

Effect of Ethanol Leaf extract of *Chromolaena odorata* on hepatic markers in streptozotocin-induced diabetic wistar albino rats.

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ABSTRACT

Chromolaena odorata is one of the medicinal plants that have served all through the ages as the mainstay in the treatment and preservation of human health. This research was designed to determine the effect of ethanol leaf extract of *Chromolaena odorata* on hepatic markers (ALT, ALP, AST, GGT and bilirubin) in streptozotocin-induced diabetic wistar albino rats. All chemicals and reagents used in this study were of analytical grade. Diabetes was induced through intraperitoneal injection of streptozotocin (STZ) single dose of 70mg/kg body weight (b.w). Forty eight (48) rats were randomly distributed into six (6) groups of 8 rats each as follows: Group 1 were diabetic rats that were given 0.5ml normal saline, rats in group 2 were diabetic rats treated with 0.5mg glibenclamide, rats in group 3 were neither induced nor treated while rats in groups 4-6 were diabetic rats treated with graded doses of 250 mg/kg, 350mg/kg and 450mg/kg b.w of ethanol leaf extract of *Chromolaena odorata*, respectively. Blood glucose level and hepatic markers were assayed using standard biochemical methods. The study lasted for 21 days. After an overnight fast, blood samples were collected from the animals after anaesthesia via cardiac puncture. STZ injection led to increase in glucose and bilirubin levels as well as the activities of ALT, ALP, AST and GGT. Interestingly, rats in groups 4, 5 and 6 treated with graded doses of 250 mg/kg, 350mg/kg and 450mg/kg b.w of ethanol leaf extract of *Chromolaena odorata* recorded significant ($p < 0.05$) decrease in the activities of these enzymes and level of bilirubin when compared with the positive control rats (group 1). Also, there were no significant differences ($p > 0.05$) when group 3 rats (normal rats) and group 2 (treated with glibenclamide) were compared with groups 4-6 rats treated with graded doses of ethanol extract extracts group. In conclusion, the results from this research indicated that ethanol leaf extract of *Chromolaena odorata* lowered the activities of liver enzymes and level of bilirubin in STZ-induced diabetic wistar albino rats. The findings in this study suggest that *Chromolaena odorata* ethanol root extract possess hepatoprotective potentials and hence can be used to ameliorate hepatic dysfunction-associated diseases. Further study is however advocated to unravel the mechanism of action of this plant.

Keywords: *Chromolaena odorata*, medicinal plants, streptozotocin, Diabetes mellitus, hepatic markers.

INTRODUCTION

The importance of medicinal plants in traditional and modern medicines cannot be over-estimated. Medicinal plants serve

as important sources of new chemical compounds with potential therapeutic effects [1]. World Health Organization has

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advocated traditional medicine as safe remedies for management of various ailments [2]. Plants are major source of therapeutic compounds and are the essential foundation of medicine since prehistoric time. Herbal and natural products have been used in folk medicine for centuries throughout the world [2]. Medicinal plants are able to provide therapeutic effects due to the various chemical compounds inherent in them [3]. Several reports on different pharmacological roles of some medicinal plants are documented such as antioxidant [4], anti-inflammatory [5], antidiabetic [6,7], anticancer [8], hepatoprotective [9-11], antibacterial [12], antimalarial [13,14], lipid-lowering and cardioprotective [15,16].

