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Sclerocarya Birrea (Anacardiaceae) Stem-Bark Extract Stimulates Protein Kinase Akt And AMPK Pathways in The Liver in a Diet-Induced Obesity Mouse Model

KEYWORDS: Mouse, sclerocarya birrea, type 2 diabetes mellitus, insulin resistance, DIO mice, hyperglycemia, hyperinsulinemia, AKT, AMPK, SREBP-1, PPARα.

* Eugène S. Attakpa	Alphonse Sezan	Lamine Baba-Moussa
Laboratoire de Biomembranes et Signalisation Cellulaire, Département de Physiologie Animale, Faculté des Sciences et Techniques 01 BP : 4521 Université d'Abomey Calavi Cotonou (Rép. du Bénin). * *Corresponding Author	Laboratoire de Biomembranes et Signalisation Cellulaire, Département de Physiologie Animale, Faculté des Sciences et Techniques 01 BP : 4521 Université d'Abomey Calavi Cotonou (Rép. du Bénin).	Laboratoire de Biologie et de Typage Moléculaire en Microbiologie, Faculté des Sciences et Techniques/Université d'Abomey-Calavi, 05 BP 1604 Cotonou (Rép. du Bénin).

Bialli Seri	Messanvi Gbéassor	Naim Akhtar Khan
Laboratoire de Neurosciences, Unité de Formation Biosciences 22 BP 582 Abidjan 22 Université de Cocody-Abidjan (Rép. de Côte-d'Ivoire).	Centre de Recherche et de Formation sur les Plantes Médicinale, Département de Physiologie Animale, Faculté des Sciences, Université du Bénin BP : 1515 (Lomé-Togo).	Unité Propre de Recherche de L'Enseignement Supérieur EA 4183 Lipides et Signalisation Cellulaire, Faculté des Sciences de la Vie, Université. Université de Bourgogne-Dijon (France)

ABSTRACT *Background: The present study was undertaken to evaluate the anti-diabetic of sclerocarya birrea (A. Rich) Hochst in a diet-induced obesity (DIO) mouse model. Obesity-induced contributes to the development of obesity-related metabolic disorders such as insulin resistance, type 2 diabetes, fatty liver disease, and cardiovascular disease. In this study, we investigated whether sclerocarya birrea can reduce obesity-induced and metabolic disorders such as insulin resistance and hepatic steatosis.*

Methods: Male C57BL/6 obese mice fed a high-fat diet for 10 weeks were administered orally by gastric intubation sclerocarya birrea extract at a dose of 200 or 300 mg/kg body weight, for a further 10 weeks and were compared with HFD-control group. Diets were prepared freshly every week and stored at 4oC. The food and the water were provided ad libitum and replenished every 3-4 days. During the study, serum glucose level was measured at the same time in the morning. Serum insulin was determined using an ELISA kit, according to the manufacturer's instructions. Serum glucose was determined by the glucose oxidase method using a glucose analyser. After liver lipid extraction according to the method of Folch et al., liver TG content was measured by using a commercially available kit. Free fatty acids (FFA) were separated on silica gel by thin layer chromatography (TLC). The purified fractions of FFA and TG were quantified by gas liquid chromatography. As well as liver and kidney functional parameters (ALT, AST, creatinine, alkaline phosphatase) were assessed. The expression of the liver protein was analyzed by Western blot.

Results: At the end of the study, insulin sensitive tissue (liver) collected to investigate anti-diabetic effects and examine the plant's molecular mechanisms. sclerocarya birrea also reduced Serum glucose. Noteworthy, 200 mg/kg sclerocarya birrea efficiently reduced glycaemia although plasma insulin levels were similar to diabetic controls while preventing hepatic steatosis (Sclerocarya birrea diminished hepatic triglyceride, free fatty acids) in DIO mice. Western immunoblot analysis, demonstrated that sclerocarya birrea stimulated two pathways: the insulin dependent Akt and the insulin independent AMPK ones. The improvement of hepatic steatosis observed in DIO treated mice was associated with a decrease in the hepatic content of SREBP-1, a transcription factor involved in de novo lipogenesis. Sclerocarya birrea treatment had tendency to increase hepatic PPARα levels beyond those of control DIO animals. Sclerocarya birrea treatment did not alter liver (ALT, AST) or renal (creatinine and alkaline phosphatase) functional parameters in the blood, thereby attesting to the lack of toxicity of the plant extract in diet-induced obesity mice.

Conclusion: These data suggest that sclerocarya birrea exerts potential anti-diabetic action by improving insulin sensitivity and mitigating high-fat diet-induced obesity and hyperglycemia. They also validate the safety and efficacy of this plant.

