RESEARCH ARTICLE

To study renoprotective effect of *Callistemon Citrinus* leaves ethanolic extract on fructose induced metabolic syndrome in experimental animals

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ABSTRACT:

In this study, we studied the ethanolic extract of callistemon citrinus leaves and examined its effects on high fructose-induced metabolic syndrome (MetS) in experimental animals. MetS groups were given a 10% w/v fructose solution to drink ad libitum throughout 9 weeks, while normal animals received regular water. EECC extract was administered to treated groups via gavage for the last 6 weeks of the experiment. Fructose administration in liquid form increased body weight, decreased insulin sensitivity, raised blood glucose levels, and caused atherogenic dyslipidemia linked to kidney oxidative stress and structural damage. MetS rats were treated with EECC extract at doses of 250 and 500mg/kg body weight/day, which significantly improved the fructose-induced changes. This study concluded that the ethanolic leaves extract of Callistemon citrinus has hypoglycemic and hypolipidemic properties, antioxidant and renoprotective abilities against fructose-induced metabolic syndrome in rats.

Key words: Metabolic syndrome, Callistemon citrinus, Fructose, Renoprotective

1. Introduction

Metabolic syndrome is the collective term for a number of problems that raise the risk of atherosclerotic cardiovascular disease, including peripheral vascular diseases, insulin resistance, myocardial infarction, cerebrovascular accidents, and type II diabetes mellitus. Central obesity, insulin resistance, atherogenic dyslipidemia, and hypertension are a few of the metabolic illnesses that make up the metabolic syndrome.[1] MetS is the aetiology of chronic kidney disease (CKD), which develops apart from hypertension and diabetes. The suggested pathophysiologic aetiology of Metabolic Syndrome (MetS) is obesity.[2] MetS is a significant risk factor for chronic kidney disease (CKD), affecting 20–25% of the population. Among other aspects of renal pathophysiology, MetS influences microalbuminuria, glomerular hyperfiltration, RAAS podocyte destruction, and profibrotic factors.
For the morbidity and mortality of cardiovascular events, such as stroke, myocardial infarction, sudden cardiac death, and thrombosis, MetS is a commonly seen risk factor. It is also crucial to the progression and initial development of chronic kidney disease (CKD). As the prevalence of MetS increases, nephrologists are becoming increasingly aware of the effects it has on the kidneys.[3] C. citrinus (Linn.), more often known as "Crimson Bottle Brush," is an evergreen shrub or tree in the Myrtaceae family. Its mid-green, acutely pointed leaves can grow up to 15 metres in height and 1.5 to 1.3 metres in circumference.[4] The spikes of vivid red flowers on this evergreen plant. Little, woody capsules that resemble bead bracelets on the bark follow the flowers and can persist for years. C. citrinus, also referred to as the "crimson bottle," is an evergreen shrub native to Australia.For its initial shape and form, refer to the bottlebrush as Red bottlebrush or Lemon bottlebrush. The plant has antidibetic, Hypolipidimic and antioxidant activity,[5] thrombolytic, free radical scavenging activity,[6] oxidative stress, cardioprotective activity[7] and in the treatment of obesity[8]

2. Materials and Methods:

2.1 Plant Material:

Callistemon citrinus was collected on oct 2023-24 from the region of pune city Callistemon citrinus Dum. Cours. A member of family Myrtaceae, Skeels. Bull. Bur. PI. Industr. U.S.D.A 282,49 (1913) wfo 0000239474; Curtis, Bot. Mag. 8:t.260 (1794) wfo 0000242379, is correctly identified by referring Herbarium, The plant was botanically authenticated and vouched specimen was disposited in the herbarium of the department of Botany and Research Center by Dr. S D. Randive and Prof. Dr. M. N Jagtap

2.2 Preparation of *callistemon citrinus* extract:

Leaves of Callistemon citrinus were collected from nigdi pune region of maharashtra (19◦ 41′ 11.3″ N latitude and 101◦ 12′ 18.4″ O longitude). Weighed fresh leaves were dried at room temperature crushed into course powder in a 1:10 ratio (1 g/10 ml of ethanol) using 96% ethanol

course powder of leaves were macerated for for five days, callistemon citrinus leaf extract was stored at room temperature in the dark. At 45 °C, the extract was finally concentrated in a rotating evaporator using vacuum removal, and it was then kept at 4 °C until needed again[9]
The extract was put through qualitative chemical testing to determine the different phytoconstituents, including glycosides, saponins, carbohydrates, sterols, alkaloids, flavonoids, tannins, proteins, and triterpenoids.