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin action, insulin secretion or both [17]. Insulin deficiency leads to chronic hyperglycemia with disturbances in fat, carbohydrate and protein metabolism. It is a global menace; with varying prevalence rate from country to country. However, India, China and United States of America (USA) are the top three countries in terms of number of diabetic patients [18]. Some factors like ageing, consumption of calorie-rich diet, obesity and sedentary lifestyle contribute to tremendous increase in the number of diabetics worldwide [17]. There are two main types of DM: type 1 and type 2, each with distinct pathogenesis [19]. However, the common feature to both is hyperglycemia and various life threatening complications [19, 20]. Type 1 diabetes mellitus is caused by insulin deficiency due to autoimmune destruction of pancreatic β -cells. Destruction of pancreatic β -cells progresses to absolute deficiency in insulin. This condition develops rapidly in young people and had been found to occur in any age group [21]. Note that, pancreatic β -cells destruction involves autoimmune mechanisms; hence, type 1 DM is also known as autoimmune type 1 diabetes mellitus. Type 2 diabetes also known as non-insulin dependent or adult-onset is characterized by insulin

resistance in the peripheral tissue and an insulin secretory defect of the β -cell. This is the most common form of DM and is highly associated with a family history of diabetes, old age, obesity and lack of exercise [22]. It is more common in women, especially women with a history of gestational diabetes. Type 2 diabetes is characterized by derangement of carbohydrate, protein and fat metabolism [23]. Insulin resistance and hyperinsulinemia eventually leads to impaired glucose tolerance [22]. Diabetes mellitus is associated with an increased risk of cardiovascular and hepatic diseases mediated via oxidative stress. Reactive oxygen species can directly damage lipids, proteins or Deoxyribonucleic acid (DNA) and modulate intracellular signaling pathways. Due to the many limitations of already existing hypoglycemic agents, search for newer drugs with fewer side effects and lower cost is imminent [24]. Several researchers have reported that some plants possess antidiabetic properties and this justifies the use of herbs by rural dwellers in the management of diabetes mellitus [6, 11, 25, 26, 27].

Chromolaena odorata is one of the medicinal plants that have served all through the ages as the mainstay in the treatment and preservation of human health. It's a tropical and subtropical species of flowering shrub in the family *Asteraceae*. It is native to America, from Florida and Texas in the United States [28]. It is a rapidly growing perennial herb that has been introduced to tropical Asia, West Africa, and parts of Australia. They are mostly spread by the wind, but can also cling to fur, clothes and machinery, enabling long distance dispersal. The plant can regenerate from the roots [29]. *Chromolaena odorata* contains carcinogenic pyrrolizidine alkaloids [30]. It is toxic to cattle [29]. The plant is larvicidal against all major mosquito vectors [31].

Chromolaena odorata has several local names. For instance, in mid-western Nigeria, it is called "Akintola" or "Awolowo"; in eastern Nigeria, it is called

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several names among which are "Obialofulu" and "Queen Elizabeth" indicating that the spread of weed might have been widely noticeable during the period of the queen's visit to Nigeria in 1956. There is no known local name for it in the Hausa language which probably is due to its scarcity in northern Nigeria [32].

Chromolaena odorata has been reported to have antispasmodic, antiprotozoal, antitrypanosomal, antibacterial and antihypertensive activities. It has also been reported to possess anti-inflammatory, astringent and diuretic activities [3]. In the south-eastern part of Nigeria, the leaves are used for wound dressing, skin infection and to stop bleeding. Some specific phenolic compounds have been isolated from the plant and the medicinal values of this plant is credited to these component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body [3].

Streptozotocin (STZ) is widely used to induce diabetes in various laboratory animals as it is particularly toxic to the pancreatic insulin-producing beta cells in

mammals [33]. Alloxan and streptozotocin are the most prominent diabetogenic chemicals in diabetic research. Both are cytotoxic glucose analogues and their mechanism of beta cell selective action is identical [33, 34]. Streptozotocin inhibits insulin secretion and causes a state of insulin-dependent diabetes mellitus. Both effects can be attributed to its alkylating potency and beta cell specificity. STZ is transported into the cell by glucose transport protein GLUT2 but it is not recognized by other glucose transporters [33].

Most medicinal plants presently employed by local herbalists are used without much scientific information and evaluation of vital organs like liver. It is therefore important to access and document the ethno-medicinal claims of these medicinal plants. In view of the various traditional medical applications of *Chromolaena odorata*, scientific investigation will help in establishing its efficacy especially those which can be used in the treatment and management of several ailments. The aim of this research was to determine the effect of ethanol leaf extract of *Chromolaena odorata* on hepatic markers in streptozotocin induced diabetic wistar albino rats.