Introduction

According to World Health Organization (WHO), diabetes has become the fourth or fifth cause of death in most developed countries. Because of the high cost of conventional treatments with synthetic drugs, traditional treatment with plants becomes an alternative. Several plants have been used by traditional and ancestral medicine men in African countries for the treatment of several pathologies including digestive disorders, weakness, liver complaints, obesity, urinary troubles, diabetes, skin infections, fever, diarrhoea and insomnia [1-2]. The medicinal properties of this plant depend on the part of the plant concerned (root, leaf stalk and pulp or fruit) and the extract used (ethanolic, butanolic etc.). Butanol extracts of *Zizyphus spina-christi* leaves which are rich in saponin improved the oral glucose tolerance and potentiated glucose-induced insulin release in type II diabetic rats [3]. In a cross-sectional survey conducted in Dakar, 43% of patients attending consultancy

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at the hospital declared using *Sclerocarya birrea* [4]. This shows the wide use of the plant for the treatment of various diseases, although investigations on biological effects are lacking. *Sclerocarya birrea* stem-bark extracts have been shown to exert hypoglycaemic effects in animal models. In rats with streptozotocin-induced diabetes, acute administration of S. birrea stem-bark extract reduces blood glucose levels [5, 6]. Chronically, the efficacy of *Sclerocarya birrea* stem-bark extract administered for 5 weeks was shown to be similar to metformin treatment with regards to lowering of glycaemia [7]. In the consensus model of glucose-stimulated insulin secretion, glucose phosphorylation initiates its metabolism [8], ultimately leading to plasma membrane depolarisation [9] and the ensuing cytosolic calcium rise inducing insulin exocytosis [10]. Additional signals, generated by mitochondrial metabolism, amplify the calcium signal [11]. Elevation of plasma insulin was reported in stem-bark-treated diabetic rats [6]. Regarding the in vivo hypoglycaemic effect of *Sclerocarya birrea* stem-bark, chronic treatments have been reported only with organic extracts [6, 7], whereas aqueous extracts have been shown to be effective in acute conditions [5].

We therefore sought to confirm the plant's antidiabetic potential *in vivo* and to further investigate the mechanisms by which this plant can improve systemic glucose and lipid homeostasis. For this purpose, we chose the diet-induced obesity (DIO) mouse model. Indeed, mice fed chronically with a high fat diet develop obesity, hyperglycemia and hyperlipidemia [12]. The DIO model thus adequately reflects the fact that a high-fat diet is the major environmental factor causing overweight and participating in the metabolic syndrome to type 2 diabetes continuum in humans [13].

To begin elucidating the mechanisms of action of *Sclerocarya birrea* responsible for the observed systemic metabolic effects in the DIO mouse model, we analyzed the signaling pathways involved in glucose uptake, lipid metabolism in the liver.

MATERIALS AND METHODS

Preparation of plant extract

One kilogram of air-dried stem-bark of *sclerocarya birrea* was minced, powdered and macerated in 3l distilled water for 48 h at room temperature. The water extracts were filtered through Whatman no. 3 filter paper and were concentrated under reduced pressure at 35°C, yielding 160 g of a dark-brown (16%) *sclerocarya birrea* extract. For each series of experiments, the extract was weighed and dissolved in distilled water to obtain a 30 mg/ml stock solution.

S. birrea ((A. Rich.)Hochst.) (Anacardiaceae) (Fresh stem-bark) was identified by the Principal Botanist of National Herbarium of Benin of University of Abomey-Calavi. A voucher specimen documenting the collection was deposited at the National Herbarium (Abomey-Calavi) under the reference AP-2078 HNB.

Animals and diets

The study was performed on male mice C57BL/6J (Charles River, Les Oncins, France). Mice were housed individually in wood chip-bedded plastic cages at constant temperature (25°C) and humidity (60±5%) with a 12-h light-dark cycle. They were fed with commercial chow diet for 2 days to stabilize their metabolic condition and allow them to adjust to the new environment.

Mice werefed a high-fat diet (45% calories from lard and soybean oil) (Research Diet, New Brunswick, NJ); 21% energy as fat; 48% as carbohydrate; 17% as protein, and 0.15% as cholesterol) for a total of 20 weeks. Then, the mice were randomly divided into 4 groups (n=12/group); one group received standard chow diet throughout the 20-week study (CHOW control group), one group received a high fat diet (HFD) throughout the 20-week study (DIO-control group). The other 2 groups received HFD for 10 weeks until they became obese and insulin resistant. *sclerocarya birrea* extract at a dose of 200 or 300 mg/kg body weight, were administered orally by gastric intubation.

