Ethanolic extract of *Chrysophyllum albidum* stem bark prevents alloxan-induced diabetes

**ABSTRACT**

**Background:** *Chrysophyllum albidum* (C. albidum) is used in folklore for the treatment of diabetes. The present study was designed to investigate the effect of the ethanolic extract of *Chrysophyllum albidum* stem bark on alloxan-induced diabetic rats.

**Methods:** Normal and alloxan-induced diabetic rats were randomly divided into groups and treated with ethanolic extract of *C. albidum* stem bark for 7-28 days. Metformin (150mg/kg) was used as the standard control. At the end of the treatment, blood sample was collected for glucose test, while serum was extracted for triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDLC), and high density lipoprotein (HDL-C) evaluation. The pancreas was excised and evaluated for superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and malondialdehyde (MDA) and histological damage.

**Results:** Blood glucose, serum TG, TC, LDL-C levels were significantly increased whereas HDL-C levels were significantly decreased in diabetic rats. Also, pancreatic levels of CAT, SOD, GSH were significantly increased (p<0.05) whereas MDA levels were significantly (p<0.05) decreased in diabetic rats. However, the levels of these parameters were restored significantly (p<0.05) in diabetic rats treated with 100, 200 and 400mg/kg of ethanolic extract of *C. albidum* stem bark.

**Conclusion:** Ethanolic extract of *C. albidum* stem bark has potentials as remedy for diabetes. The findings in the presents study support the use of *C. albidum* in folklore for the treatment of diabetes.

**INTRODUCTION**

Hyperglycemia and dyslipidemia, among other disorders, are metabolic syndromes associated with a dysfunctional endocrine system clinically referred to as diabetes mellitus (DM). Diabetes mellitus (DM) is a common endocrine disorder affecting more than 200 million people worldwide. The dilapidating action of DM qualifies it as a disease of major public health concern and epidemiological survey showed that it is the seventh leading cause of death worldwide. Many distinct types of DM exist and the etiology being a complex interaction of genetics, environmental factors, and life-style choices. In addition to hyperglycemia the metabolic deregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system. Diabetes tends to damage cell membranes which results in elevated production of free radicals. The generation of free radicals appears to play a critical role in the pathogenesis of diabetes mellitus. Orthodox medicines have played vital function in the management of diabetes however, situations which include toxicities, medication cost and treatment failures, created an alternative quest for herbal formulation remedies for the alleviation and management of DM.

Herbs are sources of potential therapeutic agents against various diseases due to their biodiversity and presence of a wide array of bioactive phytochemicals and secondary metabolites.

*Chrysophyllum albidum* (C. albidum) is a plant used in folklore medicine due to its therapeutic values. The bark is used for the treatment of yellow fever and malaria, while the leaf is used as an emollient and for the treatment of skin eruption, stomach ache and diarrhoea. The leaf and seed cotyledon have been reported to possess anti-hyperglycemic and hypolipidemic effects. It has antimicrobial, anti-nociceptive, anti-inflammatory and anti-oxidant activities. It serves as a natural anti-oxidant booster to remove free radicals from oxidative stress associated disorders. The fleshy pulp of the fruit is eaten especially as snacks and its fruit has been found to have higher ascorbic acid content than oranges and guava. Tannins, flavonoids, terpenoids, proteins, carbohydrates and resins are the phytochemicals that have been reported in *C. Albidum*. The stem bark has been used for the treatment of diabetes with no scientific evidence. Therefore, the present study was designed to evaluate the anti-diabetic potentials of the ethanolic extract of *C. albidum* stem bark in alloxan-induced diabetic rats.

**MATERIALS AND METHOD**

**Drug and chemicals**

Metformin was used as a standard control in the study and alloxan monohydrate was used in the induction of diabetes and both were obtained from a registered pharmacy.
Plant
Collection and identification of plant material
The stem bark of *C. albidum* was obtained March, 14th 2016 from Obelle town Ikwere Local Government Area of Rivers State. It was botanically identified by Mr Kola Adeleke of the department of Pharmacognosy, Madonna University, Nigeria.