MATERIALS

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade and

standard. Commercial test kits were sourced from products of Randox, UK.

Collection of Biological Materials

The present study was carried out using the leaf of *Chromolaena odorata* and albino rats. Fresh leaves of *Chromolaena odorata* were collected from Abakaliki Local Government in Ebonyi State and was authenticated in the *Herbarium* Unit of Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria by Mr. Nwankwo Onyebuchi, a plant Taxonomist. Forty-Eight (48) albino wistar rats were purchased from the Department of

Zoology, University of Nigeria, Nsukka, Enugu State, Nigeria. They were acclimatized for a period of two weeks prior to commencement of experiment. They were maintained at room temperature, 12hr day/night period and fed *ad libitum* on water and growers mash; weighed prior to commencement of experiment and daily till the end of the experiment.

METHODS

Preparation of Plant Material

Fresh leaves of *Chromolaena odorata* were harvested and washed under tap water to remove contaminants and air

dried under shade for three weeks, after which it was milled to powder form using grinding machine.

Extraction of Plant Material

A known quantity, 500g of ground leaves of

Chromolaena odorata were macerated in

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1500ml of ethanol with thorough shaking at regular interval for 72h at room temperature (26-28°C). The resulting solution was filtered using Whatman No.1 filter paper. The filtrates were

concentrated using rotary evaporator to obtain slurry of the extract. The semi-pastry extract was stored in the refrigerator and used for the study.

Determination of Liver Function Status

Serum Gamma-glutamyltransferase (γ -GGT) activity was determined as described by Szasz [35]. Aspartate Amino Transaminase (AST), Alanine Amino Transaminase (ALT) and Alkaline Phosphatase (ALP) activities

were assayed using Reitman and Frankel [36] method. Bilirubin level was determined by the method of Jendrassik and Grof [37].

Experimental Design

Forty eight (48) Wistar albino rats were used in this study. They were randomly distributed into six (6) groups of 8 rats each. The treatment and blood glucose determination lasted for 21 days. All meals were stopped by 7pm on the 21st day. After an overnight fast, blood samples were collected from the animals following chloroform anaesthesia and sacrifice/opening up of the animals using syringes and needles via inferior vena cava

and cardiac puncture, into already labelled K2 EDTA, plain and lithium heparin bottles without undue pressure to either the arm or the plunger of the syringe. The samples were mixed by gentle inversion. The samples in the lithium heparin and plain bottles were centrifuged at 5000r/min for 10mins to obtain plasma and serum. The plasma and serum supernatants were separated into sterile plain bottles and were used for biochemical assays.

Animal grouping

Group 1: (Positive control) Rats in this group were induced with diabetes without treatment and received 0.5ml of normal saline daily.

Group 4: Diabetic rats treated with 250 mg/kg body weight of the crude ethanol leaf extract of *Chromolaena odorata* daily.

Group 2: (Standard control) Rats in this group were induced with diabetes and treated with 0.5mg/kg body weight of glibenclamide daily.

Group 5: Diabetic rats treated with 350 mg/kg body weight of the crude ethanol leaf extract of *Chromolaena odorata* daily.

Group 3: (Negative control) Rats in this group were not induced with diabetes and were not treated but received 0.5 ml of normal saline daily.

Group 6: Diabetic rats treated with 450 mg/kg body weight of the crude ethanol leaf extract of *Chromolaena odorata* daily.

Induction of Diabetes

The baseline blood glucose levels were determined before the induction of diabetes. Rats were fasted overnight and experimental diabetes induced by intraperitoneal injection of streptozotocin (STZ) with a single dose of 70mg/kg body weight. STZ was dissolved in 0.1M citrate

buffer at pH of 4.5. After three days, rats with blood glucose level greater than 250mg/dl that exhibit hyperglycemia were selected for the experiment. The Accu-Check one-touch blood glucose monitoring meter and test strips were used for the assay.