2.3 Animals:

Male albino Wistar rats weighing 180-200 g were used for present study. These animals were housed in standard environmental conditions and provided with a standard pellet diet and unrestricted access to water. The study received approval from the institutional animal ethics committee (Registration No. 884/OP/05/ac/CPCSEA), and strict adherence to CPCSEA guidelines was maintained throughout the care and experimentation process.

2.4 Preliminary phytochemical screening:

In our research, we performed Preliminary phytochemical screening on the EEAI, revealing the presence of a diverse range of phytoconstituents. Specifically, we identified sterols, glycosides, alkaloids, flavonoids, carbohydrates, amino acids, and proteins within the plant's extract.

2.5 Thin layer chromatomatography:

The ethanolic extracts of *Callistemon Citrinus* underwent thin-layer chromatography, utilizing 250 µm thickness plates (TLC Silica gel 60 F254, Merck, Germany).The spots were developed using a solvent system consisting of Chloroform:Methanol (9:1).

2.6 Experimental animals and protocol:

Male albino Wistar rats, with an initial weight range of 200–250 g, were sourced from Crystal Biological Solutions. The rats were accommodated in a temperature-controlled room maintained at 22 ± 3°C, with a 12-hour light-dark cycle, and they had access to water ad libitum. Following one week of acclimatization, the rats were randomly divided into five groups (n = 6/each group) as given below:

- NC; Normal control group
- FC; Fructose-drinking control group
- F+C.C1; Fructose-drinking group treated with 250 mg/kg b.w. of C.C extract.
- F+ C.C2; Fructose-drinking group treated with 500 mg/kg b.w. of C.C extract
- F+MT; Fructose-drinking group treated with 300 mg/kg b.w. of Metformin (the positive control)[10]

During the nine weeks of the experiment, the fructose-drinking groups were given (10% w/v) fructose solution ad libitum[11], while the normal animals were given regular water. For the final

six weeks of the trial, treatment groups received gavages containing *Callistemon Citrinus* leaves extract. Over the course of nine weeks, whole blood samples obtained by tail tipping method following a 12-hour fast were used to measure fasting blood glucose (FBG) levels using the[12] method. Glucose oxidase-peroxidase reactive strips and an ACCU-CHEKVR Performa glucometer (Roche diagnostics, France) were used in the test. Every rat was also weighed at both the beginning and end. Sample collection and biochemical analysis. At the conclusion of this study, blood samples were obtained from the retro-orbital plexus while the animals were under anesthesia induced by a combination of ketamine (100 mg/kg).

2.7 Preparation of kidney and serum samples:

Before being put under anaesthesia for the experiment, the animals were fasted for the whole night. All animal blood has been collected in EDTA-containing tubes. The whole blood samples were centrifuged at 3000rpm for 10 minutes. The blood serum was collected and stored at around 20 degrees Celsius to evaluate variables associated with renal impairment, insulin, lipid profile, and total protein. The kidneys were removed from the rats right away, rinsed with ice-cold physiological saline, patted dry, and weighed. The renal levels of reduced glutathione (GSH) and malondialdehyde (MDA) were measured in the blood serum, while the histological changes were assessed in the right kidney.

The Relative kidney weigh (RKW) is calculated according to following formula:

RKW= (AKW/FBW) x 100

AKW Absolute kidney weight and FBW final body weight (Body weight of rat on day of sacrifice)

2.8 Estimatation of serum insulin level and insulin sensitivity index:

The collected blood samples were centrifuged at 3000 rpm for 10 minutes to separate the serum, which was then preserved in Eppendorf tubes at a temperature of -20°C.This stored serum was later utilized for the analysis and evaluation of various biochemical parameters.The estimation of serum insulin was conducted at Yashwantrao Chavan Memorial Hospital in Pimpri-Chinchwad, Pune. Additionally, the estimation of MDA and GSH was carried out at SCITESLA, located in Navi Mumbai.

