The aim of this study was to comparatively evaluate the antidiabetic effect of mistletoe and *Moringa oleifera* in streptozotocin-induced diabetes Wistar rats. Fifty-four Wistar rats were used in the study. The animals were randomly divided into six groups (n=9). Diabetes was induced in animals in groups 2-4 by single intraperitoneal administration of streptozotocin at 70 mg/kg bw dissolved in citrate buffer (0.1m, pH 4.5). Mistletoe and *Moringa oleifera* were administered to animals in groups 2 and 3, respectively. After expiration of the study, the animals were sacrificed and the pancreas was excised, weighed and homogenized for analysis of insulin, malondialdehyde (MDA), glutathione (GSH) and superoxide dismutase (SOD) using respective diagnostic kits. The results showed that after one week of treatment, 77.78% and 88.9% of the animals in mistletoe and moringa treated diabetic groups became normoglycemic, respectively. There was also a 16.5% and 3% increase in the average body weight for moringa and mistletoe in the third week and 23% and 20.5% increase in the sixth week. The insulin level of mistletoe and moringa treated hyperglycemic groups normalized to near normal. Pancreatic MDA levels in moringa and mistletoe treated groups were significantly lower, while pancreatic SOD and GSH concentrations increased in the extract treated group. It is concluded that moringa and mistletoe possess hypoglycemic properties that can be very useful in the management of diabetic hyperglycemia.

**INTRODUCTION**

Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by glucose intolerance and fasting hyperglycemia (1). Type 1 diabetes is immune mediated and accounts for about 10% of all diabetic cases, affecting approximately 20 million people worldwide (2). Type 2 insulin-resistant diabetes accounts for about 90% of all diabetics. It afflicts an estimated 6% of adult population, and its
worldwide prevalence is expected to grow by 6% per annum, possibly reaching a total of about 200-300 million cases by 2010 (3).

Diabetes is associated with disabling and life threatening complications such as retinopathy, nephropathy, hepatopathy and coronary artery disease (4). Chronic hyperglycemic oxidative stress is implicated in the pathogenesis of these complications (5). Previous reports showed that antioxidants may protect people from the disabling effects of diabetes by mopping up free oxygen and superoxide radicals (5). It has also been reported that depletion of antioxidant appears to be a major risk factor for developing complications, and that antioxidant supplements lowered the risk (6). Besides, impaired insulin levels or action in diabetes predispose to dyslipidemia and increased risk of atherosclerosis (7).

Evidence suggests that cellular injury caused by free radicals contributes to the development of diabetes mellitus (4). Free radicals are either generated by cellular metabolism such as glycolysis, mitochondrial respiration and xenobiotic detoxification or by exogenous factors such as redox reaction. Some are extremely reactive and therefore interact with some ‘vital’ macromolecules including lipids, nucleic acids and proteins (6). The cells have numerous defense systems (enzymatic and non-enzymatic antioxidants) to counteract the deleterious effects of reactive oxygen species (ROS) and free radicals. Moreover, diabetes also induces changes in tissue contents and the activity of antioxidant enzymes (8).

Some herbal mixtures contain mistletoe extract and some other plant ingredients are used in treating one ailment or the other. These mixtures are cador, mistletoe tea, helixor, moringa tea and viable herbal solutions.

The study was aimed at investigating the antihyperglycemic effects of the leaves of African mistletoe and Moringa oleifera in streptozotocin (STZ) induced diabetes adult Wistar rats.

MATERIALS AND METHODS

Plant materials

Collection of Moringa oleifera and mistletoe leaves

Mature fresh leaves of Moringa oleifera and mistletoe were harvested from a farm at Ogbomoso. Samples of the collections were compared to the voucher specimen at the herbarium of Pure and Applied Biology Department, LAUTECH, Ogbomoso, for identification.