Doses of *sclerocarya birrea* were selected based on previous animal studies [5, 6] and use by traditional practitioners. At the end of the treatment period, mice were fasted overnight, killed, and the blood was collected in heparinized tubes and plasma was prepared immediately by centrifugation (3,000 r.p.m., 4 °C, 15 min). Serum glucose was determined by the glucose oxidase method using a glucose analyser (Beckman Instruments, USA). The determination of insulin was performed in samples that were stored at -80°C.

The general guidelines for the care and use of laboratory animals, recommended by Benin's ethic commission in experimental research with animals according to the international conventions; Researcher Authorization number n °: 21 CAE 069.

Serum Insulin, adiponectin, and leptin determination

Serum insulin was determined using an ELISA kit (LINCO Research Inc, St. Charles, MO, USA), according to the manufacturer's instructions. Levels of Adiponectin and leptin (Millipore; St-Charles, MO) were determined using radioimmunoassay kits according to manufacturer specifications.

Determination of liver lipids

After liver lipid extraction according to the method of Folch et al. [14], liver TG content was measured by using a commercially available kit (Boehringer, France). Free fatty acids (FFA) were separated on silica gel by thin layer chromatography (TLC) using the following solvent: hexane/diethyl ether/acetic acid/methanol at 90:20:2:3 (v/v/v/v). The purified fractions of FFA and TG were quantified by gas liquid chromatography [15] using an internal standard, C17:0 for FFA and TriC15:0 for TG, with a Becker gas chromatograph (Becker instruments, Downers Grove, IL).

ALT, AST, creatinine, and alkaline phosphatase determination

As well as liver and kidney functional parameters (ALT, AST, creatinine, alkaline phosphatase). Transaminase activity (aspartate aminotransferase (AST) and alanine, aminotransferase (ALT)) was determined using the Reitman and Frankel [16] method; ALT was measured by monitoring the concentration of pyruvated hydrazone that is formed with 2,4-dinitrophenylhydrazine while AST was measured by monitoring the concentration of oxaloacetate hydrazone that is formed with 2,4-dinitrophenylhydrazine. Alkaline phosphatase (ALP) activity was determined using the colorimetric method of Rec [17]

Western Blot analysis:

Frozen tissue (liver) samples were homogenized in RIPA lysis buffer (50 mM Hepes, 150 mM NaCl, 5 mM EGTA, 2 mM MgCl2, 5% glycerol, 1% Triton-X 100, 0.1% SDS, pH 7.4) containing protease and phosphatase inhibitors (2

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mM PMSF, 10 Mm NaF, 100 µM Na-orthovanadate, 1mM Na-pyrophosphate). After homogenization, samples were centrifuged at 12000 g for 12 min at 4oC and supernatant was then removed and stored at -80oC until analysis. Total protein content of each sample was quantified using the Bradford method. Equal amounts of protein (50 µg) were subjected to electrophosphoresis on 10% SDS-polyacrylamide gels and transferred to nitrocellulose membrane (Millipore, Bedford, MA). Membranes were first blocked for 2 hours at room temperature in 5% nonfat dry milk in TBST (20 mM Tris; pH 7.6, 137 mM NaCl, 0.1% Tween-20), then incubated with primary antibodies overnight at 4oC. The membranes were washed three times with TBST and then incubated with the secondary antibody for 1 hour at room temperature. The signal was revealed by ECL Plus Western blotting detection system (Perkin Elmer, Woodbridge, Canada). Membranes were probed with the following antibodies; p-AKT (Ser 473), AKT, p-AMPKα (Thr 172), AMPK, ß-actin (1:1000 dilution, 5% BSA, Cell Signaling Technology, Danvers, MA); Signaling Technology, Danvers, MA); PPARα, SREBP-1 (1:200 dilution; 5% milk, Santa Cruz Biotechnology,inc. Santa Cruz, CA). The anti-rabbit IgG HRP-conjugated secondary antibodies or anti-mouse IgG HRP-conjugated secondary antibodies were used at 1:4000 dilutions in 5% milk in TBST (Cell Signaling Technology).

Statistical analysis

Results are shown as means ± SEM. The significance of the differences between mean values was determined by two-way ANOVA (STATISTICA, Version 4.1, Statsoft, Paris, France), followed by the least significant difference (LSD) test. Differences were considered significant at $P < 0.05$.

