A meliorating effects of Cissus populnea aqueous stem bark extract on some parameters in diabetic Wistar rats

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Abstract

This study investigated the effects of aqueous stem bark of Cissus populnea (C.p) extract on some parameters in diabetic Wistar rats. Fresh stem bark of C.p was air dried, pulverized, and mixed with distilled water at room temperature for 72 h; then filtered (11 μm) and the dried filtrate (C.p extract) was used for the animals treatment; which was in 5 groups of 6 rats each: Group 1: normal control, Group 2: treated with 200 mg/kg body weight (b.w) of C.p extract; Group 3: treated with 500 mg/kg b.w of C.p extract, Group 4: treated with 100 mg/kg b.w metformin, while Group 5 untreated, after groups 2-5 were induced with diabetes mellitus with alloxan, the study was for 21 days. Treatment of groups 2 and 3 with C.p extract restored the blood glucose to level comparable to group 1, p > 0.05, but significantly different to group 5, p < 0.05. Treatment of group 3 with C.p extract stabilized the antioxidant enzymes activity without significant difference p > 0.05, when compared to groups 1 and 4; but significantly differed from group 5, p < 0.05. The pancreatic histology of groups 2 and 3 appeared normal, while group 5 had tissue atrophy. The results showed that C.p extract had potentials to modulate glucose, MDA and antioxidant enzymes activity, with no pathological effect on the pancreas. Therefore, further work is suggested for possible extrapolation to diabetic treatment.

Keywords: Cissus populnea, Diabetes mellitus, Antioxidant enzymes, Pancreas

1. Introduction

Diabetes mellitus is a known global disease that affects millions of people, which manifests primarily by altering the blood sugar. It leads to a lifelong threatening conditions. Definite cure for this disease is still elusive, and only being managed by many drugs (Ogurtsova et al., 2018), which most have been associated with a number of side effects (Ahangarpour et al., 2014). In some cultures though, the management has been by folk medicine, utilizing plant extracts. Lack of adequate management leads to complications (Fowler, 2008). The highly needed attention to diabetes is paramount because it has become a major health problem with...
increasing world prevalence. It has been reported that over 451 millions was estimated in 2017 globally to have been affected by the disease, with a prediction of about 693 million by 2045; the situation has become a huge financial burden to many nations (Cho et al., 2018). There is therefore, the need to search further for newer anti-diabetic agents with better efficacy for its effective treatment. Hence, the quest for more alternatives from medicinal plants.

Cissus populnea a tropical, woody climber, which is usually found in West Africa—particularly Nigeria—that belongs to the family of Vitaceae/ Ampelidaceae, and of genus Cissus (Burkill, 2000; and Geidam et al., 2004); has been associated with many medicinal activities (Ojekale et al., 2006; Moody et al., 2003; Kone et al., 2004; Atawodi et al., 2002; and Akomolafe et al., 2013). The Tiv people of Benue state, Nigeria have majorly used the plant in folk medicine for its anti-diabetic effect, though without any scientific proof of its efficacy and tolerability to the metabolizing organs. Although, it has been reported that the plant has no deleterious effect on hepto-renal organs of rabbits on prolonged administration (Adebowale et al., 2013; and Ojekale et al., 2007). The use of the plant in folk medicine for its anti-diabetic effects has afforded these work to ascertain scientifically the acclaimed medicinal effect, also considering the limited literature about the plant use as an anti-diabetic remedy. We therefore, sought to investigate the anti-diabetic effect, antioxidant enzymes activity, the malondialdehyde level, and assessment of limited pancreas histology of Cissus populnea aqueous stem bark extract in alloxan-induced diabetic Wistar rats.

2. Materials and methods

2.1. Plant sample collection and extract preparation

Cissus populnea stem bark was purchased from Wannune market in Tarka L.G.A of Benue state, Nigeria. The plant sample was identified in the Department of Plant Science and Biotechnology, University of Nigeria Nsukka; with Herbarium Number, (UNH No: 902). Metformin was bought from a standard pharmacy.

Preparation of aqueous stem bark extract of Cissus populnea (C.p extract): Fresh stem bark of Cissus populnea was chopped in bits, rinsed thoroughly with distilled water and then air dried for four (4) weeks, thereafter, the dried stem bark was pulverized into fine powder, and kept dry in an air-tight container prior to aqueous extraction. The extraction involved mixing of 1000 g of the fine powdered sample in 1 litre of distilled water, and allowed to stand for 72 h at room temperature. The mixture after 72 h was filtered with Whatmann’s paper (11 μm), the filtrate was then concentrated to dryness in water bath; then labeled aqueous stem bark extract of Cissus populnea, (C.p extract); and was stored for administration to animals (Ojekale et al., 2007).