Insulin sensitivity index was estimated by homeostasis model of assessment of insulin resistance

HOMA-IR = [fasting insulin (μIU/mL) × fasting glucose (mmol/L)]/22.5

2.9 Determination of lipid profile and renal dysfunction parameters (serum):

TG, TC, urea, uric acid, creatinine levels in blood serum were evaluated by the commercially available kit reagents (Erba)

2.10 Measurement of total protein, reduced glutathione and malondialdehyde in Blood serum

Plasma Total protein (TP) concentration was determined by the method of Bradford (Bradford 1976) using bovine serum albumin (BSA) as a standard. The estimation of MDA and GSH was carried out at SCITESLA, located in Navi Mumbai.

2.11 Preparation of renal homogneate and histological study in the kidney:

The kidney of the euthanized animals were carefully collected and preserved in buffered formalin (4%). Subsequently, kidney tissue segments, each measuring 1 gm. weight, They were subsequently paraffin-embedded, and these tissue sections underwent staining with hematoxylin and eosin (H&E) to aid in the examination of histological modifications. The investigation was conducted under ×100 magnification using an optical microscope from Zeiss, Germany[13]

2.12 Statistical analysis:

The results of this study are reported as mean±standard error of the mean (SEM). When comparing outcomes at a specific time point (the end of the treatment period), the statistical analysis was carried out using a one-way ANOVA and Dunnett's multiple comparison post-test. The interaction between time and treatment was examined using a two-way analysis of variance (ANOVA) and Bonferroni's multiple comparison post-test. At $p<.05$, $p<.01$, and $p<.001$, the results were deemed statistically significant, extremely significant, and very highly significant, respectively.
The data is presented as the mean ± standard error of the mean (SEM) for measurements, and statistical significance was evaluated at a significance level of $p < 0.05$. Statistical analysis was conducted through one-way analysis of variance (ANOVA), followed by Dunn's multiple comparison test, utilizing GraphPad Prism 9.0software. This robust statistical approach allows for a comprehensive examination of the data and the identification of significant differences between groups[14]

3. Results

In vivo study:

3.1 Effect of *Callistemon citrinus* ethanolic extract on body weight fasting, blood glucose and insulin sensitivity

[Table 1] shows the result when feeding normal and fructose-drinking rats every day in gestion of 250 and 500 mg/kg b.w. of Callistemon citrinus ethanolicliquid extract of leaves on their body weight, ultimate FBG [Table 2], plasma insulin levels, and insulin resistance. Following 9 weeks of feeding Wistar rats an oral solution containing 10% w/v fructose, the FC group showed significant increases in FBG and total body weight. Additionally, compared to normal control animals, FC rats had considerably greater plasma insulin concentrations and an insulin

resistance index (HOMA-IR) [Table 3]. Rats given fructose at doses of 250 and 500 mg/kg body weight were shown to have significantly reduced body weights, final FBG levels, and plasma insulin levels as well as improved insulin sensitivity.

I) Body weight

Table1: Effect of administration of EECC on body weight in high fructose-Diet induced metabolic syndrome in experimental animals

	NORMAL	DC+HFD	HFD+MET	HFD+EECC (250mg/kg)	HFD+EECC (500mg/kg)
INITIAL	113.5± 3.063222704	178.1666667± 8.642594775	187.5± 3.818813079	155.6666667± 4.702245327	155.8333333± 5.387743292
AFTER INDUCTION	144.1666667± 5.940912762	205.8333333± 7.682953714	215± 4.654746681	198.6666667± 3.460892627	195.3333333± 5.517648452
AFTER TREATMENT	215± 9.309493363	275± 13.8443731	240.3333333± 4.447221355	259.1666667± 13.44226337	240± 7.302967433

