>WK6</th>
<th>% reduction after 6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>1.0ml</td>
<td>144.1±6.9</td>
<td>144.4±4.2</td>
<td>144.8±5.9</td>
<td>144.3±6.5</td>
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<td>144.1±7.9</td>
<td>144.0±7.9</td>
<td>0.02</td>
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<tr>
<td>ZO</td>
<td>200mg</td>
<td>183.4±8.1</td>
<td>176.3±7.0</td>
<td>176.1±4.7</td>
<td>170.6±4.3</td>
<td>165.8±3.0</td>
<td>163.8±2.0</td>
<td>154.9±6.4</td>
<td>15.6</td>
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<tr>
<td>ZO</td>
<td>250mg</td>
<td>184.1±8.8</td>
<td>174.7±5.1</td>
<td>169.4±4.8</td>
<td>168.4±2.1</td>
<td>164.8±1.1</td>
<td>158.7±1.9</td>
<td>150.9±7.9</td>
<td>18.0</td>
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<tr>
<td>ZO</td>
<td>300mg</td>
<td>183.0±8.3</td>
<td>173.0±4.0</td>
<td>166.9±1.5</td>
<td>163.7±1.4</td>
<td>160.3±1.2</td>
<td>156.1±0.8</td>
<td>146.7±6.9</td>
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<tr>
<td>GL</td>
<td>2.5mg</td>
<td>183.7±7.4</td>
<td>166.1±2.1</td>
<td>161.3±2.7</td>
<td>151.8±2.2</td>
<td>144.9±2.9</td>
<td>135.8±2.6</td>
<td>141.6±1.9</td>
<td>22.9</td>
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<tr>
<td>GL</td>
<td>3.8mg</td>
<td>183.3±7.7</td>
<td>164.8±2.3</td>
<td>158.7±2.6</td>
<td>154.6±2.7</td>
<td>141.0±1.4</td>
<td>137.8±3.7</td>
<td>133.7±3.7</td>
<td>27.1</td>
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<tr>
<td>GL</td>
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<td>182.9±8.3</td>
<td>163.6±1.8</td>
<td>155.0±3.1</td>
<td>146.1±2.9</td>
<td>136.0±3.8</td>
<td>126.8±3.9</td>
<td>122.4±1.4</td>
<td>33.1</td>
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</table>

Values given represent the Mean±SD of 9 observations. NS = Normal saline represents Non Diabetic Control, ZO = Zingiber officinale and GL = glibenclamide represents Diabetic control. P < 0.05, FLSD = 4.428
Table 3: Effect of the increasing dosage of *Z. officinale* extracts on serum cholesterol of alloxan diabetic *Rattus novergicus*.

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<th>Treatment</th>
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<th>WK1</th>
<th>WK2</th>
<th>WK3</th>
<th>WK4</th>
<th>WK5</th>
<th>WK6</th>
<th>% reduction after 6 weeks</th>
</tr>
</thead>
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<td>91.1±6.2</td>
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<td>91.1±4.2</td>
<td>91.0±5.2</td>
<td>0.0</td>
</tr>
<tr>
<td>ZO</td>
<td>200mg</td>
<td>129.1±5.0</td>
<td>132.2±5.6</td>
<td>130.4±0.4</td>
<td>125.7±3.5</td>
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<td>111.9±3.4</td>
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<td>104.7±5.7</td>
<td>99.7±3.2</td>
<td>22.9</td>
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<tr>
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<td>119.1±2.9</td>
<td>110.8±2.4</td>
<td>105.8±2.1</td>
<td>101.6±3.5</td>
<td>97.3±2.5</td>
<td>91.0±3.7</td>
<td>29.5</td>
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<td>GL</td>
<td>5.0mg</td>
<td>129.4±3.7</td>
<td>115.8±2.8</td>
<td>109.3±2.0</td>
<td>102.9±2.3</td>
<td>96.0±3.4</td>
<td>97.6±5.3</td>
<td>86.8±3.1</td>
<td>32.9</td>
</tr>
</tbody>
</table>

Values given represent the Mean±SD of 9 observations. NS = Normal saline represents Non Diabetic Control, ZO = *Zingiber officinale* and GL = glibenclamide represents Diabetic control. P < 0.05, FLSD =3.67
Discussion

Hypoglycaemic effects

Diabetes mellitus is probably the fastest growing metabolic disease in the world and as knowledge of the multifactorial/heterogeneous nature of the disease increases so does the need for more challenging and appropriate therapies (21). Traditional plant remedies have been used for centuries in the treatment of diabetes (22), but only a few have been scientifically evaluated. Alloxan is known for its selective pancreatic islet $\beta$–cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals (23, 24). Generalised increase in the level of blood glucose during diabetes have been consistently reported both in animal models (25,26,27,28) and humans especially those suffering from insulin dependent diabetes mellitus (29). Ginger extract reduced blood glucose levels in a dose dependent manner, producing its best effects at 300mg/kg which corroborates some earlier reports (30, 31, 32, 32). Ginger extracts may act by stimulating insulin production from the pancreatic islets or by increasing peripheral utilization and inhibition of the proximal tubular reabsorption mechanism for glucose in the kidney which have a glucose lowering effect (33). It is not unlikely that ginger extracts might have corrected the hyperglycaemic induced imbalance in glucose metabolism by normalizing the blood glucose homeostasis through enhanced utilization and inhibition of glucose synthesis by increasing the NADP+ and NADPH ratio in the body, the consequence of which is a drop in glucose level in the blood of hyperglycaemic rats (34). The mechanism of the hypoglycaemic effects still remains speculative, further studies are required to unravel the mechanism of its hypoglycaemic action.

Hypolipidaemic effects

Alteration in serum lipids profile are known in diabetes, which are likely to increase the risk of coronary heart disease (35, 36, 37). The marked hyperlipidaemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots (38) Hypercholesterolemia has been reported to occur in alloxan diabetic rats (39, 40). Ginger extract reduced the elevated total serum cholesterol and lipids levels in diabetic rat models in a dose dependent manner, producing its best effects at 300mg/kg which is in line with some earlier reports (41, 42, 43). The mechanism of hypolipidaemic action may be due to decreased oxidative load, by directly scavenging the reactive oxygen metabolites, due to the presence of various antioxidant compounds (44). or by increasing the synthesis of antioxidant molecules.

Conclusions

In conclusion, the levels of total serum cholesterol, total serum lipids and blood glucose levels which were actually raised in alloxan induced diabetic rats can be lowered by ginger aqueous extracts. This hypoglycaemic and hypolipidaemic effects may be protective against the development of atherosclerosis, hyperlipidaemia and hyperglycaemia common in diabetes mellitus.
References