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HYPOGLYCAEMIC AND BIOCHEMICAL PROPERTIES OF *CNESTIS FERRUGINEA*

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### Abstract

Increasing incidences of diabetes in Africa has prompted the search for safe and readily available alternative herbal remedies for the treatment of diabetes mellitus. *Cnestis ferruginea* was extracted with methanol and ethylacetate and the extracts obtained were tested for hypoglycaemic activities in streptozotocin (STZ)-induced diabetic rats and mice. The extracts (250mg/kg body weight) were administered orally for 10 consecutive days to STZ-induced diabetic rats while a single dose (250mg/kg body weight) of the extracts were administered to STZ-induced diabetic mice. Fasting blood glucose (FBG) levels were determined in the two groups of animals after extract administration. There was significant reduction in FBG ( $P < 0.005$ ) by MCF and ECF within 4 hrs of extract administration in a time- dependent manner. Furthermore, administration of MCF and ECF for 10 days significantly lowered FBG in STZ diabetic rats ( $P < 0.005$ ) by 74% and 68%, respectively, whereas, glibenclamide - a standard antidiabetic drug reduced FBG by 60%. The levels of serum creatinine, urea, triglyceride, total cholesterol, total protein and level of lipid peroxidation were also evaluated. The extracts reduced significantly ( $P < 0.005$ ) the elevated levels of serum ALT and AST in diabetic treated rats. Similarly, both extracts significantly lowered ( $P < 0.005$ ) the levels of serum creatinine, urea, total cholesterol, triglyceride and thiobarbituric acid reactive species (TBARS). These results suggest that *Cnestis ferruginea* leaves contain a highly potent hypoglycaemic principle and could be a potential source for isolation of new orally active antihyperglycaemic compounds for attenuating secondary complications of diabetes such as atherosclerosis, liver and renal dysfunction.

**Keywords:** Diabetes Mellitus, *Cnestis ferruginea*, Lipid peroxidation, alanine aminotransferase, aspartate aminotransferase, Hypoglycaemia

**Abbreviations:** MCF - Methanol extract of *Cnestis ferruginea*; ECF - Ethylacetate extracts of *Cnestis ferruginea*; DMSO- Dimethyl sulphoxide in distilled water; ALT - Alanine amino transferase ;AST- Aspartate amino transferase; TBARS- Thiobarbituric acid reactive substances ; MDA –Malondialdehyde; LPO- Lipid Peroxidation  
FBG- Fasting Blood Glucose

### Introduction

In Africa, traditional healers use several plants as herbal remedies for the treatment of diabetes mellitus (Akhtar and Alli, 1984), thus making such plants possible sources of hypoglycaemic agents. The recommendation of World health Organization (WHO) on the use of medicinal plants for the treatment of diabetes mellitus (WHO, 1980) has made investigations of medicinal plants to become more relevant in the development of novel therapeutic management for diabetes. Some of these plants have been found to lower the blood sugar levels in experimental

animals (Iwu et al., 1990). *Viburnum dilatatum* Thunb. (Gamazumi) has been demonstrated to exhibit hypoglycaemic activity in STZ-induced diabetic rats (Iwai et al., 2004). Similarly, other plant materials such as *Urtica dioica*, green tea, *Ceiba pentandra* have been reported to have antidiabetic activities (Bnouham et al., 2003; Yokozawa et al., 2005; Djomeno et al., 2006).

*Cnestis ferruginea* DC with local names 'Okó-Ajá' or 'Gboyín-Gboyín' (Irvin, 1961) is the commonest of the 13 species of the forest shrubs and climbers of genus *Cnestis ferruginea* DC (Connaraceae). It is widely distributed in Africa and bears orange-red fruits with velveting hairs on the follicle (Irvin, 1961, Margaret, 1965, Ronal, 1974). This plant is used in traditional medicine for a variety of purposes; the leaf decoction is used by Yorubas of South West Nigeria as a laxative, enema for dysentery and gonorrhoea (Dalziel, 1937). The fruits are used locally for the treatment of tooth-ache, mouth and skin infections (Boakye-Yiadom and Konning, 1975).