2.2. Phytochemical analysis and acute toxicity study

Qualitative analysis of C.p extract was carried out with standard procedures (Sofowora, 1993; Trease and Evans, 1989; and Harborne, 1973). The lethal dose of C.p extract was investigated by method of (Lorke, 1983) with 18 rats. In the first phase, rats were divided into 3 groups, of 3 rats each, and were treated with C.p extract at doses of 500, 1000, and 2000 mg/ kg body weight orally. The animals were then observed for 24 h for signs of toxicity. In the second phase, rats were also divided into 3 groups, of 3 rats each, and were treated with C.p extract at doses of 3000, 4000 and 5000 mg/ kg body weight orally also were observed for 24 h.

2.3. Study design and animal treatment

The animals used in this study were handled according to ethical guidelines for the use of animals for research, and ethical clearance obtained from the institutional ethical clearance committee (College of Medicine Research Ethics Committee, University of Nigeria Enugu Campus) with ethical clearance number: 088/07/2020. In this study, thirty (30) male Wistar rats weighing between 150 g-200 g were used, they were purchased from the Animal House facility of the College of Health Sciences, Benue State University Makurdi, Nigeria. The animals were allowed to acclimatize for 7 days in the Departmental research laboratory; fed standard rat chow and water ad libitum, with observation of light and dark throughout the experiment.

After acclimatization, alloxan was used to induce diabetes mellitus in the rats, this is a commonly used chemical for this purpose—that acts by generating reactive oxygen species (ROS)—which preferentially accumulates in the GLUT2 glucose transporter in the pancreatic beta cells, and subsequently leads to cells degeneration and death. Therefore, alloxan is a model compound when studying diabetes mellitus as a result of ROS mediated beta cell toxicity (Lenzen, 2008).

Diabetes mellitus was induced in Wistar rats that were fasted for 12 h, by a single intraperitoneal injection of a freshly prepared solution of alloxan monohydrate (100 mg/ kg b.w (6). After 72 h of administration of
alloxan, the fasting blood glucose level was estimated with a glucometer (Accu-Check Glucometer), and animals with glucose levels above 11.9 mg/dl were considered diabetic (Otunola and Afolayan, 2015) and were included in the study.

2.4. Animal grouping and treatment

This was a twenty one (21) day experimental study in which the Wistar rats were randomly selected and divided into five groups of six per group and treated as below:

- **Group 1**: Control (Normal) was given rat chow and distilled water only for 21 days.
- **Group 2**: Treated with 200 mg/kg b.w of *C.p* extract for 21 days.
- **Group 3**: Treated with 500 mg/kg b.w of *C.p* extract for 21 days.
- **Group 4**: Treated with 100 mg/kg b.w of standard drug (metformin) for 21 days.
- **Group 5**: Diabetic control (untreated) was given rat chow and distilled water only for 21 days.

2.5. Blood glucose determination and tissue sample collections

At the end of twenty one (21) days, the blood glucose level of each rat was measured with Accu-Check Glucometer test strips; the tail tip of each Wistar rat was the source of the blood for glucose estimation. Blood glucose level was measured at the commencement of the experiment and after 21 days. Thereafter, the animals were sacrificed; the liver was harvested to obtain liver homogenate of each rat for antioxidant enzymes assay, and malondialdehyde (MDA) determination as a marker of lipid per oxidation. The pancreas was also harvested from each rat for histology.

2.6. Preparation of liver homogenate, antioxidant enzymes determination, and histology of pancreas

One (1) g of the liver tissue was weighed and placed in a laboratory mortar, the mortar was placed within ice packs, with pestle, each of the liver tissues separately was homogeneously crushed with 4 ml of phosphate buffer pH 7.4 and, thereafter centrifuged at 6000 rpm for 20 min at −4°C; and the supernatant was separated and used for the assay of the following antioxidant enzymes-superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx), using standard biochemical methods for their activity in the liver (Misra and Fridovich, 1972; Claiborne, 1985; and Rotruck et al., 1973). Also, used in estimation of levels of malondialdehyde produced during lipid peroxidation experiment using standard method (Varshney and Kale, 1990). The histology of the pancreas was performed according to conventional method (Avwioro and Iyiola, 2011).