Preparation of plant extract
The stem bark of the tree of *C. albidum* was oven dried at 48 degrees centigrade for 4 days after which it was broken down into smaller pieces with the aid of a mechanical grinder. 500g of the powder was macerated with ethanol (1900ml) for 72 hours with constant shaking. The extract was then filtered after 72 hours and the filtrate concentrated using a rotary evaporator. The yield of the extract was found to be 29.52g which was stored a refrigerator for further use.

Phytochemical analysis
The following tests were carried out; test for flavonoid, tannin, protein, carbohydrate, saponin, alkaloid, glycoside, reducing sugar and steroid. The tests were carried out based on the procedures outlined by Harborne (1998).13

Animal
Adult albino rats (130-150g) were used for this study. They were kept in the animal house, of the Department of Pharmacology and Toxicology, Madonna University, Nigeria. They were allowed to acclimatize for 1 week prior to the experiment during which they were introduced to growers mash. The rats were housed in clean gauzed cages having free access to feed and water and maintained under standard conditions.

Acute toxicity test
This was carried out in 2 phases using the modified Lorke’s method.14 In phase 1, nine rats divided into 3 groups containing 3 rats each were used. Each group of rats were administered with different doses (10,100 and 1000mg/kg) of the plant extract. The rats were placed under observation for 24 hours to monitor their behavior as well as to see if mortality would occur. Phase 2 used 3 rats divided into 3 groups of 1 rat each. The rats were administered with 1500, 2500 and 5000 mg/kg of the plant extract and observed for 24hrs for changes in behavior and mortality. The LD50 which is the square root of the highest dose with no mortality multiplied by the lowest dose with mortality could not be calculated since none of the doses killed the rats although, some changes such as sluggish behavior, reduced appetite and thirst were observed.

Preparation of diabetic rats
The rats were made diabetic by intravenously injecting of 150mg/kg body weight of alloxan monohydrate dissolved in normal saline.15 Seventy two hours after alloxan administration, the induction of diabetes was confirmed by measuring the blood glucose levels using glucometer. The rats with blood glucose levels of 250-500mg/dL were considered diabetic and employed for the study.16

Grouping of rats and drug administration
- Group A contains 45 rats divided into 3 groups of 15 rats each. Rats were treated with 100, 200 and 400 mg/kg of CA extract for 7, 21 and 28 days respectively
- Group B contains 15 rats divided into 3 groups of 5 rats and served as non-diabetic control treated with 0.2ml of normal saline for 7, 21 and 28 days respectively,
- Group C contains 15 rats divided into 3 groups of 5 rats which served as diabetic, non-treated rats for 7, 14 and 21 days
- Group D contains 15 rats diabetic rats divided into 3 groups of 5 rats which were treated with the standard drug (metformin) for 7, 14 and 28 days
- Group D contained diabetic rats treated with 100, 200 and 400mg/kg body weight of the extract for 7, 14 and 28 days respectively while

Sacrifice of the rats
On the 29th day, the rats were sacrificed under anesthesia using diethyl ether and blood was collected from the heart. Two milliliter of blood was collected into the fluoride oxalate-bottle for blood glucose analysis. Also, 4 mL of blood was collected into plain sample bottle for lipid profile evaluation. Blood glucose was evaluated using glucometer whereas serum total cholesterol, triglyceride, low density lipoprotein cholesterol and high density lipoprotein cholesterol as reported using standard laboratory test kits (Randox Diagnostics, Crumlin, UK). LDL-cholesterol was estimated as reported by Friedewald et al., 1972.17 Pancreas was exercised rinsed in cold 1.05 % KCL solution the homogenized with 0.1M phosphate buffer (pH 7.2). The homogenate was centrifuged at 1200 rpm for 15min and the supernatant was decanted and evaluated for oxidative stress markers. Pancreas total protein content was determined according to Gonall et al. 194918 whereas malondialdehyde (MDA) was assayed as reported by Buege and Aust.19 Reduced glutathione (GSH) was evaluated as reported by Sedlak and Lindsay 196820 whereas superoxide dismutase (SOD) was measured as reported by Sun and Zigma 1978.21 Catalase (CAT) was analyzed using the method of
Aebi 1984 whereas Glutathione peroxidase (GPX) activity assayed according to Rotruck et al. 1973.