The petroleum ether fraction of *Cnestis ferruginea* fruit has been shown to contain constituents such as octacosanyl stearate and 1-myristo-2-stearo-3-palmitin (Ogbechie et al., 1987) and a novel isoflavone glycoside, afrormosin-7-O-beta-D- galactoside with antimicrobial activity was isolated in the fruit testa (Parvez and Rahman, 1992). Other compounds such as squalene, myricyl alcohol,  $\beta$ -sitosterol, cyanidin, delphinidin and apigenidin (Ogbede et al., 1986) have also been isolated from the plant. Extracts of *C.ferruginea* have been shown to possess antibacterial (Boakye-Yiadom and Konning, 1975) and anticonvulsant activities (Declume et al., 1984). Since *Cnestis ferruginea* has been reported to inhibit haemoglobin glycosylation *in vitro* (Adisa et al., 2004), this study therefore, investigates *in vivo* the possible effects of methanol extracts and ethylacetate fraction of *Cnestis ferruginea* on STZ-induced diabetes and its possible deleterious effects on the liver and kidneys were also investigated. It is hoped that the study would provide an insight into the hypoglycaemic activities of *Cnestis ferruginea* and its relative safety.

## Materials and Methods

### Collection of plant materials

Fresh leaves of *Cnestis ferruginea* (D.C) (Connaraceae) were collected at the Forest Reserve Area of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria in the month of October. The leaves were immediately rinsed of debris and shade-dried for one week on laboratory trays. The dried leaves were powdered and weighed. The plant was authenticated by Mr T.K Odewo of the Herbarium, Forestry Research Institute of Nigeria, Ibadan, Oyo State, Nigeria and a voucher specimen number 106524 was allotted.

### Extraction

The air-dried, powdered leaves of *Cnestis ferruginea* (D.C) (2.5kg) were exhaustively extracted with hexane (100%, bp 40- 60°C) and the defatted dried marc was re-extracted with methanol (95% v/v) (Sigma Aldrich Chemical Co. St Louis USA), in a giant size soxhlet apparatus at 60°C continuously for 17 hrs in each case. These extracts were collected and concentrated with a rotary evaporator at 40°C. The methanol extract of *Cnestis ferruginea* (MCF) (234.9g) obtained was a greenish brown sticky residue. The methanol extract was dissolved in distilled water and partitioned with equal volume of chloroform and ethylacetate (7 x 1litre) successively to yield chloroform extract (30.5g), and ethylacetate extracts of *Cnestis ferruginea* (ECF, 24.3g), respectively.

### Animals

Male Sprague Dawley rats (16 weeks old, 170 - 250g) bred and housed at the HEJ Institute of Chemistry Animal House, University of Karachi, Karachi were kept in well ventilated cages under a 12hr light/dark cycle. The Animal Care Ethics Committee of the HEJ Institute of Chemistry, University of Karachi, Karachi approved the experimental design and the protocol conforms to the guidelines of the National Institute of Health (NIH). The animals were fed standard rat chow and given water *ad libitum* before and during the experimental period. Twenty five (25) male rats and ninety-nine (99) male mice were used throughout this study.

### Method of Blood collection

Animals were anaesthetized with 60mg/kg body weight of Pentothal sodium intraperitoneally. The animals were dissected and 3 - 4ml of blood was collected from the carotid artery with 5ml syringe. The blood was carefully transferred into a clean universal bottle for separation of the serum.

### Method of Extract Administration

The appropriate weight of the extracts were suspended in 4% dimethyl sulphoxide in distilled water (DMSO) (the vehicle) and administered orally to each animal using orogastric tube.

### Acute Toxicity Study Of *Cnestis ferruginea*

Seventy four male Wistar strain albino mice were used for the toxicity studies. These animals were divided into three (3) main groups (30, 24 and 20 mice), respectively. All these animals were fasted for 6 hours before treatments were administered. Methanolic extract of *Cnestis ferruginea* was dissolved in 4% DMSO in distilled water and administered orally to all the animals with orogastric tube. The first group (30 mice) was subdivided into six (6) groups of 5 mice. Each mouse received 100-1000mg/kg b.w. of methanol extracts of *Cnestis ferruginea*. The second main group (24 mice) was also subdivided into four (4) groups of 6 mice. And each mouse received between 2000 & 10,000mg/kg b.w. of MCF. Similarly, the third main group (20 mice) was subdivided into four (4) groups of 5 mice. And each mouse received between 10,000 – 30,000mg/kg b.w. of this extract. One of each sub groups served as control in all the main study groups. The animals were observed for manifestations of adverse effects in each case within 2 hours, 6hours, 24 hours, 48 hours and 21 days and after extract administration. Since there was no adverse effect displayed by the animals, the 250mg/kg b.w dose according to Venkatesh et al. (2003) was adopted.