RESULTS

Diet-induced-obesity (DIO) model

To induce obesity and a pre-diabetic state, DIO, group were fed with high fat diet (HFD) for a period of 20 weeks. This resulted in a significant weight gain as compared to standard CHOW fed group (Non-diabetic) (49.7 ± 0.8 g vs 40.5 ± 0.7 g respectively; p<0.05). We observed that DIO group were fed with high fat diet (HFD) had significantly more adipose tissues than standard fed group. In parallel, a state of insulin resistance was established as evidenced by mild hyperglycemia and major hyperinsulinemia in DIO mice (40.20± 16 ng/mL vs 5.6± 0.75 respectively; p<0.05). This was associated hepatic accumulation of triglycerides (22.5 ± 0.8 for DIO vs 6.71 ± 0.7 for CHOW mg/g Liver; p<0.05). Circulating Leptin/Adiponectin ratio, also indicative of insulin Resistance (Leptin (38.6 ± 1.3 ng/mL vs 30.5 ± 1.6 ng/mL respectively; p<0.05); Adiponectin (14.5 ± 0.7 ng/mL vs 18.4 ± 0.6 ng/ml respectively; p<0.05).

***Sclerocarya birrea* significantly decreases glycemia, of DIO mice after 10 weeks of treatment**

When administered for the last half of a 10-week HFD protocol, *Sclerocarya birrea* exhibited a significant effect on glycemia. The two highest doses of *Sclerocarya birrea* diminished nonfasting blood glucose levels as compared to DIO control levels (p<0.05; Fig 1). This effect was more pronounced for glycemia measured at the end of treatments.

***Sclerocarya birrea* modulates insulin levels of diet-induced-obesity (DIO) mouse model.**

Sclerocarya birrea treatment also had a major impact on insulin serum levels, reducing them when compared to DIO-control levels (p<0.05; 300 mg/kg); however, they were still higher than those of the non-diabetic animals group (p<0.05). Noteworthy, 200 mg/kg *Sclerocarya birrea* efficiently reduced glycaemia although plasma insulin levels were similar to diabetic controls (Fig 2).

***Sclerocarya birrea* diminished hepatic triglyceride (TG), free fatty acids (FFA) of diet- induced-obesity (DIO) mouse model**

A state of insulin resistance was established. This was associated hepatic accumulation of triglycerides. We observed that *Sclerocarya birrea* diminished hepatic levels of triglyceride (TG) and free fatty acid alongside the HFD (p<0.05; Figure 3). Liver steatosis was significantly improved. These results were fully compatible with the changes observed at the level of liver weight, where mice treated with 200, 300 mg/kg of *Sclerocarya birrea* had significantly smaller livers than DIO-control animals (2.45 ± 0.3 g vs 2.45 ± 0.2 g; 1.92 ±0.4 respectively; p<0.05)

Effects of *Sclerocarya birrea* treatment on liver specific enzyme markers of necrotic injury and cholestasis

Finally, *Sclerocarya birrea* treatment did not alter liver (ALT, AST) (Figure 4) or renal (creatinine and alkaline phosphatase) (Figure 5) functional parameters in the blood, thereby attesting to the lack of toxicity of the plant extract in DIO mice.

***Sclerocarya birrea* treatment on Adipose tissue weight of mice**

Systemic increases in the levels of free fatty acid and leptin and decreases in the levels of adiponectin are characteristic of obesity, and are responsible for the development of insulin resistance [18]. Since obesity has been linked to high adiposity and hyperlipidemia [19, 20, 21], we assessed in these mice the adipose tissue quantity and the obesity related parameters such as adiponectin and leptin. We observed that DIO group were fed with high fat diet (HFD) had significantly more adipose tissues than standard fed group (Figure 6). The plant extract while having more modest effects on weight of Adipose tissue. This suggests that *Sclerocarya birrea* extract may act preferentially on the liver. To begin elucidating the mechanisms of action of *Sclerocarya birrea* responsible for the observed systemic metabolic effects in the DIO mouse model, we analyzed the signaling pathways involved in glucose uptake lipid metabolism in the liver.

***Sclerocarya birrea* stimulates AKT and AMPK pathways in the liver.**

The results presented above relating to liver weight and steatosis were indicative of insulin resistance in DIO animals and improved insulin sensitivity after *Sclerocarya birrea* treatment. Both insulin-dependent Akt and insulin-independent AMPK pathways, known to be involved in hepatic glucose and lipid homeostasis, were thus assessed. As illustrated in Figure 7, *Sclerocarya birrea* treatment was found to activate both pathways. Again, when compared to control DIO mice, the 300 mg/kg/d group yielded the largest increase in the phosphorylation level of Akt (p<0.05; Fig. 7 A) and AMPK (p<0.05; Fig. 7 B). PPARα is a ligand-activated transcription factor and member of the nuclear hormone receptor superfamily. It is predominantly expressed in the liver and is one of the key regulators of glucose and lipid metabolism [22]. A recent study showed that PPARα agonists protect against not only hepatic steatosis but also obesity-induced inflammation in the liver [23].