2.7. Data analysis

The data were expressed as mean ± SEM. Differences were determined with student t-test, and ANOVA with Turkey’s post hoc test. Differences were considered significant at p < 0.05.

3. Results

3.1. Qualitative phytochemical screening of *C.p* extract revealed the presence of various phytochemicals (Table 1)

| Phytochemicals       | Bioavailability |
|----------------------|-----------------|
| Alkaloids            | +++             |
| Saponins             | +++             |
| Phytosterols         | +               |
| Phenolic             | ++              |
| Tannins              | ++              |
| Flavonoids           | +++             |
| Terpenoids           | ++              |
| Cardiac glycosides   | +               |

*Note: Key: + = Present in small quantity, ++ = Moderately present, and +++ = Abundantly present.*
3.2. Acute toxicity study (LD$_{50}$)

The Wistar rats treated with C.p extract were normal at all the experimentally tested doses; there was no sign of rough hairs, weakness, decrease in chow intake nor death recorded during the attempt to determine LD$_{50}$. Therefore, the study revealed that C.p extract is non-toxic, and has a safety index of 5000 mg/kg b.w, hence the LD$_{50}$ used was determined by method (Lorke, 1983).

3.3. Results of glucose determination

| Groups                                      | Before induction of diabetes (mmol/L) | After 21 days of treatment (mmol/L) | (P-value) |
|---------------------------------------------|--------------------------------------|------------------------------------|-----------|
| Group 1: Control (normal)                   | 4.60 ± 0.19                          | 4.51 ± 0.14                       | 0.516     |
| Group 2: Diabetic + 200 mg/kg b.w C.p extract | 4.68 ± 0.30                          | 5.57 ± 0.39                       | 0.598     |
| Group 3: Diabetic + 500 mg/kg b.w C.p extract | 4.68 ± 0.19                          | 4.80 ± 0.34                       | 0.125     |
| Group 4: Diabetic + Metformin               | 4.65 ± 0.18                          | 5.22 ± 0.37                       | 0.340     |
| Group 5: Diabetes with no treatment (diabetic control) | 4.62 ± 0.16                          | 20.98 ± 0.78                      | 0.001     |

Note: Values presented as mean ± SEM, n = 6, * = significant compared with no treatment group p < 0.05, a = significant compared with value before treatment at p < 0.05.

3.4. Results of antioxidant enzymes activity

The antioxidant enzymes activity results showed that CAT, SOD and GPx in control rats were not significantly different (p > 0.05) from those in rats treated with aqueous stem bark of C.p extract for 21 days after inducing diabetes. Further, the activities of these antioxidant enzymes were significantly lower (p < 0.05) in the diabetic untreated, group 5, compared to control and other treatment groups (Table 3).

| Groups                                      | Superoxide dismutase (µmoleSOD/min/ mg protein) | Catalase (µmoleH$_2$O$_2$/min/ mg protein) | Glutathione peroxidase (µmole/min/ mg protein) |
|---------------------------------------------|-------------------------------------------------|-------------------------------------------|-----------------------------------------------|
| Group 1: Normal control                     | 54.61 ± 5.32*                                   | 19.84 ± 1.07*                             | 0.20 ± 0.01*                                 |
| Group 2: Diabetic + 200 mg/kg b.w C.p extract | 53.34 ± 0.68*                                   | 19.10 ± 0.60*                             | 0.23 ± 0.01*                                 |
| Group 3: Diabetic + 500 mg/kg b.w C.p extract | 56.00 ± 4.56*                                   | 19.97 ± 1.44*                             | 0.23 ± 0.01*                                 |
| Group 4: Diabetic + Metformin               | 45.33 ± 4.47*                                   | 19.73 ± 0.90*                             | 0.24 ± 0.01*                                 |
| Group 5: Diabetes with no treatment (diabetic control) | 33.58 ± 0.49                                    | 14.02 ± 0.72                              | 0.16 ± 0.01                                  |

Note: Values presented as mean ± SEM, n = 6, * = significant compared with no treatment group, group 5, p < 0.05.