### Experimental Design

The animals were divided into 2 major study groups as follows:

1: Effects of MCF and ECF on STZ- induced hyperglycaemia in mice; 2: Effects of MCF and ECF on STZ-induced hyperglycaemia in rats

#### **Group 1: Effect of MCF and ECF on STZ- induced hyperglycaemic mice**

Twenty five mice were used for this study. The mice were fasted for 6hrs and were divided into 5 groups (A, B, C, D and E) of five animals each. Animals in groups B, C D and E were injected intraperitoneally with streptozotocin (50mg/kg/1.5ml; Sigma Chemical, USA) in sodium citrate buffer (pH 4.3). The blood glucose of the animals was allowed to stabilize for 3 days and animals with fasting blood glucose level above 170mg/dl were recruited for the experiment. The animals in groups A and B received 4% DMSO in distilled water and served as healthy and diabetic controls respectively; group C, D and E were given MCF, ECF (250mg/kg b.w) and glibenclamide (10mg/kg b.w) through oral gavaging, respectively. The fasting blood glucose levels of all the mice used were measured prior to extract administration. Blood specimens were collected through the tail vein at 1, 2 and 4 hrs after extracts administration and glucose level was analysed using glucose oxidase method (Barham and Trinder, 1972).

#### **Group 2: Effect of MCF and ECF on STZ-induced hyperglycaemia in rats**

Another set of twenty five (25) rats were fasted for 16 hours. Five (5) of these rats represent the healthy undiabetized control group (A). The remaining 20 rats were injected intraperitoneally with streptozotocin (50mg/kg/1.5ml; Sigma Chemical, USA) in sodium citrate buffer (pH 4.3). The blood glucose of the animals was allowed to stabilize for 3 days and animals with fasting blood glucose level above 300mg/dl were recruited for the experiment.

The hyperglycaemic animals were subdivided into 4 groups (B, C, D, and E) of 5 animals each. Groups A and B received 4% DMSO in distilled water, groups C, D and E received 250mg/kg body weight of MCF, ECF and 10mg/kg b.w of glibenclamide, respectively. The treatments were administered at regular times for 10 consecutive days (Venkatesh et al., 2003). All animals were fasted for 16 hrs before extract administration and blood samples were obtained from the tail 3 hrs after, for fasting blood glucose on days 1, 3, 7 and 10. The animals were anaesthetized with intraperitoneal injection of 60mg/kg b.w of Pentothal sodium. The animals were dissected and blood was quickly collected from the carotid artery into dry universal bottles for serum preparation. The visceral organs (liver, kidney, heart, pancrease and lungs were quickly excised and weighed. The collected blood was allowed to clot and after 30mins was spun at 3500g for 20mins. The serum obtained was separated and used to assay for alanine amino transferase (ALT), aspartate amino transferase (AST), triglycerides, cholesterol, urea and creatinine levels using a Start Fax Biochemical Analyzer (Diasys, GmbH, Ltd). The respective Kits for each biochemical analysis were purchased from Diasys Diagnostic systems Istanbul, Turkey.

### Assay Methods

#### **Determination of Serum Alanine and Aspartate aminotransferases**

Serum Alanine and Aspartate aminotransferases were assayed using commercial diagnostic kits (Diasys

Diagnostic systems Istanbul, Turkey) according to the method described by Bergmeyer et al., (1974).

#### **Determination of serum triglyceride and total cholesterol levels**

Serum triglyceride and cholesterol levels were determined using commercial diagnostic kits (Diasys Diagnostic Systems Istanbul, Turkey) according to the method described by Rifai et al., (1999).

#### **Determination of serum urea and creatinine levels**

Serum urea and creatinine levels were determined using commercial diagnostic kits (Diasys Diagnostic systems Istanbul, Turkey) according to the method described by Trinder, (1969).

#### **Determination of serum lipid peroxidation (LPO)**

Serum lipid peroxidation was determined spectrophotometrically by the thiobarbituric acid reactive substances (TBARS) method, as described by Varshey and Kale (1990) and then expressed in terms of TBARS formed per mg protein.

#### **Determination of serum total Protein**

Serum total protein was estimated spectrophotometrically according to the method described by Lowry et al., (1951) using bovine serum albumin as standard.

#### **Statistical analysis.**

The results are presented as mean  $\pm$  S.D of 5 animals per group, and the statistical levels of significance of the treated compared to control groups were calculated using the one- way analysis of variance followed by the post-hoc Duncan multiple range tests for analysis of biochemical data using SPSS (10.0) statistical software.  $P < 0.05$  was considered significant.