***Sclerocarya birrea* treatment had tendency to increase hepatic PPARα levels and tended to decrease SREBP-1 levels in the liver of DIO mice.**

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Liver PPARα content was significantly increased in Diabetic Control animals as compared to Non-diabetic. These results suggest that the reduction of hepatic steatosis responses by *Sclerocarya birrea* is, at least in part, mediated by direct up-regulation and activation of PPARα due to *Sclerocarya birrea* acting as a ligand for PPARα. As shown in Figure 8A, *Sclerocarya birrea* treatment had tendency to increase hepatic PPARα levels beyond those of control DIO animals. The levels of SREBP-1 tended to increase in DIO control animals. Sclerocarya birrea tended to decrease SREBP-1 levels compared to the DIO-control group in the liver (Figure 8B).

Discussion

The diet-induced-obesity (DIO) mouse model, which is based on the feeding of a high fat diet, therefore represents a valuable tool to investigate and validate new therapeutic avenues in the treatment of obesity and diabetes, including medicinal plants. Indeed, the findings of the present study confirmed the previously reported obesity, hyperglycemia, insulin resistance and fatty liver that are normally associated with the DIO mouse model [12]. In the present study, this antidiabetic activity was investigated in the DIO mouse model. Treatment with *Sclerocarya birrea* was administered for 10 weeks, after obesity and mild hyperglycemia were established by an initial 10 weeks of HFD feeding. The results clearly demonstrate that such treatment improves glucose homeostasis in the face of continued HFD feeding and strongly suggest that this is achieved by an attenuation of insulin resistance. Indeed, *Sclerocarya birrea* treatment significantly countered hyperglycemia, hyperinsulinemia as well as hepatic steatosis as from reductions in hepatic TG accumulation and liver weight. The plant extract did not influence the weight of WAT. This suggests that *Sclerocarya birrea* extract may act preferentially on the liver while having more modest effects on white adipose tissue.

The major organs involved in the regulation of blood glucose levels are the muscle, the liver and the adipose tissue. Therefore, the activation and the expression of key proteins involved in glucose and lipid homeostasis were assessed in these insulin sensitive tissues in order to begin elucidating the molecular mechanisms underlying the apparent systemic antidiabetic activity of *Sclerocarya birrea*. Here, we showed that glycaemia of diabetic mice was efficiently corrected by *Sclerocarya birrea* treatment. *Sclerocarya birrea* at a dose of 200 or 300 mg/kg body weight. Serum insulin levels were modulated by *Sclerocarya birrea treatment* in vivo, indicating an action on pancreatic β-cells. Noteworthy, 200 mg/kg body weight, *Sclerocarya birrea* efficiently reduced glycaemia although serum insulin levels were similar to diabetic controls. This suggests that tissues other than pancreatic β-cells also contributed to the lowering of blood glucose. Pancreatic β-cells produce and store insulin in response to physiological demand, and hyperglycaemia, within 15 min, results in the activation of a complex network of intracellular signalling pathways that trigger insulin release [24].

Indeed, *Sclerocarya birrea* treatment was shown previously to increase hepatic glycogen storage to similar extent than metformin [7]. Such liver-specific effects could possibly explain the blood glucose lowering effect observed in mice after the administration of *Sclerocarya birrea*. Among other molecules, *Sclerocarya birrea* contains epicatechin-3-galloyl ester [25] that is also present in green tea and is shown to improve glucose tolerance in diabetic mice and human subjects [26]. In the present study, acute treatment of insulin-secreting cells with *Sclerocarya birrea* modify the secretory response of insulin-producing cells. Therefore, pancreatic β-cells are likely to contribute to hypoglycaemic effects of *Sclerocarya birrea*. Obesity-induced dysregulation of adipocytokines increases lipolysis, causing an increase in free fatty acid levels [27]. These metabolic changes not only impair hepatic lipid metabolism but also trigger hepatic insulin resistance, leading to systemic aggravation of metabolic dysfunction and hepatic inflammation [28, 29].

It has been shown that agonists of the anti steatotic drug PPARα are effective in inhibiting the development of obesity-induced hepatic steatosis and insulin resistance [30], and is also implicated in hepatic inflammatory responses [31].