Statistical analysis
Data are expressed as mean ± SEM and were subjected to one way analysis of variance (ANOVA) test and Dunnett’s post hoc test. Results were considered to be significant at p<0.05

Phytochemical analysis
The phytochemicals present in the stem bark of *C. albidum* are shown in the table below

| Phytochemicals | Results |
|----------------|---------|
| Flavonoid      | ++      |
| Tannin         | ++      |
| Protein        | ++      |
| Carbohydrate   | +       |
| Saponin        | ++      |
| Alkaloid       | ++      |
| Steroid        | -       |
| Glycoside      | +       |
| Reducing sugar | -       |

Key: ++= High, += Moderate, - = Absent

Acute toxicity study

Phase one

Table 2 The acute toxicity test (LD-50) result of phase 1 using the modified Lorkes method (Lorke, 1983)

| Dose      | No of death |
|-----------|-------------|
| 10mg/kg   | 0/3         |
| 100mg/kg  | 0/3         |
| 1000mg/kg | 0/3         |

Phase two

Table 3 The acute toxicity test (LD-50) result of phase 2 using the modified Lorkes method

| Dose      | No of death |
|-----------|-------------|
| 1500mg/kg | 0/1         |
| 2500mg/kg | 0/1         |
| 5000mg/kg | 0/1         |

In phase 1, the rats that were administered different doses (10,100 and 1000mg/kg) of the plant extract after observation for 24 hours, no death was recorded neither were there noticeable behavioural changes. In the phase 2, all the rats also survived. There were no observable changes in behavior except at the highest dose (5000mg/kg) where the rat exhibited sluggish movements, lack of appetite and thirst although it didn’t die. It therefore indicated that the plant extract has a wide therapeutic index.

RESULTS

Phytochemical evaluation of the ethanolic extract of *C. albidum* stem back shows high presence of flavonoids, alkaloids and tannins (Table 1). No mortality was observed during the evaluation of the LD50 of *C. albidum* (Table 2 and 3). The body weights of non-diabetic rats treated with 100-400mg/kg of *C. albidum* extract for 7-28 days were not significantly (p<0.05) altered when compared to non-diabetic control. However body weights were significantly (p<0.05) decreased in diabetic rats when compared to control. In contrast body weight was increased in a dose and time dependent manner in diabetic rats pretreated with 100-400mg/kg of *C. albidum* extract when compared to diabetic control (Table 4). However, increases in body weights were most observed in metformin treated rats and differ significantly (p<0.05) when compared to 100 and 200mg/kg of *C. albidum* extract (Figure 1). Furthermore, 100-400mg/kg of *Calbidum* extract had no significant (p<0.05) effects on glucose levels in non-diabetic rats when compared to non-diabetic control (Table 5). On the other hand, glucose level was significantly (p<0.05) increased in alloxan-treated rats when compared to control. More diabetic rats administered with 100-400 mg/kg of the extract showed significant (p<0.05) decreases in blood glucose levels in a dose- dependent manner when compared to diabetic control. Comparatively, metformin produced significant (p<0.05) reduction in blood glucose level than 100, 200 and 400 mg/kg of the extract (Table 5). *C. albidum* extract did not produce significant (p<0.05) effects on serum TG, TC, LDL and HDL-C levels in non-diabetic rats when compared to non-diabetic control (Table 6-9). However, diabetic rats showed significant (p<0.05) elevations in glucose levels when compared to control. Nevertheless, diabetic rats treated with 100-400mg/kg of *C. albidum* extract showed decreased in serum levels of TG, TC and HDL with
Ethanolic extract of Chrysophyllum albidum, DC= Diabetic Control, Values are expressed as Mean± SEM, n=5, Values with different super script down the column differ significantly at p<0.05 ANOVA