*BW:Body weight; NC: normal control rats; HFDC: High fructose-drinking control rats; HFD+CC250: High fructose drinking rats treated with CC extract at250mg/kg b.w/day; HFD+CC500: High fructose drinking rats treated with CC extract at 500 mg/kg b.w/day and HFD+MT: High fructose drinking rats treated with metformin at 300mg/kg b.w./day. Values are expressed as Mean ± S.D. (n =6),. #P< 0.05, **P < 0.01, ***P < 0.001. Compared with disease control (ANOVA followed by multiple Dunnet's test)*

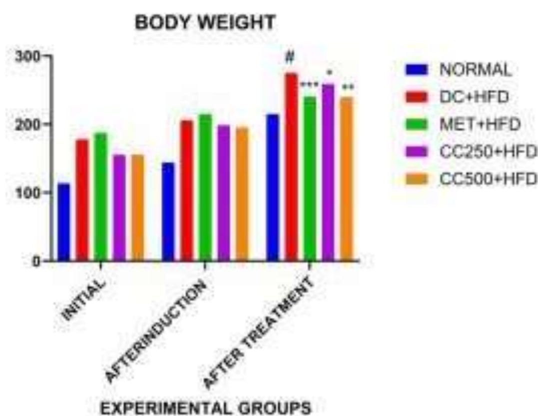

Fig.3.1. Effect of EECC on body weight in high fructose-diet induced metabolic syndrome in experimental animals.

*Body weight; NC: normal control rats; HFDC: High fructose-drinking control rats; HFD+CC250: High fructose drinking rats treated with CC extract at250mg/kg b.w/day; HFD+CC500: High fructose drinking rats treated with CC extract at 500 mg/kg b.w/day and HFD+MT: High fructose drinking rats treated with metformin at 300mg/kg b.w./day. Values are expressed as Mean ± S.D. (n =6),. #P< 0.05, **P < 0.01, ***P < 0.001. Compared with disease control (ANOVA followed by multiple Dunnet's test)*

II) Fasting blood glucose:

Table2: Effect of administration of EECC on fasting blood glucose in high fructose-Diet induced metabolic syndrome in experimental animals

	NORMAL	**DC+HFD**	**HFD+MET**	**HFD+EECC (250mg/kg)**	**HFD+EECC (500mg/kg)**
INITIAL	97.33333333± 3.200694369	105.1666667± 2.271807895	104.6666667± 2.940143609	105.5± 2.376972865	102.8333333± 1.815060452
AFTER INDUCTION	89.66666667± 2.060204952	109± 1.932183566	100.3333333± 1.308094458	101.1666667± 2.00693243	101.6666667± 1.475729575
AFTER TREATMENT	101.5± 2.404856198	127.8333333± 2.688452674#	113.1666667± 2.00693243***	110.1666667± 2.227355183*	111.8333333± 1.990254032**

*FBG Fasting Blood Glucose; NC: normal control rats; HFDC: High fructose-drinking control rats; HFD+CC250: High fructose drinking rats treated with CC extract at250mg/kg b.w/day; HFD+CC500: High fructose drinking rats treated with CC extract at 500 mg/kg b.w/day and HFD+MT: High fructose drinking rats treated with metformin at 300mg/kg b.w./day. Values are expressed as Mean ± S.D. (n =6),. #P< 0.05, **P < 0.01, ***P < 0.001. Compared with disease control (ANOVA followed by multiple Dunnet's test)*

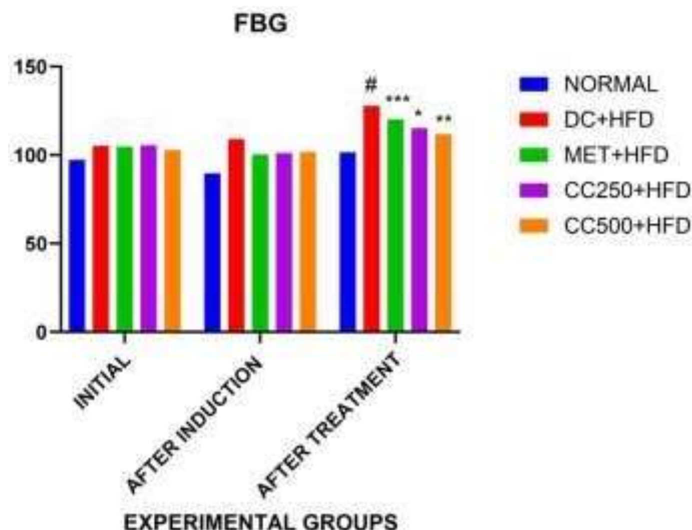

Fig.3.2. Effect of EECC on body weight in high fructose-diet induced metabolic syndrome in experimental animals.