In contrast, ingestion of this plant extract resulted in the activation of both the Akt and AMPK pathways in the liver. These pathways have been demonstrated to regulate hepatic glucose output, which plays a major role in glucose homeostasis and the pathogenesis of Type 2 diabetes [32, 33). For instance, it is known that the activation of these phosphorylation cascades can reduce hepatic glucose output by stimulating glucose storage as glycogen and by inhibiting gluconeogenesis [34, 35]. Hence, the significant reduction of hyperglycemia afforded in vivo by *Sclerocarya birrea* can likely be related to a combination of reduced hepatic glucose output and enhanced muscle glucose uptake. it is well documented that ectopic fat accumulation in the liver is a major contributor to the development of insulin resistance in this organ and that this is a crucial component in diabetes pathogenesis [36]. Conversely, liver insulin sensitivity is enhanced

by stimulating hepatic fatty acid oxidation through AMPK/ACC (37-38) and/or PPARα [39, 40] activities, on one hand, and by inhibiting cholesterol and triglyceride synthesis through regulation of SREBP-1, on the other [41, 42]. Results obtained in the control DIO mice were consistent with these concepts since the liver steatosis and increased hepatic TG levels were associated with increased SREBP-1 levels; these probably being related to hyperinsulinemia and enhanced circulating cytokines acting through the NFκB inflammatory pathway, respectively [43, 44]. On the other hand, the enhanced PPARα levels observed in DIO control animals suggest that compensatory mechanisms may have been induced in the face of sustained high intake in lipids. The results obtained in *Sclerocarya birrea* treated mice indicate that the plant extract can also improve hepatic lipid homeostasis. Firstly, as mentioned, *Sclerocarya birrea* treatment significantly attenuated liver steatosis and reduced hepatic intracellular TG. Secondly, elevated levels of PPARα were maintained and SREBP-1 levels tended to be reduced in *Sclerocarya birrea* treated mice as compared to DIO control congeners. Taken altogether, these data suggest that *Sclerocarya birrea* treatment could tip the balance towards increased oxidation of fatty acids and less lipid storage in the liver, thus improving hepatic steatosis and hence insulin sensitivity. These results therefore support our interpretation that *Sclerocarya birrea* probably exerts its antidiabetic activity by acting mainly on the liver. In conclusion, the current studies provide solid evidence that treatment with *Sclerocarya birrea*, a medicinal plant, exerts significant antidiabetic activity by improving systemic glucose and tissue lipid homeostasis.

Mechanisms point to the attenuation of insulin resistance caused by a high fat diet. This is apparently affected by reducing hepatic TG accumulation and steatosis, likely through concomitantly increased lipid β oxidation and

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decreased lipogenesis in the liver. These effects seem to be mediated whereas both insulin-dependent and -independent (AMPK) pathways are involved in the liver. Suggesting that *Sclerocarya birrea* may enhance mitochondrial β–oxidation by activating downstream transcription factors such as PPARα. More importantly, we observed that *Sclerocarya birrea* elicited PPARα ligand activity in hepatocytes. Moreover, it has been shown that PPARα activation inhibits nuclear factor-κB signaling, resulting in decreased production of inflammatory cytokines [45]. Hence, it seems that *Sclerocarya birrea* reduces hepatic steatosis and hepatic inflammation by upregulating PPARα as well as activating it, ultimately influencing circulating free fatty acid and glucose levels, which in turn protect against insulin resistance, suggesting compensatory mechanisms in the face of enhanced fatty acid intake.

ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum [46]. Increase in the serum ALP could be due to renal or intestinal damage, biliary track damage and inflammation [47].The increase could be attributed to enzyme activation by the phytochemical constituents. The ALT and AST are liver specific enzyme markers of necrotic injury and cholestasis [48]. Increase could be due to damage to the hepatic cell or heart attack [49] and may have been induced by some phytocompounds. Serum urea, uric acid, creatinine and electrolytes are markers of damage to renal function [50].

Conclusion

It is hoped that the inclusion of traditional medicine in diabetes care will contribute to reduce the dramatic burden of type 2 diabetes in this population. Our findings suggest that *Sclerocarya birrea* is a useful dietary phytochemical for improving not only obesity-induced inflammation but also obesity-related metabolic disorders such as insulin resistance and liver diseases.

Abbreviations: PPARα: *Peroxisome-proliferator–activated receptor;* SREBP-1: *Sterol regulatory element-binding proteins-1;* AMP: adenosine monophosphate ; AMPK: *AMP-activated protein kinase.*

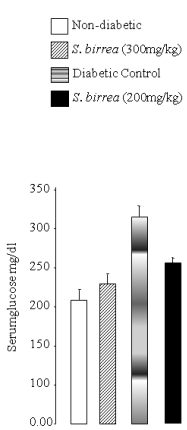

Figure 1: *Sclerocarya birrea* significantly decreases glycemia, of DIO mice after 10 weeks of treatment.

The diet-induced-obesity (DIO) mouse model, which is based on the feeding of a high fat diet (at time pre-diabetic), followed by a period of 2 weeks to stabilise the diabetic state before initiation of treatments (day 1). Daily oral administration of water (non-diabetic and diabetic control), of *S. birrea* (200 and 300 mg/kg); $p<0.05$ versus diabetic control. Values are means ± SEM, n = 12 per group of animals. Data were analyzed by two-way ANOVA.

