



Research Journal of  
**Medicinal  
Plant**

ISSN 1819-3455



Academic  
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### **Alterations in Serum Lipid Profile of Male Rats by Oral Administration of Aqueous Extract of *Fadogia agrestis* Stem**

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**Abstract:** The effects of repeated administration of aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem on serum lipid profile of male rats and their recovery tendencies for 10 days post-administration were investigated. Graded doses of 18, 50 and 100 mg kg<sup>-1</sup> body weight of the extracts were administered orally on daily basis for 28 days. Rats were sacrificed 24 h after their daily doses of 1, 14 and 28 while those for the recovery test were sacrificed 10 days after terminating their 28 daily administration. The serum lipid profile investigated included Total Cholesterol (TC), triacylglycerol (TG), high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C). The administration of the plant extract to the animals at all the doses produced significant increase ( $p < 0.05$ ) in the serum concentration of total cholesterol, triacylglycerols, high-density lipoprotein-cholesterol and low-density lipoprotein-cholesterol with no reversal towards their control by the end of 10 days post-treatment. The computed atherogenic index did not portend predisposition to atherosclerosis. The results indicated alterations in the serum lipid profile of the animals but these alterations are not sufficient enough to predispose the animals to atherosclerosis.

**Key words:** *Fadogia agrestis*, lipid profile, repeated administration, atherosclerosis, atherogenic index

### **INTRODUCTION**

The use of medicinal plants in the management of various illnesses is due to their phytochemical constituents and dates back to antiquity (Yakubu *et al.*, 2007). However, during the last decade, an increase in the use of medicinal plants has been observed in metropolitan areas of developed countries (Harnack *et al.*, 2001). Plants are extensively used to manage sexual dysfunction and have become known world wide as an instant treatment (Adimoelja, 2000; Yakubu *et al.*, 2007). Some of these herbs which include *Terminalia catapa* seeds (almond) have been shown to exhibit aphrodisiac action by receptor mediated action in the brain (Ratnasooriya and Dharmasiri, 2000); *Myristica fragrans* (nutmeg) act by nervine stimulating property (Tajuddin *et al.*, 2004) while *Tribulus terrestris* and *Fadogia agrestis* stem may be due to androgen increasing property (Gauthaman *et al.*, 2002; Yakubu *et al.*, 2005).

Medicinal plants with aphrodisiac potentials like *Asparagus racemosus* have been shown to alter serum lipids (Visavadiya and Narasimacharya, 2005) and as such, affect the normal functioning of the heart, the normal pumping action which is one of the factors responsible for normal erection of the male organ; the combination of increased inflow and decreased outflow of the blood rapidly raises intracavernosal pressure resulting in progressive penile rigidity and full erection. Lipid profile, which is altered in the serum of animals administered with extract of medicinal plants with aphrodisiac

potentials, appears to be a significant factor in the development of premature atherosclerosis. The elevation of serum total cholesterol and more importantly low density lipoprotein cholesterol have been implicated as primary risk factors for cardiovascular diseases like hypertension and atherosclerosis (Edijala *et al.*, 2005). Also, elevated serum levels of high density lipoprotein cholesterol leads to lowered atherosclerotic disease. Similarly, it has also been shown that high blood lipids are associated with hypertension and lipid peroxidation (Yakubu, 2006).

In men with hypertension, prevalence of Erectile Dysfunction (ED) is significantly higher than in the general population (Croog *et al.*, 1988; Dai *et al.*, 2000). In fact, 8-10% of untreated hypertensive patients are found to be suffering from ED once their hypertension is diagnosed (Lewis *et al.*, 2000). Hypertension is commonly associated with structural and functional modifications that take place at the level of the endothelium, vascular smooth muscle and extracellular matrix of blood vessels (Chamiot-Clerc *et al.*, 2001). However, one of such plants used in managing ED is *Fadogia agrestis*.