Statistical Analysis

Results were expressed as mean \pm standard deviation (SD). Mean values were properly determined and compared using one-way analysis of variance (ANOVA) followed by Turkey's post hoc test;

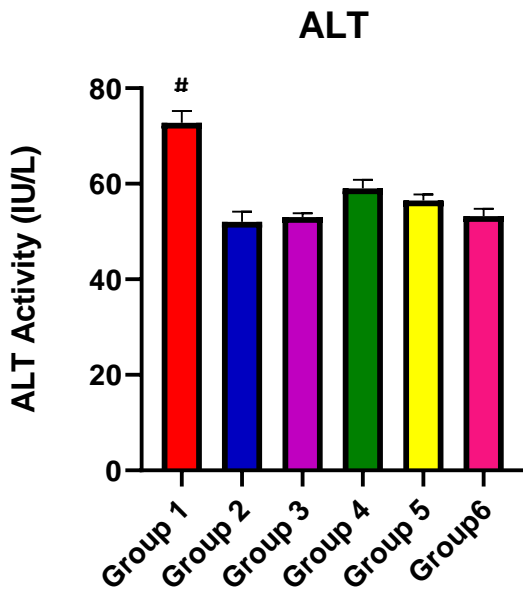
significance was accepted at $p < 0.05$. All statistical analysis was carried out using Graph Pad Prism version 5.00 for Windows.

RESULTS

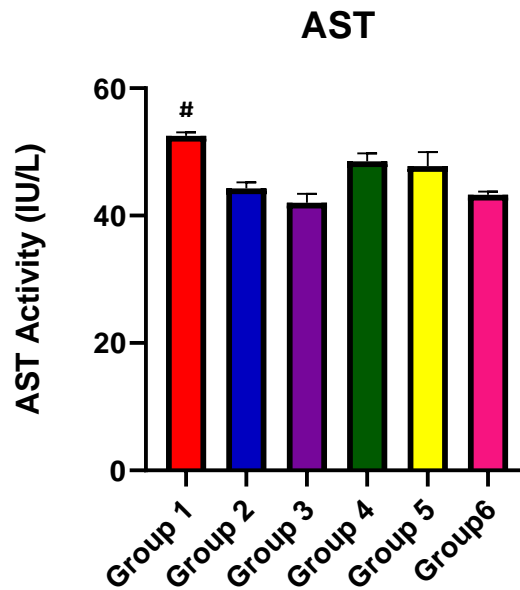
Effect of Ethanol Leaf-Extract of *Chromolaena odorata* on Liver Function Parameters in STZ-induced Diabetic Albino Rats

STZ injection led to increase in activities of ALT, ALP, AST, GGT activities and bilirubin level. Treatment with graded doses of 250 mg/kg, 350mg/kg and 450mg/kg b.w of ethanol leaf extract of *Chromolaena odorata* produced significant decrease ($p < 0.05$) in ALT, ALP, AST, GGT activities and bilirubin level

compared with the positive control groups. Also, there were no significant differences ($p > 0.05$) when group 3 rats (normal rats, negative control) and group 2 (treated with glibenclamide) were compared with groups 4-6 rats treated with graded doses of ethanol extract groups (Figure 1A-E).