3.5. Results of malondialdehyde level determination

Lipid peroxidation was significantly higher (p < 0.05) in the rats of the untreated, group 5 when compared to all other treatment groups. Furthermore, the MDA level of the rats treated with C.P extract was not significantly different (p > 0.05) from that in control rats and those treated with the standard drug metformin (Table 4).
Table 4: The Mean ± SEM of the MDA level of the Normal control, those treated with C.p extract, metformin and diabetic control (untreated) of the Wistar rats after 21 days

| Groups                                               | MDA (mmol/mg protein) |
|------------------------------------------------------|-----------------------|
| Group 1: Normal control                              | 0.58 ± 0.08*          |
| Group 2: Diabetic + 200 mg/kg b.w C.p extract        | 1.07 ± 0.03*          |
| Group 3: Diabetic + 500 mg/kg b.w C.p extract        | 0.93 ± 0.05*          |
| Group 4: Diabetic + Metformin                         | 1.04 ± 0.12*          |
| Group 5: Diabetes with no treatment (diabetic control)| 2.44 ± 0.21           |

Note: Values presented as mean ± SEM, n = 6 , * = significant compared with no treatment group, group 5, p < 0.05.

3.6. The results of pancreas histology

Plate 1 (Group 1): Pancreas of Wistar rat subjected to normal environmental and nutritional conditions, showing normal tissue architecture as demonstrated by the presence of interspersing centroacinar cells (white arrows) and secretory acinar (black arrows). Blood vessels are seen with red blood cells (white arrowheads). The pancreatic islets (white stars) appear normal. H & E B: X400

Plate 2 (Group 2): Pancreas of Wistar rat, induced with diabetes and treated with 200 mg/kg b.w of plant extract, presenting with normal tissue morphology. White stars = pancreatic islets, Black arrows = secretory acinar, white arrows = centroacinar cells, black arrowheads = red blood cells. H & E A: X100 B: X400

Plate 3 (Group 3): Pancreas of Wistar rat, induced with diabetes mellitus, and treated with 500 mg/kg b.w of the plant extract, showing normal morphology evident by the presence of interspersing secretory acinar (white arrows) and centroacinar cells (black arrows). The pancreatic islets (white stars) appear normal. Black arrowheads = red blood cells. H & E B: X400

Plate 4 (Group 4): Pancreas of Wistar rat induced with diabetes mellitus and treated with 100 mg/kg body weight of metformin, showing normal morphology, with mild atrophy of the pancreatic islets (white stars). White arrows = secretory acinar, Black arrows = centroacinar cells, white arrowheads = red blood cells. H & E B: X400
4. Discussion

The phytochemical analysis of C. p extract revealed that it contains alkaloids, flavonoids, saponins, tannins, phytosterols, cardiac glycosides, terpenoids and phenolics. Alkaloids, flavonoids and saponins were present in high concentrations, while tannins, phenolic compounds and terpenoids were moderately present. Phytosterols and cardiac glycosides were sparsely present (Table 1). Constituents are as previously reported (Aguoru et al., 2014). Alkaloids and flavonoids are free radical scavengers (Tiong et al., 2013; and Sarian et al., 2017), which prevent oxidative cell damage and have strong anti-diabetic activity. The high contents of these compounds in C. p extract could possibly be responsible for its observed therapeutic potentials (Tables 2, 3 and 4; Plates 2 and 3).

This study utilized alloxan to induce diabetes mellitus, which is the most common method, alloxan is a toxic glucose analogue that generates ROS in the presence of intracellular thiols. The beta cells of the pancreas have a low anti-oxidative defense capacity, thus the generation of ROS by alloxan possibly leads to degeneration of these cells. The radicals are responsible for possible eventual death of the beta cells, and the ensuing state of insulin-dependent alloxan induced diabetes (Lenzen, 2008). Thereby resulting in constant high blood glucose which proves a significant deteriorating effects on beta cells of the pancreas (Saleha et al., 2017; and Sayyed and Wadkar, 2018). Marked hyperglycemia was observed in all the animals treated with alloxan 72 h after the administration. Similar observations have been reported (Szkudelski, 2001; and Lenzen et al., 1996). In this study, the 21 day treatment of the diabetic rats of groups 2 and 3 with C. p extract attenuated the blood glucose to the tune of no statistical significant difference when compared to group1 $p > 0.05$, while there was a significant difference compared to group 5 $p < 0.05$. Treatment of group 4 with metformin also had a similar effect (Table 2). Since alloxan generates ROS that could impair the functioning of beta cells of pancreas; it is possible that C. p extract lowers the blood glucose level due to its antioxidant properties as a result of flavonoids and alkaloids contents (Tiong et al., 2013; and Sarian et al., 2017); as they are known to be free radical scavengers. The capacity of the free radical scavengers to suppress oxidative stress on the pancreatic beta cells might have enabled the cells to enhance its function of insulin production, leading to reduction in blood glucose.
Experimental findings have shown that plant extracts with hypoglycemic activity could mediate their action on blood glucose through different mechanisms (Eddouks et al., 2003; Musabayane et al., 2006; and Yadav et al., 2008), the mechanism of possible increase in beta cells of pancreas by activating regeneration of these cells (Shanmugasundaram et al., 1990; and Jelodar et al., 2007), may be the process through which the extract controlled the blood glucose of groups 2 and 3 (Table 2), as their pancreas did not show any deterioration compared with group 5 (Plates 2, 3 and 5).