## **Results**

### **Toxicity Test**

In all the groups of animals utilized for toxicity studies, there was no manifestation of any adverse effects and no death was recorded. Therefore, the choice of 250mg/kg bw dose (Venkatesh et al., 2003) was adopted.

### **Effect of MCF and ECF on Fasting Blood Glucose (FBG)**

After 4hrs of treatment with MCF, there was 18% reduction in fasting blood glucose level whereas treatment with ECF within the same time frame produced 11% reduction ( $p < 0.05$ ). The reduction in fasting blood glucose levels over 4 hrs in ECF, MCF and glibenclamide- treated diabetic groups were significantly different from the diabetic control at ( $P < 0.05$ ) (Table 1).

A significant reduction of 74% in fasting blood glucose was observed after a 10 day treatment with MCF compared to 64% and 60% reduction when treated with ECF and glibenclamide respectively. Reductions in fasting glucose levels produced by MCF, ECF, and glibenclamide over 10 days were significantly different from diabetic control at  $P < 0.05$  (Table 2).

### **Effect of MCF and ECF on serum Alanine and Aspartate amino transferases, Total protein and Lipid peroxidation**

Significant reduction ( $p < 0.05$ ) in the level of ALT in both MCF and ECF- treated groups were observed when compared with diabetic untreated group. Glibenclamide- treated group showed a much more reduced level of ALT, almost equal to normal (Table 3). The level of AST in both MCF and ECF- treated group reduced significantly compared to diabetic untreated group ( $p < 0.05$ ). There was however, no difference in AST level of MCF, ECF and glibenclamide-treated group ( $p < 0.05$ ).

**Table 1:** Acute treatment of STZ-induced diabetic mice with methanol extract and ethylacetate fraction of *C. ferruginea*

| Groups           | Fasting Blood glucose (mg/dl) |                           |                          |                           |
|------------------|-------------------------------|---------------------------|--------------------------|---------------------------|
|                  | Basal                         | 1hr                       | 2hr                      | 4hr                       |
| Normal           | 52 ± 3.5                      | 60 ± 5.4                  | 58 ± 6.0                 | 59 ± 5.4                  |
| Diabetic Control | 177.6 ± 6.81                  | 198.4 ± 7.65 <sup>a</sup> | 184.6±5.01 <sup>a</sup>  | 190.2 ± 4.87 <sup>a</sup> |
| Diabetic+ MCF    | 184 ± 9.20                    | 178.6 ± 3.55 <sup>b</sup> | 170.4±4.23 <sup>b</sup>  | 156.2 ± 7.04 <sup>b</sup> |
| Diabetic + ECF   | 185.2 ± 4.16                  | 184.2 ± 5.02 <sup>b</sup> | 174.8±10.84 <sup>b</sup> | 169.2 ± 8.2 <sup>c</sup>  |
| Diabetic + Glib. | 182 ± 5.3                     | 179.1 ± 4.2 <sup>b</sup>  | 170.2 ± 7.3 <sup>b</sup> | 169.1 ± 5.3 <sup>c</sup>  |

Values are mean ± S.D. of five mice in each group. Mean values with different superscript are significantly different from diabetic control within the same time frame P<0.05, MCF- Methanolic extract of *Cnestis ferruginea*, ECF- Ethylacetate fraction, Glib. - Glibenclamide

**Table 2:** Effect of 10-day treatment with MCF and ECF (250mg/kg) on fasting blood glucose in STZ-induced hyperglycaemic rats.

| Groups           | Fasting Blood glucose (mg/dl) |                          |                         |                         |                          |
|------------------|-------------------------------|--------------------------|-------------------------|-------------------------|--------------------------|
|                  | Basal                         | Day 1                    | Day 3                   | Day 7                   | Day 10                   |
| Normal           | 99.6± 8.61 <sup>b</sup>       | 100.6±5.64 <sup>d</sup>  | 99.6±3.36 <sup>a</sup>  | 99.8±5.76 <sup>b</sup>  | 99.2±4.97 <sup>d</sup>   |
| Diabetic Control | 568±8.92 <sup>a</sup>         | 567±9.92 <sup>a</sup>    | 558.2±5.13 <sup>c</sup> | 552 ± 4.83 <sup>a</sup> | 600.6±4.64 <sup>a</sup>  |
| Diabetic+ MCF    | 536±6.44 <sup>a</sup>         | 291.8±16.38 <sup>c</sup> | 336.4±8.16 <sup>b</sup> | 300.8±13.2 <sup>c</sup> | 156.2±3.05 <sup>c</sup>  |
| Diabetic + ECF   | 526.8±8.32 <sup>a</sup>       | 429.4± 7.68 <sup>b</sup> | 395.2±3.4 <sup>b</sup>  | 266.8±6.41 <sup>c</sup> | 189.8±6.13 <sup>c</sup>  |
| Diabetic + glib. | 560.8±9.70 <sup>a</sup>       | 446.6±6.42 <sup>b</sup>  | 366.6±8.87 <sup>b</sup> | 290 ± 4.19 <sup>c</sup> | 240.6±10.11 <sup>b</sup> |