Extraction of Moringa oleifera and mistletoe (Viscum album) leaves

Fresh leaves of Moringa oleifera and mistletoe were air-dried (under shade). A total of 2.4 kg and 200 g of dry leaf powder, respectively, were extracted at room temperature after dissolving it in 2000 mL and 1000 mL of distilled water for 48 hours with intermittent shaking. The dissolved leaf powder was filtered using a filtered paper (Whatmann size no. 1).

Animals

Animal breeding

Fifty-four Wistar rats were used. The animals were 6 to 10 weeks old (150-200 g). Animals were kept in cages and housed in the animal holdings of the Faculty of Health Sciences, Animal House, LAUTECH, Ogbomoso. The animals were exposed to 12-hour light, 12-hour darkness cycle at room temperature. They were maintained on animal feeds and allowed free access to water and feeds.

Animal grouping

Fifty-four Wistar rats were divided randomly into six groups (n=9): group 1, normoglycemic animals; group 2, diabetic group that received mistletoe only; group 3, diabetic group that received moringa only; group 4,
untreated diabetic group; group 5, normoglycemic group that received moringa only; and group 6, normoglycemic group that received mistletoe only.

**Induction of hyperglycemia**

Hyperglycemia was induced in 27 overnight-fasted randomly selected rats by single intraperitoneal administration of STZ at 70 mg/kg bw (9). STZ was dissolved in citrate buffer (0.1 m, pH 4.5) just prior to injection. Hyperglycemia was allowed to develop for 72 hours (10). Animals with fasting blood glucose (FBG) ≥250 mg/dL were considered hyperglycemic (11) and were included in the study. Control animals received a single intraperitoneal injection of 0.1 m citrate buffer (1 mL/kg bw; pH 4.5).

**Moringa oleifera and mistletoe treatments**

The dose of the aqueous extract of *Moringa oleifera* used in the study was supported by a literature report (11). Aqueous extract of *Moringa oleifera* was dissolved in physiological saline daily and administered orally using oral cannula to rats in groups 3 and 5 at 200 mg/kg bw (at 9.00-10.00 each day) for a maximum of six weeks.

Also, the dose of aqueous leaf extract of mistletoe used in the study was based on toxicity test. Aqueous extract of mistletoe was dissolved in physiological saline daily and administered orally using oral cannula to rats in groups 2 and 6 at 40 mg/kg bw (at 9.00-10.00 each day) for a maximum of six weeks.

**Measurement of blood glucose**

Blood glucose was estimated in overnight fasted rats at 9:00-10:00 using a one-touch ultra 2 glucometer. Blood was obtained from the dorsal vein of the tail. On day 0 of treatment and weekly, blood glucose was monitored for six weeks.

**Termination of treatment**

For the purpose of assessing the morphological and biochemical changes occurring in different groups, the treatments were applied for six weeks. After six weeks, animals were sacrificed by cervical dislocation. Laparotomy was performed; the pancreas was harvested and homogenized in phosphate buffer solution (pH 7.4) and refrigerated for estimation of insulin, malondialdehyde (MDA), glutathione (GSH) and superoxide dismutase (SOD) using respective diagnostic kits.

**Statistical analysis**

The results were analyzed as mean ± standard error of the mean (mean ± SEM). Mean values were compared using one-way analysis of variance (ANOVA). The level of statistical significance was set at *P*<0.05.

**RESULTS**

**Blood glucose**

Blood glucose was measured in different groups over the six-week period. At week 0, a week after hyperglycemia induction, all animals in the untreated diabetic, mistletoe treated and moringa treated diabetic groups remained hyperglycemic (FBG >250 mg/dL). After one week of treatment, 77.78% and 88.9% of the animals in mistletoe and moringa treated diabetic groups, respectively, became normoglycemic and their blood glucose was almost similar to the control group (p>0.05). The blood glucose level progressively normalized as the administration continued to the sixth week (Fig. 1).

