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Pharmacological evaluation of *Chloroxylon swietenia* DC bark extract on alloxan induced Diabetic nephropathy in Wistar rats

Kaur Manpreet[1], Sharma Amit[2], Kaushik Madan[3]

[1]Asst. Professor Dept. of pharmacy, SBS polytechnic and pharmacy collage Patti Tarn Taran, Punjab, India
[2]Asst. Professor Dept. of Pharmacy Manav Bharti University, Village-Laddo, Sultanpur, Kumarhatti, District Solan, Himachal Pradesh. **India**
[3]Supervisor & Head Department of Pharmacology, CT Institute of Pharmaceutical Sciences, Jalandhar-Punjab, **India**

ABSTRACT

Diabetes is now a days consider as common problem with serious issues. Diabetes cause various other dysfunctions and issues in various organs and parts of bodies, so it should be cured and treated. *Chloroxylon swietenia* DC bark extract proved to be helpful in treating diabetic problems. It is highly available and can cure various other problems caused by diabetes in bodies. In present study we have studied various properties, functions, effects, of extracts of *Chloroxylon swietenia* DC bark extract on alloxan induced Diabetic nephropathy in Wistar rats.

Key words: Diabetes, wistar rats, alloxan, nephropathy, UV Spectrophotometer, Glucose

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***Address for Correspondence**
Amit Sharma, Asst. Professor Dept. of Pharmacy Manav Bharti University, Village-Laddo, Sultanpur, Kumarhatti, District Solan, Himachal Pradesh. India

INTRODUCTION

Diabetic nephropathy is the leading cause of end stage renal failure (ESRF) worldwide, representing over 50% of patients on renal replacement therapy in some parts of the world. The condition is common in people with type 1 and type 2 diabetes, although the incidence appears to be declining, especially in type 1 diabetes. More than 1 in 3 people with type 2 diabetes have impaired kidney function[1]. As the incidence of diabetes is increasing worldwide, diabetic nephropathy has become the main cause of chronic kidney disease in patients who require renal replacement therapy in the Western world, also known as Kimmelstiel- Wilson syndrome[2]. It is characterized by glomerular hypertrophy, thickness of basement, tubular and glomerular membranes and accumulation of extracellular matrix in these membranes that finally cause tubule interstitial and glomerular fibrosis and sclerosis[3], about 3% per year after 15 years.

There are five stages of diabetic nephropathy

Stage I: Hypertrophic hyper filtration.

Stage II: The quiet stage.

Stage III: The micro albuminuria stage.

Stage IV: Chronic kidney failure (CKF) is the irreversible stage.

Stage V: Terminal kidney failure[3-4].

Herbal product represent safely and securely as compare to synthetic drug which leads toward research in herbal medicines[5-6]. The bark of Chloroxylon swietenia may be play important role in the treatment of diabetic nephropathy.Chloroxylon swietenia DC. is a member of Rutaceae family. It is a medium sized and deciduous tree with a height of about 9 -15 m and 1.0 -1.2 m girth with a spreading crown and clear bole up to 3 m. The tree is native to India and Sri Lanka and commonly known as „Ceylon Satinwood" or „East Indian Satinwood". In India, it is found wild in dry deciduous forests up to an

altitude of 1100 m, extending in the north to the Satpuras and Chota Nagpur[7]. Phytochemicals has been Investigated in this Plant such as alkaloids, coumarin, flavonoids, saponins, triterpenes, Glycosides.

Diabetic Nephropathy (History)

Diabetic nephropathy (DN) is one of the most common and serious long-term complications in diabetic patients, affecting about 40% of these patients[8-9]. According to United States renal data system, DN is the leading cause of end-stage renaldisease, and a major contributing factor to morbidity and mortality of diabetic patientsthroughout the world[10].

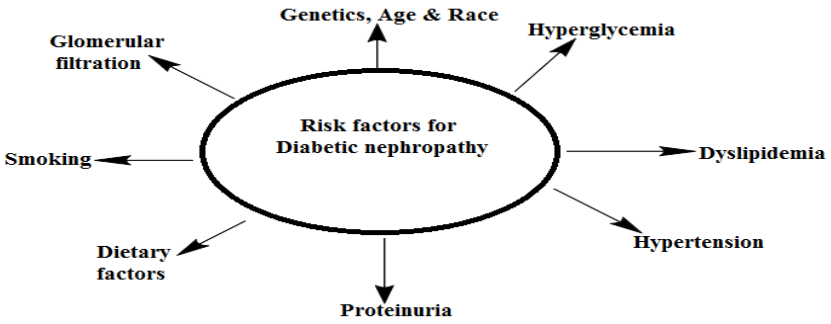


Figure 1: Risk factors for Diabetic nephropathy

Plant Profile:-

***Chloroxylon swietenia* DC** is a member of Rutaceae family. It is a medium sized and deciduous tree with a height of about 9 -15 m and 1.0 -1.2 m girth with a spreading crown and clear bole up to 3 m. The tree is native to India and Sri Lanka and commonly known as Ceylon Satinwood or East Indian Satinwood. In India, it is found wild in dry deciduous forests up to an altitude of 1100 m, extending in the north to the Satpuras and Chota Nagpur. It grows on black cotton soils, metamorphic rocks and bare rocky ground on poor soils, if they are well drained and contain a large portion of sand or gravel.