*Fadogia agrestis* (Schweinf. Ex Hiern), (Rubiaceae) (Hausa: bakin gagai; English: black aphrodisiac) is an erect shrub, 1-3 feet high. The leaves and stem are yellowish and tomentellous. It has been shown that aqueous extract of *F. agrestis* stem which contain alkaloids, saponins, flavonoids and anthraquinones also is an aphrodisiac (sex enhancing potentials) by causing increase in serum testosterone concentration (Yakubu *et al.*, 2005). *F. agrestis* is one of several plants commonly used in Nigeria in the management of erectile dysfunction without information as to whether or not it could predispose the users to atherosclerosis and its associated coronary heart disease.

Since there has been no report on the lipid profile effects of *F. agrestis*, a commonly used plant in the management of erectile dysfunction, the present study was undertaken to investigate the effect of aqueous extract of *F. agrestis* stem on male rat serum lipids. Several workers (Castelli *et al.*, 1986; Ng *et al.*, 1997; Owoyele *et al.*, 2005; Abolaji *et al.*, 2007) have used parameters of serum lipids like total cholesterol, triacylglycerol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol and computed atherogenic index as indices of predisposition to atherosclerosis and hypertension, hence their use in this study.

## MATERIALS AND METHODS

### Plant Material and Authentication

The whole plant obtained between the periods of October, 2003 and May, 2004 from the herb sellers at Kulende Market, Ilorin, Nigeria was authenticated at the Department of Horticulture and Landscape Technology, Federal School of Forestry, Jos, Nigeria. A voucher specimen was deposited at the herbarium of the Department of Horticulture and Landscape Technology, Federal School of Forestry, Jos, Nigeria under a Voucher No. 2:108.

### Experimental Animals

Apparently healthy, male, white albino rats (*Rattus norvegicus*) weighing between 220-250 g were obtained from the Small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were housed in clean metabolic cages placed in well-ventilated house conditions (Temperature: 28-31°C; photoperiod: 12 h natural light and 12 h dark; humidity: 50-55%). They were also allowed free access to rat pellets (Bendel Feeds and Flour Mills Ltd., Ewu, Nigeria) and tap water. The cages were cleaned of waste twice daily at 12 h intervals.

### Reagents

All reagents used were of analytical grade and were prepared in all glass distilled water unless otherwise specified.

### **Preparation of Aqueous Extract of *Fadogia agrestis* Stem**

The wet stem cuttings (ranging between 37.20 and 42.83 g) were oven-dried at 40°C for 48 h to a constant weight (5.96-8.76 g). The dried pieces were thereafter pulverized using an electric blender (Blender/Miller III, model MS-223, China). The pulverized plant material was stocked in a plastic container from which 5 g of the powder was extracted in 100 mL of cold distilled water (5%, w/v) with constant shaking on a Stuart Scientific orbital shaker SO1, United Kingdom for 48 h at room temperature (26-28°C). The resulting solution was then filtered using filter paper (Whatman No. 1) after which it was concentrated on a steam bath to give between 0.53 and 0.60 g of the residue (0.57±0.049 g). Appropriately calculated amount of the residues (0.37, 1.00 and 2.00% w/v) were reconstituted in cold distilled water to give the equivalent dose of 18 mg kg<sup>-1</sup> body weight (value obtained from ethnobotanical survey), higher doses of 50 mg kg<sup>-1</sup> body weight and 100 mg kg<sup>-1</sup> body weight (all doses were as used in our study of the aphrodisiac potentials of the plant) (Yakubu *et al.*, 2005). The reconstituted aqueous extract was administered orally to all animals in the different groups using metal oropharyngeal cannula.

### **Animal Grouping and Extract Administration**

A total of eighty male rats were used and were randomly grouped into four: A, B, C and D of 20 animals each after being allowed to acclimatize for 2 weeks. Rats in groups B, C and D were administered with the plant extract once daily at 24 h interval at the dose of 18, 50 and 100 mg kg<sup>-1</sup> body weight, respectively for 28 days. Group A (control), were treated just like the test groups except that the animals received 1.0 mL of the vehicle (distilled water) instead of the plant extract. Five rats from the various groups were left for 10 days post administration to recover.