**Body weight**

Weekly changes in body weight of animals from various groups showed that at week 3, there was a 29.4% increase in the average body weight of the untreated diabetic group animals, but after week 3 their average body weight started declining and by week 6 there was an 18% decrease compared to the initial average body weight at week 0. In the moringa treated diabetic group, there was a 16.5% increase at week 3, but at week 6 the increase was 23% when compared with their weight at week 0. Also, in the
mistletoe treated diabetic group, there was a 3% increase in the average body weight of animals at week 3 and a 20.5% increase at week 6 when compared to their average body weight at week 0.

Pancreatic insulin levels

Pancreatic insulin levels in the six groups at week 6 are shown in Table 1. The insulin level of mistletoe and moringa treated hyperglycemic groups were not significantly different from control ($P>0.05$). However, plasma insulin level in the untreated
The pancreatic MDA level in the moringa treated group was low and statistically significantly different from control ($P<0.05$). There was also a significant decrease in pancreatic MDA level in the mistletoe treated diabetic group ($P<0.05$), but the level of significance was not as high as that in the moringa treated group. The pancreatic MDA level in the untreated diabetic group was high and the increase was statistically significant when compared to control ($P<0.05$).

**Pancreatic SOD levels**

The pancreatic SOD levels in the mistletoe and moringa treated diabetic groups were comparable and similar to control ($P>0.05$), whereas the pancreatic SOD level in the untreated diabetic group was low and the decrease was statistically significant when compared to control ($P<0.05$).

**Pancreatic GSH level**

Percentage pancreatic GSH levels at week 6 are shown in Table 1. There was a 50% increase ($P<0.05$) of pancreatic GSH level at week 6 in the moringa treated diabetic group. In the mistletoe treated diabetic group, there was a 15% increase of pancreatic GSH level when compared to control ($P<0.05$). However, in the untreated diabetic group there was a 49% decrease in pancreatic GSH level when compared to control at week 6 ($P<0.05$).

**DISCUSSION**

In this study, treatment of STZ-induced hyperglycemic rats with aqueous leaf extract of *Moringa oleifera* at a dose of 200 mg/kg/d produced normoglycemia in 88.9% of animals by the end of the first week of treatment, and all the animals had their blood glucose brought to near normal by the end of second week. These findings when compared with hyperglycemic animals treated with mistletoe at 40 mg/kg bw/d showed a relative advantage because in mistletoe treated animals, 77.8% were normoglycemic at the end of first week, 88.9% at the end of second week and 100% normoglycemia was achieved in these animals after third week. Hypoglycemic activities of moringa leaf have been reported by Tende *et al.* (11), and that of mistletoe was documented by Gray and Flatt (12).

Hypoglycemic activity was achieved through accentuation of insulin release from β cells of the islet of Langerhans of the pancreas, prevention of glucose uptake from gastrointestinal tract, as seen in α-glucosidase or pancreatic amylase enzyme inhibitors, prevention of gluconeogenesis and glycogenolysis (13). The moringa hypoglycemic activity is reported to be due to the presence of α-glucosidase and pancreatic amylase enzyme inhibitors (14). These enzyme inhibitors prevent digestion of glucose into an absorbable product, hence the inability of blood glucose to increase after glucose intake. The presence of these inhibitors has been reported in plants like M. oleifera.
Morus alba, which was able to exhibit hypoglycemic activity (15). Also, the hypoglycemic activity of moringa leaf extract may be due to the presence of antioxidants like flavonoids, phenol, and vitamins C and E in it.

Hypoglycemic activities of mistletoe leaf extract were already documented to be due to the presence of antioxidants, which prevented destructive effect of hyperglycemia on β cells and the presence of α-glucosidase and pancreatic amylase inhibitors in it (14). Mistletoe was also documented to have some insulin secreting activities (12).