Values are mean ± S.D. of five rats in each group Mean values with different superscript are significantly different P<0.05 from diabetic control within the same time frames

**Table 3:** Effect of 10-day treatment with MCF and ECF (250mg/kg) on serum levels of ALT, AST, protein and TBARS in STZ-induced diabetic rats.

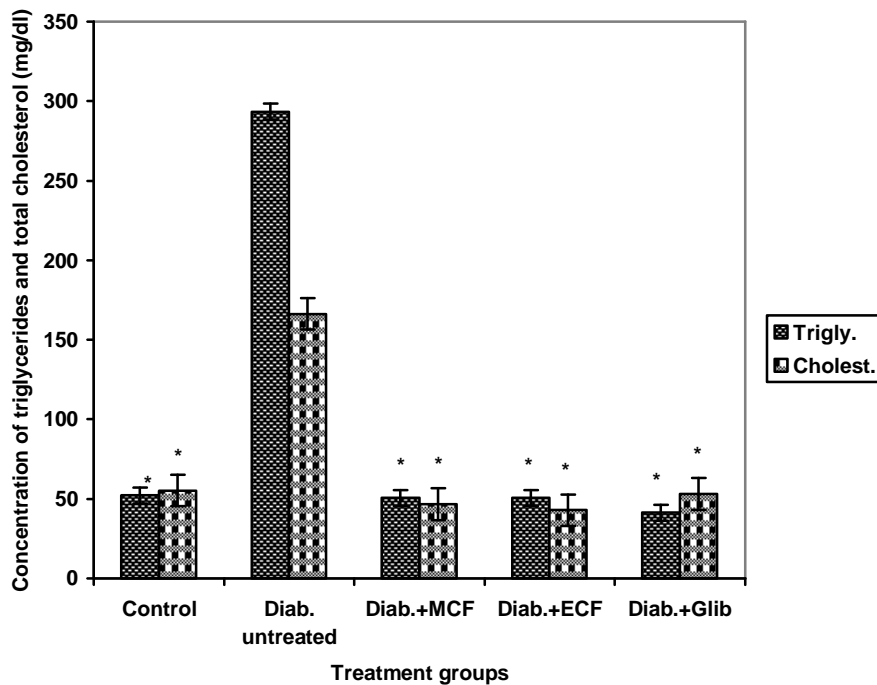
| Groups           | ALT(U/L)                 | AST (U/L)                | TBARS                    | Total protein            |
|------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                  |                          |                          | nmol/mgprotein           | (mg/ml)                  |
| Normal           | 63.4 ± 5.1 <sup>a</sup>  | 125 ± 6.6 <sup>a</sup>   | 272 ± 9.5 <sup>a</sup>   | 7.82 ± 0.5 <sup>f</sup>  |
| Diabetic         | 552.3± 52.3 <sup>b</sup> | 2891.9±16.7 <sup>b</sup> | 5400 ± 30.2 <sup>b</sup> | 5.73 ± 0.35 <sup>e</sup> |
| Diabetic + MCF   | 108.9± 2.1 <sup>c</sup>  | 120.4±5.7 <sup>c</sup>   | 439 ± 15.5 <sup>c</sup>  | 7.49 ± 0.3 <sup>f</sup>  |
| Diabetic+ ECF    | 104 ± 9.9 <sup>c</sup>   | 168.7 ± 7.4 <sup>c</sup> | 374 ± 12.4 <sup>d</sup>  | 9.11 ± 0.49 <sup>f</sup> |
| Diabetic + Glib. | 66.1± 11.5 <sup>a</sup>  | 130.9 ± 4.3 <sup>c</sup> | 729 ± 20.5 <sup>e</sup>  | 7.48 ± 0.6 <sup>f</sup>  |

Values are mean ± S.D. ; n=5; Mean values with different superscript are significantly different from diabetic control P<0.05.