Table 6: Effect of Chrysophyllum albidum on serum triglyceride level of alloxan-treated albino rats

| Dose (mg/kg) | 7days (mg/kg) | 14days (mg/kg) | 28 days (mg/kg) |
|-------------|---------------|----------------|-----------------|
| Control     | 58.1±3.61     | 57.8±4.32      | 55.7±3.51       |
| Diabetic Control (DC) | 299.9±15.2 | 300.5±12.3 | 310.2±11.8 |
| CA 100      | 59.6±4.57     | 61.9±5.11      | 58.5±4.21       |
| CA 200      | 57.6±5.33     | 60.6±6.32      | 58.7±5.23       |
| CA 400      | 60.1±6.63     | 58.3±5.56      | 57.4±4.76       |
| DC +Metformin | 100.6±9.61 | 99.1±8.90 | 100.7±6.41 |
| DC +CA 100  | 237.4±12.7    | 187.6±11.4     | 120.3±11.5      |
| DC +CA 200  | 160.9±10.3    | 131.8±10.7     | 100.7±10.6      |
| DC +CA 400  | 101.4±11.6    | 73.3±7.33      | 60.8±7.55       |

CA= Chrysophyllum albidum, DC= Diabetic Control, Values are expressed as Mean± SEM, n=5, Values with different super script down the column differ significantly at p<0.05 ANOVA

Furthermore, pancreatic levels of GSH, CAT, SOD and MDA were not significantly (p<0.05) altered in non-diabetic rats treated with the extract of C. albidum when compared to non-diabetic control (Table 7). In sharp contracts, pancreatic levels of GSH, CAT, SOD, were increased while MDA levels were decreased significantly (p<0.05) and in diabetic rats. However, the pancreatic levels of GSH, CAT, SOD, and MDA levels were significantly (p<0.05) restored and in a dose-dependent manner in C. albidum extract treated diabetic rats (Table 10).

**DISCUSSION**

Diabetes Mellitus (DM) is a metabolic disease associated with impaired glucose and lipid metabolism. It is also associated with impaired beta cell function and oxidative stress.24 The management of DM involves the use of insulin and oral hypoglycemic agents.25 However, some herbal preparations contained active medicinal constituents which are used in the management of diabetes.26 Recently, the world health organization estimated that 80% of people worldwide rely on herbal medicine for part of their primary health care. In Germany, about 600-700 plant based medicines are available and are prescribed by some 70% of German physicians.26 Therefore, the present study evaluated the effect of the ethanolic extract of C. albidum stem bark on alloxan –induced diabetic rats. In the present study, normal rats treated with 100-400mg/kg of C. albidum extract showed no changes in body weights. However, decreases in body weights were observed in diabetic rats. Interestingly, body weights were restored in diabetic rats treated with 100-400mg/kg of C. albidum extract treated. This study observed normal blood glucose levels in non-diabetic rats treated with 100-400mg/kg of C. albidum extract. On the other hand, blood glucose levels were elevated in diabetic rats. The observed increases in blood glucose levels in diabetic rats were restored in rats treated with 100-400mg/kg of C. albidum extract.

Furthermore, lipid profile which is usually altered in diabetic condition is a primary factor for the development of cardiovascular diseases.27 In the present study, serum levels of TG, TC, LDL-C and HDL-C were normal in rats treated with 100-400mg/kg of C. albidum extract. However, serum levels of TG, TC, LDL-C, and HDL-C were altered in diabetic rats, but were restored in diabetic rats treated with 100-400mg/kg of C. albidum extract. Studies have shown that diabetes is associated with oxidative stress characterized by altered levels of SOD, CAT, GSH, GPX and MDA.28 The by-products of lipid
Effect of *Chrysophyllum albidum* on total cholesterol level of alloxan-treated albino rats

| Dose (mg/kg) | 7 days (mg/kg) | 14 days (mg/kg) | 28 days (mg/kg) |
|-------------|----------------|-----------------|-----------------|
| Control     | 9.0±7.32       | 9.1±8.33        | 8.9±8.45        |
| Diabetic Control (DC) | 355.9±12.1 *   | 360.2±13.7 *    | 377.9±13.0 *    |
| CA 100      | 91.8±7.00      | 90.2±7.31       | 87.5±8.90       |
| CA 200      | 92.0±7.52      | 88.4±8.62       | 86.0±7.57       |
| CA 400      | 90.9±8.61      | 86.9±7.52       | 84.9±8.61       |
| DC + Metformin | 120.7±11.0 b   | 110.0±11.3 b    | 90.7±28.32 b    |
| DC + CA 100 | 257.8±12.7 c   | 209.7±13.8 c    | 159.3±10.37 c   |
| DC + CA 200 | 200.0±12.0 d   | 150.4±11.0 d    | 120.4±10.6 d    |
| DC + CA 400 | 155.6±10.6 e   | 130.8±10.8 e    | 90.9±7.52 e     |

CA = *Chrysophyllum albidum*, DC = Diabetic Control, Values are expressed as Mean± SEM, n=5, Values with different super script down the column differ significantly at p<0.05 ANOVA.