Antioxidant activity has been shown to play a crucial role in protecting against hepatic and pancreatic damage by boosting the antioxidant defense system (Loki and Rajamohan, 2003). Antioxidant enzymes are critical part of cellular protection against ROS mediated diseases and ultimately oxidative stress.

This study also reported decreased significant differences in the activities of SOD, CAT and GPx in group 5 when compared with normal group 1 rats \( p < 0.05 \); whereas their activity was stabilized in groups 2 and 3 after 21 days of treatment with C. p extract to a point comparable to normal group1, with no significant differences \( p > 0.05 \); metformin had a similar effect on group 4. Increase in liver SOD, CAT and GPx activity after treatment with plant extract has also been reported (Moodley et al., 2015).

Depletion of antioxidant enzymes in diabetic condition has been reported (Sindh et al., 2004), as seen in this study, there was a decrease in activities of these enzymes in the liver homogenate of group 5, with very low value compared to other groups, reflecting a decrease in the antioxidant defense system, which administration of the C. p extract to groups 2 and 3, could stabilize to the level of normal group 1; a similar study had shown that insulin could correct antioxidant enzyme activity in diabetic rats (Sindh et al., 2004), our results therefore, revealed the potency of C. p extract as a potential antioxidant enzyme promoter equivalent to standard drug. A another related study also showed that myrtle oil could regenerate decreased CAT and SOD in diabetic rats (Sepici-Dince et al., 2007), finding similar to effect of C. p extract on the antioxidant enzymes activity in this study.

On the other hand, MDA was also assessed in the liver homogenate, to evaluate the capability of the C. p extract to attenuate or prevent lipid peroxidation, increased MDA has been reported in diabetes (Erdyas et al., 2004; and Mahreen et al., 2010). In this study, there was an increase in MDA in group 5 compared to others (Table 4). The C. p extract showed attenuation of lipid peroxidation by decreasing MDA levels in the groups 2 and 3 to a point comparable to normal group 1, which was without a significant difference \( p > 0.05 \) (Table 4); reflecting the potentials of C. p extract as an antioxidant defense system. This observation is similar to effect of metformin in group 4.

The histology of the pancreas of group 5 showed marked tissue atrophy and necrosis as demonstrated by the degeneration of both nuclear and cytoplasmic components (Plate 5). However, C. p extract treatment of groups 2 and 3 proved normal pancreatic tissues comparable to normal control group1 (Plates 2, 3 and 1). This may be as a result of increase in the antioxidant defense system by the C. p extract. Group 4, treated with standard drug, metformin, showed mild atrophy of pancreatic islets (Plate 4), a reflection of mild side effect on the pancreas. The reduction in blood glucose and MDA, stabilization of SOD, CAT and GPx activity to point of normal control, and possible regeneration of pancreatic cells in the Wistar rats treated with C. p extract for 21 days compared with diabetic untreated, could be associated with high therapeutic potentials of the C. p extract.

5. Conclusion

This study has shown that C. p extract alleviates hyperglycemia, oxidative stress and prevents degeneration of pancreas in the diabetic animals effectively, probably by its antioxidant effect at dosage of 200 and 500 mg/ kg b.w without dose dependent effect. The treatment with 100 mg/ kg b.w of metformin was effective, though with mild atrophy of pancreatic islet cells. The C. p extract is therefore, a potential therapeutic agent needed to be harnessed for possible extrapolation to effective treatment of diabetic mellitus, considering its none toxic effect to the pancreatic cells, ability to reduce blood sugar, lower MDA, and stabilise antioxidant enzymes.

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Conflict of interest
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