**Table 4:** Serum levels of creatinine and urea in STZ-induced diabetic rats treated for 10 days with MCF and ECF (250mg/kg).

| Groups          | Creatinine (mg/dl)       | Urea (mg/dl)             |
|-----------------|--------------------------|--------------------------|
| Normal          | 0.7±0.07 <sup>c</sup>    | 132.2 ± 8.9 <sup>a</sup> |
| Diabetic        | 3.6±0.32 <sup>a</sup>    | 455.7 ± 5.6 <sup>b</sup> |
| Diabetic + MCF  | 1.4±0.07 <sup>b</sup>    | 179.1 ± 8.1 <sup>c</sup> |
| Diabetic + ECF  | 1.3 ± 0.12 <sup>cb</sup> | 158.7 ± 7.1 <sup>c</sup> |
| Diabetic+ Glib. | 1.1±0.20 <sup>cb</sup>   | 87.7 ± 4.3 <sup>d</sup>  |

Values are mean ± S.D of five rats in each group; Mean values with different superscript are significantly different from diabetic control, P<0.05.



**Fig. 1:** Effect of 10-day treatment of STZ-induced hyperglycaemic rats with *Cnestis ferruginea* extracts (250mg/kg) on serum Triglycerides and Total cholesterol. \*Significantly different from diabetic untreated values (p<0.05)

**Table 5:** Effect of 10-day treatment of STZ-induced diabetic rats with *Cnestis ferruginea* extracts (250mg/kg) on total body and visceral organ weights

| Group              | Initial (b.w) (g) | Final (b.w) (g)        | % change weight (g) | Liver (g)              | Kidney (g)             | Heart (g)              | Pancreas (g)            | Lungs (g)               | wt of liver ----- x 100<br>wt of animal |
|--------------------|-------------------|------------------------|---------------------|------------------------|------------------------|------------------------|-------------------------|-------------------------|---|
| <b>Control</b>     | 202.5±12          | 208±10 <sup>a</sup>    | 5.5                 | 8.3 ±0.6 <sup>a</sup>  | 2.2±0.03 <sup>a</sup>  | 0.98±0.04 <sup>b</sup> | 1.0±0.02 <sup>c</sup>   | 2.2± 0.03 <sup>a</sup>  | 4.0                                     |
| <b>Diab</b>        | 203.5± 10         | 190.4±6.2 <sup>b</sup> | -13.1               | 5.5±0.7 <sup>b</sup>   | 1.6±0.07 <sup>b</sup>  | 0.6±0.07 <sup>c</sup>  | 0.4±0.08 <sup>d</sup>   | 1.13±0.08 <sup>c</sup>  | 2.90                                    |
| <b>Diab + MCF</b>  | 203.8±14          | 168.6±8.7 <sup>c</sup> | -35.2               | 5.85±0.7 <sup>b</sup>  | 1.4± 0.34 <sup>b</sup> | 0.6± 0.07 <sup>c</sup> | 0.3± 0.11 <sup>d</sup>  | 1.03± 0.07 <sup>c</sup> | 3.47                                    |
| <b>Diab + ECF</b>  | 202.4± 13         | 188.4±13 <sup>b</sup>  | -14.1               | 7.60± 0.9 <sup>a</sup> | 1.6± 0.12 <sup>b</sup> | 0.7± 0.11 <sup>c</sup> | 0.26± 0.05 <sup>d</sup> | 1.1± 0.11 <sup>c</sup>  | 4.04                                    |
| <b>Diab + Glib</b> | 204±9             | 186±8.9 <sup>b</sup>   | -18                 | 6.26±0.2 <sup>b</sup>  | 1.6±0.07 <sup>b</sup>  | 0.6±0.04 <sup>c</sup>  | 0.53±0.04 <sup>d</sup>  | 0.96±0.07 <sup>c</sup>  | 3.36                                    |

Values are mean ± S.D of five rats in each group. Mean values with different superscript are significantly different from control P<0.05,

Also, there was significant reduction in the concentrations of TBARS in the MCF, ECF and glibenclamide-treated diabetic group. The concentrations of TBARS in these three were however, still higher than in the healthy group. Furthermore, total protein concentrations in the serum of MCF, ECF and glibenclamide-treated animals were significantly different (p<0.05) from diabetic untreated groups. MCF, ECF and glibenclamide produced 31%, 59%, and 31% increase in serum proteins, respectively when compared with diabetic untreated animals.