The extract and distilled water were administered daily at the same point time (08:00-08:45 h) throughout the duration of the experiment. The animals were allowed free access to rat pellets and tap water after the daily doses. Five rats each from groups A, B, C and D were sacrificed 24 h after 1, 14 and 28 daily doses while the remaining rats were sacrificed 24 h after 10 days of recovery period designated as day 38. The animals were strictly handled in line with the Principles of Laboratory Animal Care of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

### **Preparation of Serum**

The procedure described by Yakubu (2006) was used in the preparation of serum. Briefly, the rats were anaesthetized in ether vapour. When they became unconscious, their neck areas were cleared of fur to expose the jugular veins. These veins which were slightly displaced from the neck region (to prevent interstitial fluid from contaminating the blood) were cut with sterile scapel and made to bleed into clean, dry centrifuge tubes. The centrifuge tubes containing the blood were left at room temperature for 10 min after which they were centrifuged at 33.5 x g for 15 min with Uniscope Laboratory Centrifuge (model SM800B, Surgifriend Medicals, Essex, England). The resulting sera were then aspirated using Pasteur pipettes into clean, dry, sample bottles and were then stored frozen overnight before being used for the lipid assay.

### **Determination of Serum Lipids**

Serum total cholesterol concentration was estimated by the method described by Fredrickson *et al.* (1967) while serum Low-Density Lipoprotein Cholesterol (LDL-C) content was estimated by the method described by Demacker *et al.* (1984). Serum High-Density Lipoprotein Cholesterol (HDL-C) concentration was determined by the method described by Albers *et al.* (1978). Triacylglycerol concentration in the serum was estimated by the method described by Fossati and Prencipe (1982) while the method described by Ng *et al.* (1997) was used to compute the atherogenic index.

### Statistical Analysis

Data obtained were analyzed using Two-way analysis of variance complemented by Bonferroni post-test. Differences were considered statistically significant at  $p < 0.05$  (Mahajan, 1997).

## RESULTS

Extract administration at 50 and 100 mg kg<sup>-1</sup> body weight produced significant ( $p < 0.05$ ) increase in serum total cholesterol and triacylglycerol contents throughout the 28 days (Table 1, 2). While that of 18 mg kg body weight did not become significant ( $p > 0.05$ ) on serum total cholesterol concentration until after day 14 (Table 1), the same dose produced significant ( $p < 0.05$ ) increase in serum triacylglycerol concentration throughout the period of administration (Table 2).

There was also significant increase ( $p < 0.05$ ) in the serum High-Density Lipoprotein Cholesterol (HDL-C) content following the administration of the plant extract in all the dose groups (Table 3). The same pattern of increase ( $p < 0.05$ ) was obtained by the end of the 10 days post-treatment period (Table 3).

Extract administration for 28 days at 100 mg kg<sup>-1</sup> body weight produced significant ( $p < 0.05$ ) increase of about 2.5 times the control value in serum Low-Density Lipoprotein Cholesterol (LDL-C) content. However, the recovery period revealed significant ( $p < 0.05$ ) increase in serum LDL-C at higher doses (50 and 100 mg kg<sup>-1</sup> body weight) while the least dose produced reduction in the serum lipid (Table 4).

Table 1: Effect of administration of aqueous extract of *Fadogia agrestis* stem on serum total cholesterol concentration (mmol L<sup>-1</sup>) of male rats

| Doses (mg kg <sup>-1</sup><br>body weight) | Days        |             |             |             |
|--|-------------|-------------|-------------|-------------|
|  | 1           | 14          | 28          | 38          |
| Control                                    | 12.80±0.25  | 12.80±0.24  | 12.90±0.21  | 13.10±0.55  |
| 18   | 13.10±0.40  | 13.90±0.14  | 17.16±1.40* | 16.50±0.10* |
| 50   | 15.50±0.40* | 14.90±0.25* | 21.84±1.40* | 19.50±0.35* |
| 100  | 16.50±0.50* | 16.20±0.13* | 24.18±1.77* | 21.80±0.65* |

Values are mean of 5 replicates±SD; \*Compared with control,  $p < 0.05$

Table 2: Effect of administration of aqueous extract of *Fadogia agrestis* stem on serum triacylglycerol concentration (mmol L<sup>-1</sup>) of male rats

| Doses (mg kg <sup>-1</sup><br>body weight) | Days        |            |             |             |
|--|-------------|------------|-------------|-------------|
|  | 1           | 14         | 28          | 38          |
| Control                                    | 5.80±0.60   | 5.95±0.60  | 6.00±0.45   | 5.50±0.75   |
| 18   | 8.60±1.70*  | 7.55±0.90* | 12.00±0.25* | 7.10±0.30*  |
| 50   | 10.10±1.30* | 8.30±0.44* | 15.85±0.05* | 8.40±0.22*  |
| 100  | 7.30±0.75*  | 9.40±1.02* | 17.20±0.90* | 13.20±0.18* |