STZ diabetes increases in pancreatic oxidative stress are associated with the progression of the disease (16), as confirmed in this study. STZ is a nitric oxide (NO) donor; and NO has been reported to mediate destruction of pancreatic islet cells, probably via DNA damage (17). In addition to NO, STZ also generates ROS (from the action of STZ on the mitochondria and from increased xanthine oxidase activity) (18). Thus, scavenges of NO (17) and ROS (18) have been reported to exert beneficial effects against DNA damage and β cell toxicity induced by these substances. Lipid peroxidation is usually associated with hyperglycemia (13). In the absence of insulin when β cells are destroyed, as seen in untreated hyperglycemic animals, the tissue turned to the use of fatty acids and acetyl-CoA (13). There was an increase in MDA levels in the pancreas of untreated hyperglycemic animals. The increase in MDA levels was significantly different from the control in moringa treated animals (P<0.05). The ability of some constituents of moringa leaf extract such as oleic acid enhanced the release of insulin (19) and early attainment of hypoglycemia in this group was able to prevent lipid peroxidation. The above reason can explain the low MDA levels seen in the pancreas and blood of moringa treated group and this decrease was significantly different from control and untreated hyperglycemic animals. In mistletoe treated group, the pancreatic MDA level was low and significantly different from control. This is in agreement with the findings of Meki et al. (20) that mistletoe strongly inhibits lipid peroxidation and prevents tissue damage in liver and kidney.

SOD is a natural antioxidant produced by cells (20). The tissue SOD level is inversely proportional to plasma glucose level (16), as shown in this study. The pancreatic SOD level in animals from the untreated hyperglycemic group was low and this decrease was significantly different from control (P<0.05). The increase in pancreatic SOD levels in mistletoe and moringa treated hyperglycemic groups, though higher than control, was not statistically significant (P>0.05). This showed that neither mistletoe nor moringa was able to increase endogenous SOD. This finding is contradictory to that reported by Shi et al. (21) that mistletoe increases endogenous SOD. The antioxidant activity of leaf extract of moringa (14) and mistletoe (22) must be due to some antioxidants like flavonoids, and vitamins C and E present in them. The ability of the extract of mistletoe and moringa to ensure attainment of normoglycemia within third and second week, respectively, was able to prevent the oxidant effect of hyperglycemia on tissues. Tissue destruction in hyperglycemic state is usually due to the release of oxygen radicals and ROS (4).

Glutathione synthesis begins with the formation of γ-glutamyl-cysteine, a reaction catalyzed by γ-glutamylcysteine synthase and subsequent addition of the glycerin moiety by glutamyl synthase (23). Glutathione (γ-glutamyl-cysteinyl-glycine) plays a pivotal role in the defense against oxidative stress (24). In human blood, the bulk of glutathione is found inside erythrocytes (2 mmol/L), whereas the plasma glutathione concentration is extremely low (10 mmol/L). This is in agreement with the report of Dominiguez et al. (25) on 32% decrease in tissue glutathione level in type 1 diabetes and that of Elhadd et al. (26) who report on 30% decrease in tissue glutathione level in type 2 diabetes. The depletion is not due to a decrease in the rate of glutathione synthesis, but occurs despite unaltered or accelerated rates of glutathione synthesis and this depends on the blood glucose levels. Depletion of glutathione arises from its increased utilization in diabetes (25,26). The pancreatic GSH level in moringa treated diabetic group was high and the increase was significantly different from control and untreated diabetic group (P<0.05). Although there was a significant increase in
the pancreatic GSH level in mistletoe treated animals, the difference was not as high as that in the moringa treated diabetic group.

Moringa contains vitamin C that is as much as that of 7 oranges in weight and β carotene that is 4 times of carrot weight by weight (19). This high antioxidant level enhances the activities of moringa compared to mistletoe.

Accordingly, it is concluded that aqueous leaf extract of moringa and mistletoe exerts a hypoglycemic effect. This also underscores the potential of these herbal therapies in the management of diabetic hyperglycemia.

REFERENCES