#### Effect of MCF and ECF on serum urea and creatinine.

MCF and ECF produced 61% and 64% reductions in the level of creatinine compared to the diabetic control. The percentage reduction produced by glibenclamide was not statistically different from ECF (P<0.05) (Table 4). Also, MCF and ECF produced 61% and 65% reduction in the level of urea, respectively, though the percentage reduction of urea in MCF and ECF -treated groups were not statistically different. Glibenclamide however, showed a higher reduction in the level of urea when compared with diabetic- untreated and even normal (Table 4).

#### Effect of MCF and ECF on serum triglycerides and cholesterol

Administration of STZ caused a significant elevation of serum triglycerides and cholesterol in diabetic untreated rats when compared to control. But treatment with MCF, ECF and glibenclamide separately significantly reduced the levels of serum cholesterol and triglycerides by not less than 80% (Figure 1).

#### Effect of MCF and ECF on body and organ weights

Treatment with MCF and ECF produced significant reduction in total body weight of the animals (P<0.05). The liver was slightly enlarged compared to diabetic untreated but not completely regenerated as in the control groups. The weight of heart, kidney, pancreas, of MCF and ECF-treated groups were not significantly different from the weight of these organs in diabetic untreated groups but are significantly different from healthy control (p< 0.05) (Table 5).

## Discussion

Although different types of oral hypoglycaemic agents are available along with insulin for the treatment of diabetes mellitus, patients' demand for the use of natural products with antidiabetic activity is on the increase. Insulin cannot be used orally and the continuous use of synthetic drugs has its risk of side effects and toxicity (Holmann, 1991; Chattopadhyay, 1993). Therefore, the need for the use of effective herbal drugs with less side effects and relatively cheap is imperative (Choi et al., 1991; Erenmehsoghi et al., 1995). STZ-induced hyperglycaemia has been described to be a useful experimental model for the study of hypoglycaemic agents (SZkudelski, 2001). Its mechanism of action is by the destruction of pancreatic insulin secreting B-cells, leaving less active cells thereby resulting in a diabetic state (SZkudelski, 2001).

In this study, the methanol and ethylacetate extracts of *C. ferruginea* exhibited significant hypoglycaemic activities in STZ-induced diabetic rats and mice respectively. Both extracts produced a significant blood glucose lowering effect ( $P < 0.05$ ) as from 1 hour up to 4 hours after the extract administration, and on days 1, 3, 7, and 10 (Tables 1 and 2). There is no significant difference ( $P > 0.05$ ) in potency of MCF and ECF except in their hypoglycaemic activity after the 4<sup>th</sup> hour. Although we are yet to investigate the mechanism by which the blood glucose is lowered, the result suggests that the MCF and ECF extracts of *C. ferruginea* could be acting in similar manner as glibenclamide, (standard antidiabetic) by stimulation of the surviving  $\beta$ -cells to release more insulin. Furthermore, the results show that MCF exhibited higher hypoglycaemic effects in rats and mice respectively, compared to the effect of ECF. The reason for this could likely be due to the presence of a combination of ECF and some other potent secondary metabolites present in MCF but which were excluded during extraction with ethylacetate. The *C. ferruginea* has been shown to contain isoflavone (Parvez and Rahman, 1992), coumarin (Vickery and Vickery, 1980 not listed?), anthocyanins (Ogbede et al., 1986) which have been ascribed to be responsible for the hypoglycaemic activity of some plants. It is therefore possible that the presence of isoflavone in these extracts has contributed to the reduction in blood glucose.