Values are mean of 5 replicates±SD; \*Compared with control,  $p < 0.05$

Table 3: Effect of administration of aqueous extract of *Fadogia agrestis* stem on serum high-density lipoprotein cholesterol (HDL-C) concentration (mmol L<sup>-1</sup>) of male rats

| Doses (mg kg <sup>-1</sup><br>body weight) | Days       |            |             |             |
|--|------------|------------|-------------|-------------|
|  | 1          | 14         | 28          | 38          |
| Control                                    | 5.70±0.80  | 5.90±0.04  | 5.80±0.42   | 5.50±0.11   |
| 18   | 8.50±0.95* | 7.30±0.35* | 7.88±0.28*  | 7.10±0.10*  |
| 50   | 8.40±0.55* | 8.10±0.65* | 10.57±0.07* | 8.20±0.12*  |
| 100  | 6.30±0.25  | 9.40±0.40* | 11.92±0.21* | 12.50±0.13* |

Values are mean of 5 replicates±SD; \*Compared with control,  $p < 0.05$

Table 4: Effect of administration of aqueous extract of *Fadogia agrestis* stem on serum low-density lipoprotein cholesterol (LDL-C) concentration (mmol L<sup>-1</sup>) of male rats

| Doses (mg kg <sup>-1</sup><br>body weight) | Days       |            |             |            |
|--|------------|------------|-------------|------------|
|  | 1          | 14         | 28          | 38         |
| Control                                    | 5.40±0.55  | 5.55±0.45  | 5.58±0.36   | 5.30±0.81  |
| 18   | 5.50±0.10  | 5.90±0.07  | 7.50±0.60*  | 4.10±0.30* |
| 50   | 5.50±0.35  | 5.70±0.15  | 10.14±0.48* | 7.10±0.33* |
| 100  | 7.80±0.65* | 7.40±0.25* | 13.20±0.75* | 9.20±0.23* |

Values are mean of 5 replicates±SD; \*Compared with control, p<0.05

Table 5: Effect of administration of aqueous extract of *Fadogia agrestis* stem on computed atherogenic index (LDLC/HDL-C) of male rats

| Doses (mg kg <sup>-1</sup><br>body weight) | Days       |            |            |            |
|--|------------|------------|------------|------------|
|  | 1          | 14         | 28         | 38         |
| Control                                    | 0.95±0.07  | 0.94±0.11  | 0.96±0.09  | 0.96±0.07  |
| 18   | 0.65±0.05* | 0.81±0.05* | 0.95±0.02  | 0.58±0.03* |
| 50   | 0.65±0.05* | 0.70±0.05* | 0.96±0.06  | 0.87±0.03* |
| 100  | 1.24±0.26* | 0.79±0.06* | 1.11±0.04* | 0.74±0.04* |

Values are mean of 5 replicates±SD; \*Compared with control, p<0.05

Compared with the control, extract administration at 18 and 50 mg kg<sup>-1</sup> body weight produced significant reduction (p<0.05) in the atherogenic index while that of 100 mg kg<sup>-1</sup> body weight resulted in significant increase (p<0.05) in the index. The recovery period produced significant reduction (p<0.05) in the atherogenic index in all the dose groups (Table 5).

## DISCUSSION

Alterations in the concentration of major lipids like cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides can give useful information about the functioning of the heart (Chawla, 1999; Abolaji *et al.*, 2007) as it relates to the organ's predisposition to atherosclerosis and its associated coronary heart diseases. Elevated levels of all lipids except the HDL-C are associated with an increased risk of atherosclerosis. High levels of triglycerides and LDLs are associated with coronary artery diseases. In the present study, significant proportionate increases in the concentration of various serum lipids have been observed following the administration of aqueous extract of *F. agrestis* stem for 28 days. These patterns were not different during the recovery period.