*FBG Fasting Blood Glucose; NC: normal control rats; HFDC: High fructose-drinking control rats; HFD+CC250: High fructose drinking rats treated with CC extract at250mg/kg b.w/day; HFD+CC500: High fructose drinking rats treated with CC extract at 500 mg/kg b.w/day and HFD+MT: High fructose drinking rats treated with metformin at 300mg/kg b.w./day. Values are expressed as Mean ± S.D. (n =6),. #P< 0.05, **P < 0.01, ***P < 0.001. Compared with disease control (ANOVA followed by multiple Dunnet's test)*

III) Insulin sensitivity

Table3: Effect of administration of EECC on body weight in high fructose-Diet induced metabolic syndrome in experimental animals

Groups	Final FBG	Insulin(mg/dl)	HOMA-IR
NORMAL	89.666± 2.060	1.046± 0.027	0.283333333± 0.025516879
DC+HFD	109± 1.932	2.77± 0.207	0.978333333± 0.05160211
MET(300mg/kg)+HFD	100.33± 1.308	1.851± 0.198	0.47± 0.041553179
CC250(mg/kg)+HFD	101.66± 2.940	1.986± 0.137	0.675± 0.046025355

CC500(mg/kg)+HFD	100.83± 2.104	1.811± 0.222	0.476666667± 0.04601932

*HOMA-IR: homeostasis model assessment of insulin resistance; FBG: fasting blood glucose; NC: normal control rats; HFDC: High fructose-drinking control rats; HFD+CC250: High fructose drinking rats treated with CC extract at250mg/kg b.w/day; HFD+CC500: High fructose drinking rats treated with CC extract at 500 mg/kg b.w/day and HFD+MT: High fructose drinking rats treated with metformin at 300mg/kg b.w./day. Values are expressed as Mean ± S.D. (n =6),. #P< 0.05, **P < 0.01, ***P < 0.001. Compared with disease control (ANOVA followed by multiple Dunnet's test)*

3.2 Effect of *Callistemon citrinus* ethanolic extract on renal dysfuntion (Urea, Uric acid, Creatinine(blood serum)

[Table 4] shown the serum urea, uric acid, creatinine level in the disease control group displayed a notable increase compared to the normal control group. In contrast, a significant decrease was noated in the group treated with metformin. Furthermore, the administration of EECC at both doses(250mg/kg and 500mg/kg) as resulted in a significant reduction in the urea, uric acid, creatinine levels compared to the disease control group.

Groups	**Urea**	**Uric Acid**	**Creatinine**
NORMAL	28.08± 0.034832935	2.238333333± 0.088030929	0.521666667± 0.007031674
DC+HFD	53.845± 0.141768591#	4.611666667± 0.213875613#	0.613333333± 0.014063349#
MET(300mg/kg)+HFD	29.73333333± 0.198958399***	3.326666667± 0.03765339***	0.546666667± 0.010852547***
CC(250mg/kg)+HFD	46.01± 0.061913919*	3.503333333± 0.006666667**	0.56± 0.012110601*
CC(500mg/kg)+HFD	31.32166667± 0.298601369**	3.395± 0.030632227**	0.521666667± 0.014240006**

*Table 4: Urea; GSH:Uric acid;Creatinine;; NC: normal control rats; FC: fructose-drinking control rats; HFD+CC250: Heigh fructose drinking rats treated with C.C extract at250mg/kg b.w/day; HFD+CC500: Heigh fructose drinking rats treated with C.C extract at 500 mg/kg b.w/day and HFD+MT: Heigh fructose drinking rats treated with metforminat 300mg/kg b.w./day. Values are mean ± SEM (N=6).#P< 0.05, **P < 0.01, ***P < 0.001. Compared with disease control (ANOVA followed by multiple Dunnet's test)*