(A)



(B)

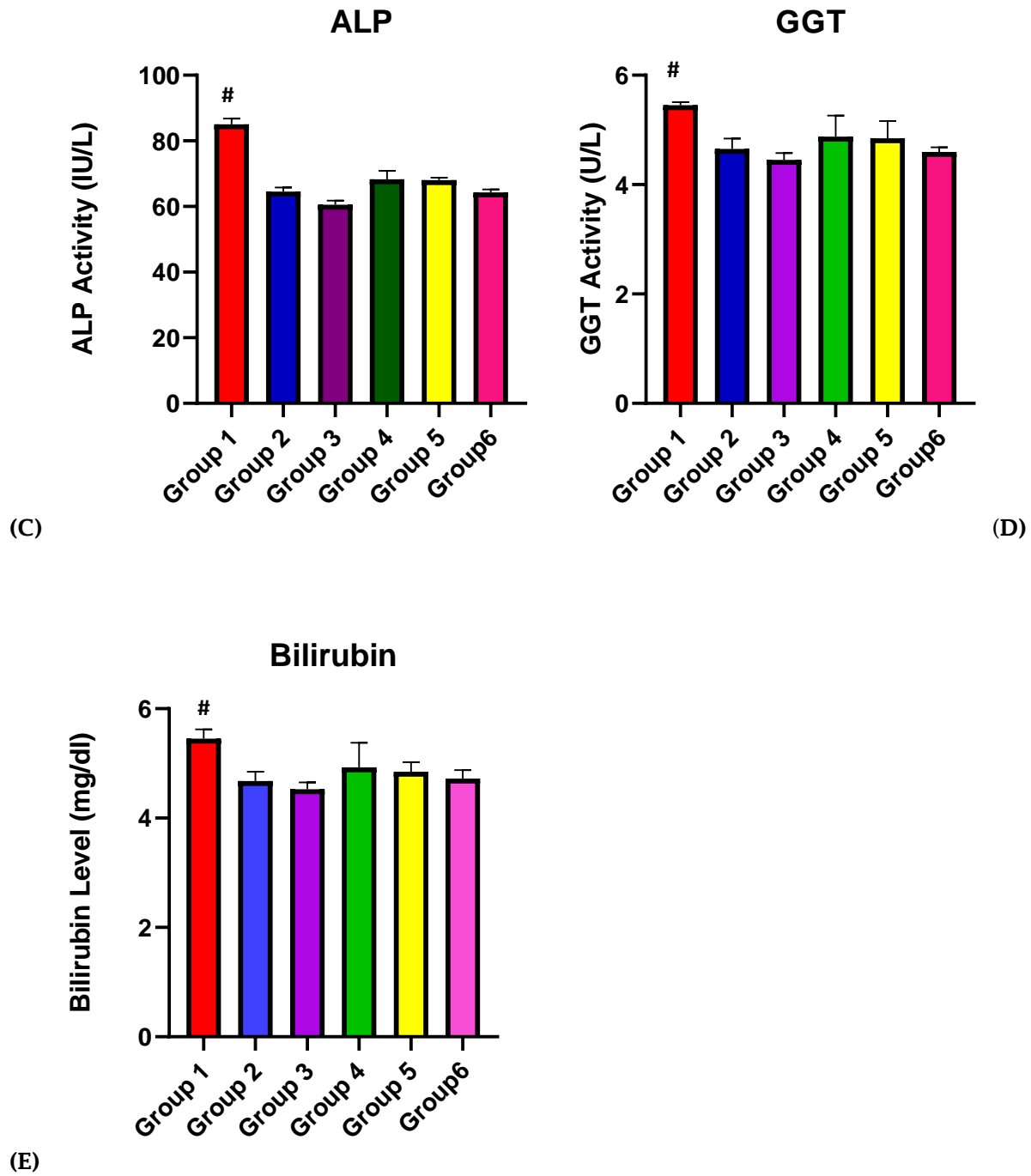


Figure 1 (A-E): Effect of Ethanol Leaf-Extract of *Chromolaena odorata* on Liver Function Parameters in STZ-induced Diabetic Albino Rats (n=6). '#' represents significant difference (p<0.05) of positive control from treated groups. There were no significant differences (p>0.05) among treated groups but all were significantly different from positive control (bars without '#').