Effect of *Chrysophyllum albidum* on high density lipoprotein cholesterol level of alloxan-treated albino rats

| Dose (mg/kg) | 7 days (mg/kg) | 14 days (mg/kg) | 28 days (mg/kg) |
|-------------|----------------|-----------------|-----------------|
| Control     | 42.3±3.32      | 43.4±3.12       | 44.6±4.42       |
| Diabetic Control (DC) | 10.7±0.27 a    | 11.9±0.77 a     | 13.4±0.98 a     |
| CA 100      | 42.1±3.22      | 40.1±3.60       | 40.6±3.70       |
| CA 200      | 43.8±3.71      | 40.9±4.21       | 42.8±3.51       |
| CA 400      | 43.7±3.60      | 41.6±3.33       | 41.9±3.42       |
| DC + Metformin | 30.0±2.75 b   | 37.3±3.71 b     | 40.3±3.56 b     |
| DC + CA 100 | 15.5±1.20 c    | 22.0±1.22 c     | 27.5±3.33 c     |
| DC + CA 200 | 21.7±2.30 d    | 27.5±2.60 d     | 34.1±3.71 d     |
| DC + CA 400 | 27.0±2.55 b    | 32.0±3.50 b     | 42.9±4.57 b     |

CA = *Chrysophyllum albidum*, DC = Diabetic Control, Values are expressed as Mean± SEM, n=5, Values with different super script down the column differ significantly at p<0.05 ANOVA.

Effect of *Chrysophyllum albidum* on low density lipoprotein cholesterol level of alloxan-treated albino rats

| Dose (mg/kg) | 7 days (mg/kg) | 14 days (mg/kg) | 28 days (mg/kg) |
|-------------|----------------|-----------------|-----------------|
| Control     | 36.7±2.33      | 37.4±2.11       | 32.0±2.03       |
| Diabetic Control (DC) | 229.9±9.88 *   | 288.9±10.1 *    | 302.6±10.3 *    |
| CA 100      | 38.6±2.00      | 38.6±3.90       | 35.6±2.70       |
| CA 200      | 37.9±2.33      | 35.4±3.10       | 31.9±3.62       |
| CA 400      | 35.4±2.41      | 34.7±2.51       | 32.6±3.11       |
| DC + Metformin | 70.9±5.29 b   | 52.5±3.40 b     | 36.8±3.51 b     |
| DC + CA 100 | 195.5±9.88 c   | 142.0±8.61 c    | 140.7±7.66 c    |
| DC + CA 200 | 146.3±7.00 d   | 97.8±5.44 d     | 66.5±5.43 d     |
| DC + CA 400 | 108.8±7.90 b   | 84.5±6.33 b     | 50.9±4.33 b     |

CA = *Chrysophyllum albidum*, DC = Diabetic Control, Values are expressed as Mean± SEM, n=5, Values with different super script down the column differ significantly at p<0.05 ANOVA.

peroxidation such as conjugated dienes and MDA are increased in patients with obesity, metabolic syndrome and DM. In the current study, there were no effects observed on pancreatic levels of SOD, GSH, CAT, GPX and MDA in normal rats treated with 100-400mg/kg of *C. albidum* extract. Nevertheless, pancreatic levels of SOD, GSH, CAT, GPX and MDA were altered in diabetic rats. However, pancreatic levels of SOD, GSH, CAT, GPX and MDA were restored in 100 - 400mg/kg of *C. albidum* extract treated diabetic rats.