The increase in serum cholesterol concentration of the extract dosed group (Table 1) might be due to increase in the concentration of acetyl CoA arising probably from increased  $\beta$ -oxidation of fatty acids, since acetyl CoA is a key substrate in the biosynthesis of cholesterol (Rang *et al.*, 1995). Such increase in serum cholesterol concentration may be harmful as it may help increase the incidence of atherosclerosis and hypertension (Enas, 1999). Such increase in atherosclerosis and hypertension may aggravate erectile dysfunction (Feldman *et al.*, 1994).

Triacylglycerols are the main storage form of fatty acids. The significant increase in serum triacylglycerol concentration following the administration of the extract (Table 2) might be due to accelerated lipolysis. This may imply depletion in the store of fatty acids. Adebayo *et al.* (2006) have shown that triacylglycerol has clinical value in assessing coronary heart disease. Similarly, Patsch (1993) and Ng *et al.* (1997) have shown that cardiovascular diseased patients had markedly elevated levels of triacylglycerols but reduced HDL-C levels apparently caused by the metabolism of triacylglycerol-rich lipoproteins on the HDL-C, particularly the subfraction HDL<sub>2</sub> which has a negative association with cardiovascular disease risk (Miller *et al.*, 1981). However, such an inverse relationship is not the case in this study, instead, there exist a direct relationship. This direct relationship further indicate that the plant extract may not potentate cardiovascular risk.

HDL-Cholesterol is considered to have anti-atherogenic properties, since there is negative correlation between HDL-cholesterol and risk of cardiovascular disease. HDL-C transports cholesterol from peripheral tissues to the liver thereby reducing the amount stored in the tissue and the possibility of developing atherosclerotic plaques and hence termed good cholesterol. The increase in High-Density Lipoprotein Cholesterol (HDL-C), employed (Table 3) may also be clinically beneficial to the animal. It has been demonstrated that an increase in the concentration of HDL-C correlates inversely with coronary heart disease (Philip, 1995; Mayes, 1996). Since HDL-C removes cholesterol to the liver for excretion, the increase in HDL-C will be appropriate for the increased total cholesterol (Table 3) and thus reduce the risk of coronary artery disease.

Low-Density Lipoprotein Cholesterol (LDL-C) are primary carriers of plasma cholesterol. LDL-C is referred to as bad cholesterol because it builds up slowly in the walls of arteries feeding the heart and brain. As a result of this, it forms plaque that clots the arteries thereby causing atherosclerosis and increasing the risk of high blood pressure (Jackson, 1996) which may eventually lead to stroke. The significant increase in serum LDL-C (Table 4) is quite understandable since an increase in serum total cholesterol (Table 1) should normally result in similar increase in serum LDL-C. The increase in LDL-C, notwithstanding, the association between elevated levels of LDL-C with an increased risk of coronary heart disease (Woo *et al.*, 2002) may not constitute threat to the health and well being of the animals since there was also corresponding increase in the serum concentration of HDL-C.

The ratio of LDL-cholesterol to HDL-cholesterol referred to as atherogenic index has also been used as indicator of cardiovascular diseases (Panagiotakos *et al.*, 2003). According to Ng *et al.* (1997), the cut-offs for high risk of atherosclerosis was put at atherogenic index of greater than 5. Since the values for all the extract dosed groups are less than 5, it could be inferred that the extract does not possess positive risk for atherogenesis. Therefore, the atherogenic index as computed (Table 5) might be an indication that the extract of *F. agrestis* stem may not predispose to atherogenesis and associated coronary heart diseases despite the various alterations produced on the serum lipids. The fact that the patterns exhibited during the period of administration (day 28) are not significantly different from those obtained during the recovery periods may be an indication of permanent alteration in the lipid metabolism and that the animals could not recover by the 10 days recovery period.

In conclusion, this study has shown that aqueous extract of *Fadogia agrestis* stem has stimulatory effect on the lipid metabolism by increasing the concentrations of the serum lipids. The permanent alterations in the serum lipids which are of proportionate increase, notwithstanding, are not sufficient enough to predispose the heart of the animals to atherosclerosis and its associated coronary heart disease.

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