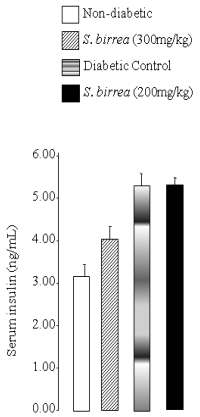

Figure 2: Effects of *Sclerocarya birrea* treatment on insulin levels of diet- induced-obesity (DIO) mouse model.

The diet-induced-obesity (DIO) mouse model, which is based on the feeding of a high fat diet (at time pre-diabetic), followed by a period of 2 weeks to stabilise the diabetic state before initiation of treatments. After days of indicated daily treatments, fasted mice were killed, and plasma insulin levels were determined. Serum insulin. The parameters were determined as described in Research Methods and procedures section. Values are means ± SEM, n = 12 per group of animals. Data were analyzed by two-way ANOVA.

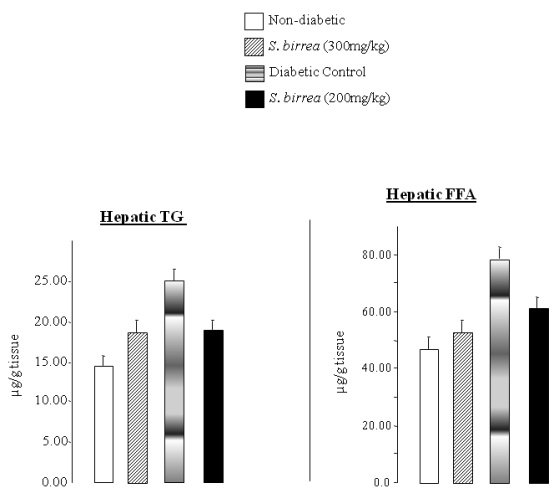

Figure 3: Effects of *Sclerocarya birrea* treatment on hepatic triglyceride (TG) and free fatty acids (FFA) of diet- induced-obesity (DIO) mouse model.

The diet-induced-obesity (DIO) mouse model, which is based on the feeding of a high fat diet (at time pre-diabetic), followed by a period of 2 weeks to stabilise the diabetic state before initiation of treatments. After days of indicated daily treatments, the lipid parameters were determined as described in Research Methods and procedures section. Values are means ± SEM, n = 12 per group of animals. Data were analyzed by two-way ANOVA.

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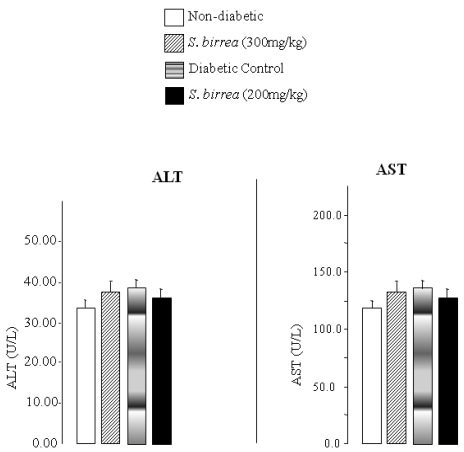

Figure 4: Effects of *Sclerocarya birrea* treatment on liver specific enzyme markers of necrotic injury and cholestasis.

The diet-induced-obesity (DIO) mouse model, which is based on the feeding of a high fat diet (at time pre-diabetic), followed by a period of 2 weeks to stabilise the diabetic state before initiation of treatments. Serum alanine amino transferase (ALT) and aspatate amino transferase (AST) were determined as described in Research Methods and procedures section. Values are means ± SEM, n = 12 per group of animals. Data were analyzed by two-way ANOVA.

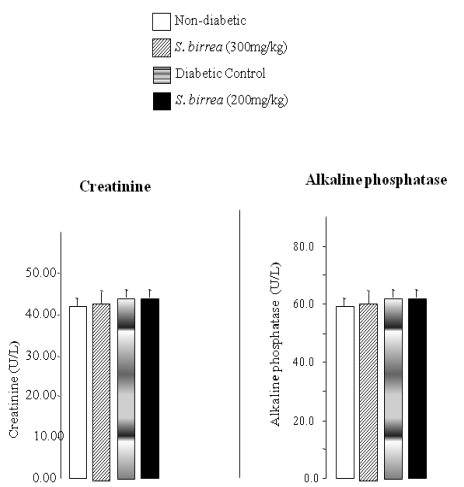

Figure 5: Effects of *Sclerocarya birrea* treatment on markers of damage to renal function (Creatinine) and marker enzyme for the plasma membrane and endoplasmic reticulum marker enzyme for the plasma membrane and endoplasmic reticulum (Alkaline Phosphatase).