Alloxan, a β-cytotox toxic glucose analogue is commonly used for the development of animal model of type-I diabetes mellitus (IDDM). Alloxan is rapidly taken up by the pancreatic β-cells through GLUT2 receptors. The observed increases in glucose levels of diabetic rats could be attributed to alloxan selective inhibition of glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta cell, and its ability to induce free radical formation, resulting in the selective necrosis of beta cells. Furthermore, this study observed decreases in glucose levels in diabetic rats treated with metformin. Metformin is a standard drug used for the treatment of diabetics. The exact molecular mechanism of its action remains unknown. However, studies have shown that it can inhibits liver gluconeogenesis, facilitates glucose uptake into peripheral tissues, such as striated muscle and acts in the gut also, it increases insulin sensitivity. In this study, the extract of *C. albidum* might have exhibits similar mechanism as metformin in decreasing glucose levels in treated diabetic rats. Also, the effects of *C. albidum* extract could be attributed to its ability to increase insulin production by pancreatic cells. Also *C. albidum* extract might have decrease the release of glucagon or stimulates direct glycolysis in peripheral tissues or reduces glucose absorption from the gastrointestinal tract. *C. albidum* extract contains phytochemicals which include terpenoids that are known to reduce glycaemia through many mechanisms which include insulin like activity, inhibition of gluconeogenesis and glycoanalysis. The alterations in pancreatic levels of GSH, CAT, SOD, and MDA are signs of oxidative stress and lipid peroxidation via the generation of free radicals. Therefore, the effects of *C. albidum* extract observed on GSH, CAT, SOD, and MDA in diabetic rats could be attributed to its antioxidant effect through the inhibition of free radical production and its ability to regenerate antioxidants. *C. albidum* extract contains flavonoids, tannins and phenolics which have antioxidant activities. Studies have also shown that *C. albidum* extract contains high ascorbic acid contents which might have contributed to its antioxidant effect observed in the present study. These phytochemicals might have protected pancreas beta cells against oxidative stress, by increasing the endogenous defensive capacity of the pancreas to combat oxidative stress and by direct scavenging of free radicals.
Table 10  Effect of Chrysophyllum albidum on pancreas oxidative stress markers of alloxan-treated albino rats

| Dose (mg/kg) | MDA nmole/mg protein | SOD U/mg protein | CAT U/mg protein | GSH µg/mg protein | GPX U/mg protein |
|-------------|----------------------|------------------|------------------|-------------------|------------------|
| Control     | 0.14±0.02            | 27.8±3.00        | 20.6±2.00        | 14.8±2.12         | 25.0±3.52        |
| Diabetic Control (DC) | 3.21±0.55 *        | 6.71±0.12 *      | 4.70±0.08 *      | 4.91±0.73 *       | 7.22±0.76 *      |
| CA 100      | 0.13±0.05            | 28.1±2.00        | 21.7±3.70        | 15.0±2.63         | 25.1±3.52        |
| CA 200      | 0.12±0.07            | 28.8±2.33        | 21.9±3.51        | 15.6±3.51         | 25.6±2.01        |
| CA 400      | 0.13±0.22            | 30.0±3.12        | 22.8±3.42        | 15.9±2.12         | 26.0±3.63        |
| DC + Metformin | 1.20±0.45 b        | 24.7±2.32 b      | 18.7±2.00 b      | 12.5±1.21 b       | 23.1±3.72 b      |
| DC + CA 100 | 2.40±0.21 b c       | 10.8±0.11 c      | 7.51±3.33 c      | 6.12±0.72 c       | 12.6±1.51 c      |
| DC + CA 200 | 2.00±0.51 d         | 15.7±1.32 d      | 12.6±2.10 d      | 8.75±0.57 d       | 17.6±0.42 d      |
| DC + CA 400 | 1.53±0.21 e         | 20.8±3.11 e      | 17.0±1.73 e      | 12.0±1.21 b       | 23.4±2.00 b      |

CA = Chrysophyllum albidum, DC = Diabetic Control, Values are expressed as Mean± SEM, n=5, Values with different super script down the column differ significantly at p<0.05 ANOVA

Conclusion: The oral administration of the ethanolic extract of Chrysophyllum albidum stem back demonstrated anti-hyperglycemic, anti-hyperlipidemic and anti-oxidative effects on alloxan-induced diabetic rats. The present study, hypothesize that ethanolic extract of Chrysophyllum albidum contains essential medicinal substances that could be used as remedy for diabetes. However, further investigation to isolate the active medicinal substances and elucidate the exact mechanism of action is very imperative.

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CONFLICTS OF INTEREST
None

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