The role of the liver in the pathogenesis of type 2 diabetes is attracting increasing attention. Fortunately, circulating concentrations of a number of variables appear to give insight into the extent of liver fat accumulation injury. Among these are  $\gamma$ -glutamyl transferase, alanine aminotransferase (ALT) and aspartate amino transferase (AST). Of the three, ALT is the most specific marker of liver pathology and appears to be the best marker of liver fat accumulation (Tiikkarnen et al., 2003). Of recent, Sattar et al., (2004) demonstrated that elevated levels of ALT within the "normal" range predict the incidence of diabetes. Based on the simplicity of ALT measurement and its availability in routine clinical practice, it was suggested that this enzyme activity could be included in future diabetes prediction algorithms (Sattar et al., 2004). In the light of this, we have measured the ALT and AST levels in the serum of STZ-induced diabetic rats that were treated for 10 days. The results obtained from this study indicate a significant elevation in the levels of these enzymes in diabetic, untreated rats compared to normal rats. Interestingly, treatments with MCF and ECF for 10 days significantly lowered the elevated levels of these enzymes in the treated rats. The increased serum levels of AST and ALT have been attributed to the damaged structural integrity of the liver, because these are cytoplasmic enzymes and are released into circulation after cellular damage (Recknagel et al., 1989). From these results, it is obvious that STZ produced marked hepatic damage to the rats as evidenced by the elevation of serum ALT and AST. Treatment with MCF and ECF markedly reversed this liver damage as observed by the lowered serum levels of AST and ALT. The result therefore, suggests that both extracts contain active principles that can possibly reduce or reverse hepatic damage (Table 3).

Reactive oxygen species not only contribute to the symptoms of diabetes but also induce some of the secondary complications of diabetes by oxidative injury. The free radicals generated can react with polyunsaturated fatty acids (PUFAS) on cell membrane causing peroxidation resulting in the release of products such as malondialdehyde, hydroperoxides and hydroxyl radicals. An elevated LPO levels indicate serious damage to cell membranes, inhibition of several enzymes and cellular function (Thirunavukarasu et al., 2001). In the present study, we observed an increase in level of TBARS in the serum of STZ-induced diabetic rats which is in consonance with previous studies that have used the TBARS assay as index of LPO (Bastar et al.; 1998, Kakkar et al., 1998, Adaramoye and Adeyemi, 2006). However, serum LPO was significantly reduced ( $p < 0.05$ ) in STZ-induced diabetic rats treated with MCF and ECF when compared with untreated rats (Table 3). The ability of MCF and ECF to reduce TBARS level further confirms the radical scavenging property of *Cnestis ferruginea* extracts (Adisa et al., 2004).

Diabetic nephropathy is one of the most serious chronic complications of diabetes mellitus, and renal dysfunction is reflected by proteinuria, decreased creatinine clearance (Cr), increased serum urea nitrogen and creatinine levels (Baron, 1978). In this study, treatment of diabetic rats for 10 days resulted in significant reduction of serum urea, and creatinine (Table 4). This result is consistent with the reports of Yokozawa et al. (2006) in which Wen-pi-tang - a Chinese prescription was found to attenuate STZ-induced diabetic nephropathy in rats. The results obtained showed an increase in the levels of triglycerides and total cholesterol in the serum of STZ-



induced diabetic rats compared to normal (Figure 1). This increase may have occurred in the diabetic rats as a result of the lack of insulin which activates the lipase enzymes responsible for hydrolyzing the stored triglycerides and releasing large amounts of fatty acids and glycerol into the circulating blood. Consequently, the excess of fatty acids in the plasma could have promoted the hepatic conversion of some fatty acids into phospholipids and cholesterol, the main product of lipid metabolism (Damasceno et al., 2002). These substances associate with the triglyceride excess formed in the liver to cause atherosclerosis as observed in individuals with severe diabetes (Laakso, 1996; Macmahon, et al., 1998). Interestingly, treatment with MCF and ECF reduced the serum cholesterol by 72% and 74.2% and triglyceride levels by 82% and 82%, respectively. This implies that ECF and MCF most likely possess anti-atherogenic properties or hypocholesterolaemic activity and that *C. ferruginea* could improve the metabolic disorder of lipids due to secondary complications of diabetes mellitus (Odetola et al., 2006). However, MCF and ECF did not improve the weight loss caused by diabetes eventhough these extracts have lowered the blood glucose levels, creatinine, urea, cholesterol and triglyceride levels. The toxic effects of STZ treated group was further reduced by the extracts. In conclusion, leaves of *C. ferruginea* have been shown in this study to possess hypoglycaemic and hypocholesterolaemic activities. Although, we obtained no significant difference in the hypoglycaemic activities of MCF and ECF, these effects could be attributed partly to the possible combination of some bioactive agents in the ethylacetate fraction and other secondary metabolites present in the methanolic fraction which were excluded in the ethylacetate fraction. These fractions also attenuated the extent of liver and kidney damages resulting from treatment with STZ. However, further studies are required to determine the exact active principle eliciting the hypoglycaemic effects in *C. ferruginea* and to isolate and carry out a structural elucidation of these compounds. The inability of these extracts to restore the weight loss in the different vital organs and subsequent total body weight is an area for further studies.

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