3.4 Effects of *Callistemon Citrinus* Ethanolic extract on relative kidney weight, GSH, MDA, total protein (Tissue homogenates)

The variations in relative kidney weight, GSH, and MDA levels in renal homogenate, as well as total protein and kidney dysfunction markers in plasma, between normal and fructose-drinking

rats. Consuming fructose-enriched water for 9 weeks significantly lowered GSH and total protein levels, but raised relative kidney weight and MDA concentrations. Administration of EECC to fructose-drinking groups for 6 weeks resulted in significant increases in GSH and total protein levels, as well as lower relative kidney weight. Furthermore, the acquired data (Table) demonstrated that the gavage of EECC significantly increased the level of GSH to a value greater than the NC level.

Groups	**Kidney weight**	**GSH**	**MDA**	**Total protein**
NORMAL	0.57±0.01	26.88± 0.282	7.25± 0.269	7.133333333± 0.068150161
DC+HFD	0.66±0.02#	20.853± 0.257#	31.233± 0.261#	6.033333333± 0.249902203#
MET(300mg/kg)+HFD	0.56±0.02 ***	22.225± 0.073****	31.233± 0.261****	6.563333333± 0.057600154****
CC(250mg/kg)+HFD	0.58±0.0**	23.675± 0.156**	9.603± 0.195**	6.588333333± 0.088030929**
CC(500mg/kg)+HFD	0.56±0.01***	25.333± 0.284 0.284***	8.518±0.160 0.160***	6.625± 0.12574445***

*Table 3: Kidney weightl; GSH: reduced glutathione; MDA: malondialdehyde; total protein; NC: normal control rats; FC: fructose-drinking control rats; HFD+CC250: Heigh fructose drinking rats treated with C.C extract at250mg/kg b.w/day; HFD+CC500: Heigh fructose drinking rats treated with C.C extract at 500 mg/kg b.w/day and HFD+MT: Heigh fructose drinking rats treated with metforminat 300mg/kg b.w./day. Values are mean ± SEM (N=6) #P< 0.05, **P < 0.01, ***P < 0.001, ****p<0.0001 . Compared with disease control (ANOVA followed by multiple Dunnet's test)*

3.5 Effect of callistemon *citrinus ethanolic* extract on Lipid profiles TG, TC

[Table 4] Shown the serum triglyceride, total cholesterol, level in the disease control group displayed a notable increase compared to the normal control group. In contrast, a significant decrease was evident in the group treated with metformin. Furthermore, the administration of EECC at both doses (250 and 500 mg/kg) resulted in a significant reduction in triglyceride, total cholesterol, when compared to the disease control group.

Groups	TG(mg/dl)	TC(mg/dl)
NORMAL	85.185±16.638	65.51±1.524
DC+HFD	294.88±59.60#	104.15±1.905#
MET(300mg/kg)+HFD	184.51±37.193****	73.79±1.238****

CC(250mg/kg)+HFD	150.46±29.901**	75.511±1.483**
CC(mg/kg)500+HFD	141.98±28.344***	70.843±1.850***

*Table 4: TC: total cholesterol; TC: triglycerides; NC: normal control rats; FC: fructose-drinking control rats; HFD+CC250: fructose drinking rats treated with C.C extract at250mg/kg b.w/day; HFD+CC500: fructose drinking rats treated with C.C extract at 500 mg/kg b.w/day and HFD+MT: fructose drinking rats treated with metforminat 300mg/kg b.w./day. Values are mean ± SEM (N=6) #P< 0.05, **P < 0.01, ***P < 0.001, ****p<0.0001 . Compared with disease control (ANOVA followed by multiple Dunnet's test)*

3.6 Histology of Kidney

The consumption of a high fructose diet led to histopathological changes in the kidney of rats in the HFD group, including morphological alterations, notably glomerular hypertrophy and tubular dilatation alterations. These findings indicate significant damage to the kidney tissue as a result of the high fructose intake. The administration of metformin (300 mg/kg) and CC (250 and 500 mg/kg) notably mitigated these alterations, leading to a significant improvement in the histological structure, as depicted in [Figure 3.3].