The diet-induced-obesity (DIO) mouse model, which is based on the feeding of a high fat diet (at time pre-diabetic), followed by a period of 2 weeks to stabilise the diabetic state before initiation of treatments. Serum Creatinine (ALT) and Alkaline Phosphatase (ALP) were determined as described in Research Methods and procedures section. Values are means ± SEM, n = 12 per group of animals. Data were analyzed by two-way ANOVA.

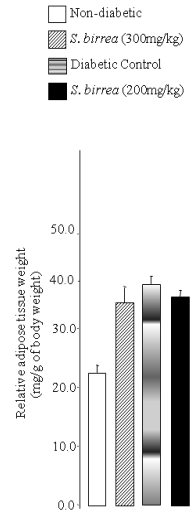

Figure 6: Effects of *Sclerocarya birrea* treatment on Adipose tissue weight of diet-induced-obesity (DIO) mouse model.

The diet-induced-obesity (DIO) mouse model, which is based on the feeding of a high fat diet (at time pre-diabetic), followed by a period of 2 weeks to stabilise the diabetic state before initiation of treatments. Values are means ± SEM, n = 12 per group of animals. Data were analyzed by two-way ANOVA.

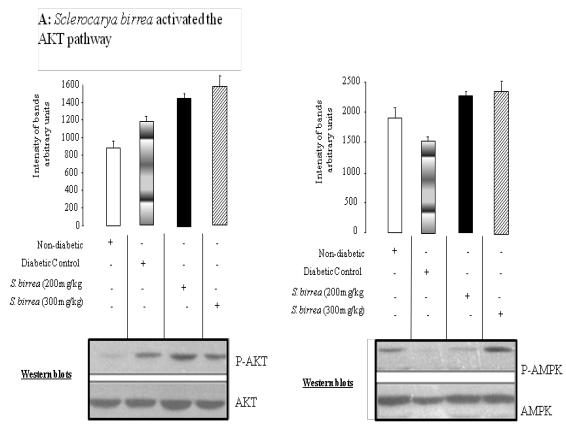

Figure 7: *Sclerocarya birrea* activated the AKT and AMPK pathways of DIO mice.

The diet-induced-obesity (DIO) mouse model, which is based on the feeding of a high fat diet (at time pre-diabetic), followed by a period of 2 weeks to stabilise the diabetic state before initiation of treatments. Samples of liver tissue (50 µg protein) from mice fed Non-diabetic, Diabetic control and Diabetic control + *Sclerocarya birrea* (200, 300 mg/Kg) were homogenized. *Sclerocarya birrea* activated the AKT pathway (A) and AMPK pathway (B). The phosphorylated forms were analyzed by western blot as described in the materials and methods section. Values are means ± SEM, n = 12 per group of animals. Data were analyzed by two-way ANOVA.

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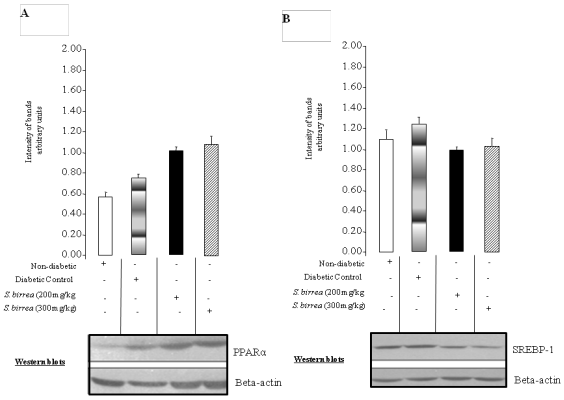

Figure 8: ***Sclerocarya birrea*** **treatment had tendency to increase hepatic PPARα levels** ***and*** **tended to decrease SREBP-1 levels in the liver of DIO mice.**

The diet-induced-obesity (DIO) mouse model, which is based on the feeding of a high fat diet (at time pre-diabetic), followed by a period of 2 weeks to stabilise the diabetic state before initiation of treatments. Samples of liver tissue (50 µg protein) from mice fed Non-diabetic, Diabetic control and Diabetic control + *Sclerocarya birrea* (200, 300 mg/Kg) were homogenized. *Sclerocarya birrea* *Sclerocarya birrea* treatment had tendency to increase hepatic PPARα levels (A) and tended to decrease SREBP-1 levels in the liver of DIO mice. (B). The phosphorylated forms were analyzed by western blot as described in the materials and methods section.Values are means ± SEM, n = 12 per group of animals. Data were analyzed by two-way ANOVA.

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