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Group 1: induced and treated with 0.5ml normal saline (positive control); Group 2: induced and treated with 0.5mg/kg of glibenclamide; Group 3: No induction nor treatment (Negative control); Group 4: diabetic rats treated with 250mg/kg of

ethanol leaf-extract of *Chromolaena odorata*; Group 5: diabetic rats treated with 350mg/kg of ethanol leaf-extract of *Chromolaena odorata*; Group 6: diabetic rats treated with 450mg/kg of ethanol leaf-extract of *Chromolaena odorata*

DISCUSSION

Diabetes mellitus (DM) is a metabolic disorder that is characterized by high blood glucose level and derangement in vital organs of the body. Liver is an important organ involved in excretory, synthetic and metabolic functions and hence a target organ that needs to be checked during treatment of DM. This study was designed to evaluate the effect of treating diabetic rats with graded doses of ethanol leaf extract of *Chromolaena odorata* on the liver. Streptozotocin (STZ) is a popular diabetogenic compound [38]. Expectedly, injection with STZ produced increase in ALT, ALP, AST, GGT activities and bilirubin level indicating hepatobiliary damage. Previous studies have reported increase in ALT, ALP, AST, GGT activities and bilirubin levels in rats injected with STZ [7, 16].

Interestingly, treatment of the diabetic rats with graded doses of 250 mg/kg, 350mg/kg and 450mg/kg b.w of ethanol leaf extract of *Chromolaena odorata* significantly decreased ($p < 0.05$) ALT, ALP, AST, GGT activities and bilirubin level compared with the positive control groups. This result corroborates previous reports about the hepatoprotective effect of medicinal plants [9, 39, 10, 6, 40, 41, 42]. In the present study, the decreased activities of these liver enzymes were similar to the effects produced by a known standard drug (glibenclamide) in group 2 (standard control). This shows that the extract can be used to ameliorate hepatic damages caused by diabetes and its related diseases.

Liver is a vital organ and site of metabolism of xenobiotics and drugs. Therefore, liver malfunction will impair the function and metabolism of drugs and xenobiotics in the blood [43].

The results from this research indicated that ethanol leaf extract of *Chromolaena odorata* lowered the activities of liver

Markers of liver function capacity are the aminotransferases (ALT, AST), ALP, GGT, bilirubin and albumin. Aminotransferases are cytosolic enzymes widely distributed in tissues with highest concentration in liver and heart with ALT being more specific to the liver. Damage to the membrane architecture of cells due to exposure to toxicants will lead to their spillage into blood [43]. Increase in the serum activities of ALT, AST, GGT and ALP signify hepatic impairment [41, 42, 44, 45, 46]. ALT and AST are sensitive indicators of hepatocellular injury but they lack specificity as they are also present in muscle (cardiac and skeletal), kidney, and RBCs. These aminotransferases may be increased in patients presenting with cirrhosis, chronic hepatitis, alcoholic hepatitis, acute viral hepatitis and toxic ischemic injury [47]. ALP is found in the liver, bone, kidney, intestine and placenta [48]. Serum ALP is thus a mixture of different ALP isoenzymes which can be fractionated by electrophoresis. In normal serum, ALP comprises the liver and bone moieties; however, bone ALP is heat labile. Liver ALP, found on canalicular surfaces, is raised in any condition of biliary obstruction (intrahepatic and extra-hepatic). In hepatocyte injury, ALP is often normal or marginally elevated [49]. GGT activity is normal in bone disease and hence used to corroborate ALP increase in biliary disease [48]. Serum bilirubin is used in distinguishing causes of jaundice [48]. In prehepatic jaundice due to haemolysis, unconjugated bilirubin is increased with little or no increase in conjugated bilirubin. In hepatic and post-hepatic jaundice, there is increased conjugated bilirubin [50].

CONCLUSION

enzymes and level of bilirubin in STZ-induced diabetic wistar albino rats. The findings in this study suggest that

Chromolaena odorata ethanol root extract possess hepatoprotective potentials and hence can be used to ameliorate hepatic

dysfunction-associated diseases. Further study is however advocated to unravel the mechanism of action of this plant.

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