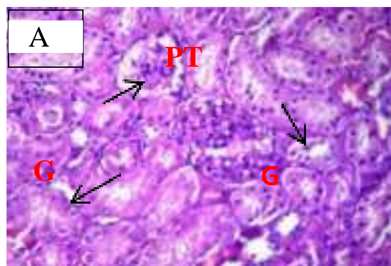

Fig1: NC

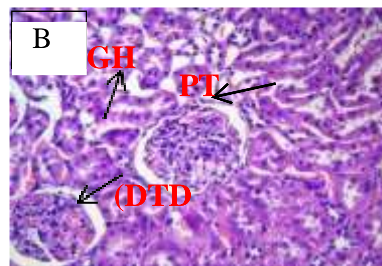

Fig2: HFDC

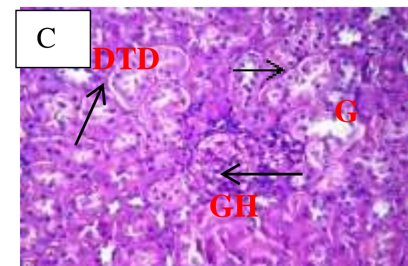

Fig3: HFD+MET(300mg/kg)

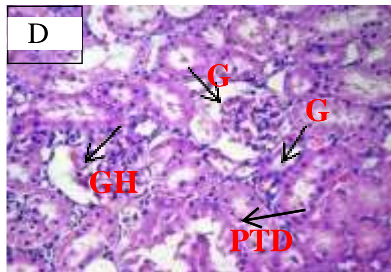

Fig4: HFD+EECC(250mg/kg)

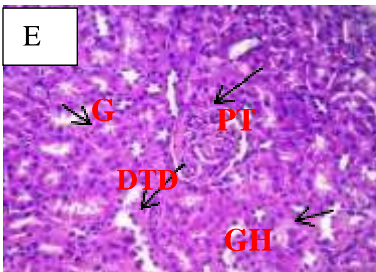

Fig4: HFD+EECC(500mg/kg)

Fig.3.3. Effect of EECC on histopathological kidney tissue in high fructose-diet induced metabolic syndrome in experimental animals.

Histopathological comparisom of the renal tissues sections in normal and Heigh fructose-drinking rats. Photomicrographs showing sections from renal tissue (× 400 magnifications, scale bar: 100µm) [A] NC: normal control rats; [B] HFDC: Heigh fructose-drinking control rats; [D] HFD+CC250: Heigh fructose drinking rats treated with CC extract at250mg/kg

b.w/day; [E] HFD+CC500: Heigh fructose drinking rats treated with CC extract at 500 mg/kg b.w/day and [C] HFD+MT: Heigh fructose drinking rats treated with metformin at 300mg/kg b.w./day. Values are mean ± SEM (N=6) G: glomeruli in Bowman's capsules; GH: glomerular hypertrophy; GN: glomerular necrosis; DT: distal tubules; PT: proximal tubules; PTD: proximal tubules dilatation; DTD: distal tubules dilatation.

4. Discussion

Previous phytochemical studies on *Callistemon Citrnus* show the consistent presence of phenolic acid, flavonoids, Terpenoids, steroids, polyphenols,(Gallic acid, Rutuine, 1, 8-cineole, limonene, alpha terpinol, alpha pinene, Beta terpinene, P-cymene, steroids, carbohydrates, quercetin, ellagic acid, flavonoids, P-coumeric acid)[15 16 8 5] Fructose consumption in human subject and in experimental animals has been associated to many of the components of metabolic syndrome such as insulin resistance, glucose intolerance, obesity and hypertension[17]. Moreover, when ingested in large amounts as part of a hypercaloric diet, it can provokes a raise in total and visceral fat mass associated with accumulation of ectopic fat in the liver and skeletal muscle[18]. In the present study, after 9weeks of fructose feeding as a liquid solution (10% w/v) to Wistar rats, significant ($p<.01$) augmentations in body weight [Table 1] and fasting blood glucose[Table 2] were noted in FC group. Moreover, plasma insulin concentrations and insulin resistance index (HOMA-IR) were significantly ($p<.001$) higher in FC rats than those in normal control animals [Table 3], demonstrating that experimental MetS rats have obesity, hyperglycaemia, hyperinsulinemia with insulin resistance[Table3], which are previously reported in this animal model[19 20 21] Treating fructose-drinking rats with ethanolic extract of *Callistemon citrinus* leaves considerably lowered body weight, final FBG and plasma insulin levels as well as ameliorated insulin sensitivity. The hypoglycaemic effect of L. guyonianum extract may be attributed to the gallic acid and vanillic acid. Indeed, gallic acid was found to ameliorate hyperglycaemia and hepatic carbohydrate metabolism in rats fed a high-fructose diet[22]. On the other hand, vanillic acid was found to provoke a significant decreases in the values of fasting plasma glucose, insulin and blood pressure in diabetic hypertensive rats[23] Fructose is a highly lipogenic nutrient implicated in the onset of a profound metabolic dyslipidemia, which appears to result from hepatic and intestinal overproduction of atherogenic lipoprotein particles[24 17]. Oxidative stress and lipid peroxidation are regarded as some of the basic mechanisms of tissue damage caused by MetS. In this study, the intake of heigh fructose-enriched water by experimental rats reduced drastically GSH level and augmented considerably MDA concentration in their renal homogenates [Table 5], indicating the development of oxidative stress and increased peroxidative deterioration of lipids in renal tissues of these insulin resistant rats, which was previously reported[5 25]. Administration of ethanolic leaves extract of Callistemon citrinus to heigh fructose-drinking groups for the last 6 weeks of experimental period resulted in considerable increase in GSH level associated with reduced level of lipid peroxidation intermediate, MDA [Table 5]. Furthermore, the obtained results showed that the gavage of *Callistemon citrinus* laves extract at 500mg/kg b.w. to normal rats increased remarkably the GSH content in renal tissue to value higher than the NC level [Table 5]. Antioxidant activity of

this halophyte was attributed to its richness in phenolics, more probably in flavonoids and their combined activity[26].

Several studies have suggested a close relationship between metabolic syndrome and renal damage[27] [28]. In our study, experimental MetS animals have exhibited increased relative kidney weights associated with augmented levels of urea, creatinine and uric acid [Table 4]. The enhanced plasma levels of urea, creatinine and uric acid could be attributed to reduced clearance of these substances, reflecting a decline in the glomerular filtration rate[29] and/ or an increased net tubular absorption. Furthermore insulin resistance induced by long term exposure to a high fructose diet was suggested to be associated to renal structural damage, evidenced by kidney inflammation, glomerular hypertrophy, sclerosis and tubulointerstitial injury[30] [31] [32] which were also observed in our MetS rats (Figure 2). Administration of ethanolic extract of *Callistemon citrinus* leaves to fructose-drinking groups for 6 weeks resulted in considerable reduction of relative kidney weight associated with lowered concentrations of urea, creatinine and uric acid [Table 4]. A moderate decline of the renal histological damage was also observed in treated animals [Figure 3.3].

As illustrated in [Table 5], a clear decrease in total protein levels was observed in all heigh fructose-drinking groups compared to the normal value. The noted hypoprotinemia might be explained by a drastic decline in hepatic protein anabolism secondary to insulin resistance or may be caused by a microproteinuria state due to a renal failure, which was suggested in previous experimental studies. The renoprotective ability of callistemon citrinus against heigh fructose-induced MetS in rats may be related to Phenols and Flavonoids[33] have suggested that diosmin alleviate hypertension and nephropathy in MetS rat model via potent antioxidant capacity, anti-hyperlipidemic effects and attenuation of inflammation. On the other hand[34]have postulated that polyphenols, phenolic acids, flavonoids, Terpenolids may be effective to regulate glycaemia and kidney damage

CONCLUSION:

The data here demonstrated, that ethanolic extract of Callistemon citrinus leaves possesses hypoglycaemic, antioxidant and renoprotective abilities against fructose-induced metabolic syndrome in rats. These activities are probably due to the richness of the extract in phenolic acids and more particularly in flavonoids. Our results represent a scientific argument for the folk medicine use of the plant in treating diabetes and prevention of this disease by delaying the progression of the metabolic syndrome

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