Table1: Vernacular Name of *Chloroxylon Swietenia* DC

Synonym	**English**	**East indian Satinwood**
	Hindi	Bhirra
	Telugu	Billudu
	Tamil	Purasu
Family:	Rutaceae	
Subfamily:	Flindersioideae	
Genus:	*Chloroxylon*	
Species:	*C. swietenia*	
Traditional Uses	Antifungal, anti-inflammetry, anti-rheumatism, anti-diabeti, anti-microbial, ant-ioxidant, insecticidal, anthelminitic, analgesic.	

Figure 2 : *Chloroxylon swietenia*DC

MATERIAL AND METHODS

Collection of plant material

The plant material ***Chloroxylon Swietenia* DC** investigated in the present study was collected from Satpura, Madhya pardesh in the months of June- July, 2016. The plant was identified and authenticated by Sri Venkateswar University Tirputi in December 2016, Voucher number 1042. The protocol was dually approved by IAEC under Protocol no: **IAEC-CTIPS / 2017/ VIII / 0054 (PCL-M).**

Table No: 2 List of Instruments used

S. No.	Name	Model	Make
1.	UV Spectrophotometer	UV- 1800	Shimadzu UV- Spectrophotometer
2.	Auto-analyzer	ERBA CHEM-Touch	Transasia Bio Medical Ltd.
3.	Cooling micro centrifuge	RM-1215	REMI electrotechnik Ltd. Vasai -401208 (India)
4.	Digital Balance	AV213	OHAUS
5.	Heating mental	-	Perfit
6.	Rotary vacuum evaporator	PI-68	Popular India
7.	Metabolic cages	CMR-01	Orchid Scientific

Table No: 3 List of Active pharmaceutical ingredient

S. No.	Active pharmaceutical ingredients/ bulk	Manufacturers
1.	Alloxan	Loba Chemie Ltd, India
2.	Glibenclamide	Sanofi India Ltd, (B. No. A150027)

Table No: 4 List of Chemical and reagent

S. No.	Chemical	Manufacturers
1.	Trichloroaceticacid (TCA)	Fisher Scientific
2.	Thiobarbituric acid (TBA)	Avarice Laboratories Pvt. Ltd., GB nagar; India
3.	Sodium hypdroxide(NaOH) pellets	LobaChemie Ltd, India
4.	Fehling's solution A	Qualigens fine chemicals
5.	Fehling's solution B	Avarice laboratories Pvt. Ltd. , GB nagar; India
6.	Benedict's reagent	Ranbaxy fine chemicals Ltd. New Delhi
7.	Million's reagent	Nice chemicals Pvt. Ltd. Cochin
8.	Conc. Sulphuric acid (H_2SO_4)	Nice chemicals Pvt. Ltd. Cochin
9.	Potassium dihydrogen phosphate (KH_2PO_4)	Ranbaxy fine chemicals Ltd.
10.	Ninhydrin solution	LobaChemie Ltd, India
11.	Acetic anhydride	LobaChemie Ltd, India
12.	Sodium nitropruside	LobaChemie Ltd, India
13.	Pyridine	Nice chemicals Pvt. Ltd. Cochin
14.	Benzene	Nice chemicals Pvt. Ltd. Cochin
15.	Ammonia	Nice chemicals Pvt. Ltd. Cochin
16.	Glacial acetic acid	Nice chemicals Pvt. Ltd. Cochin
17.	Ferric chloride ($FeCl_3$)	Nice chemicals Pvt. Ltd. Cochin
18.	Lead acetate	LobaChemie Ltd, India
19.	Mayer's reagent	LobaChemie Ltd, India
20.	Hydrocholoric acid (HCl)	Nice chemicals Pvt. Ltd. Cochin
21.	Nitric acid (HNO_3)	Molychem, Mumbai
22.	Glucose solution	Molychem, Mumbai
23.	Normal saline	Nirma Ltd, India

Table No: 5 Kits used for estimation of diabetic nephropathy

S.No.	Name	Make, Importer/ Supplier
1.	Glucose Trinder end point/ fixed point estimation kit	ERBA Diagnostic Mannheim GmbH, Germany
2.	Cholestrol DES (CHOD-PAP method with LCF, end point) kit.	
3.	Triglycerides DES (GPO-Trinder method, end point) kit	ERBA Diagnostic Mannheim GmbH, Germany
4.	Creatinine (CRE) (Jaffe's Method, Initial Rate) kit	
5.	BUN kit	
6.	Erba wash kit	

Preliminary Phytochemical Screening

Test for carbohydrates:

Fehling test:

1 ml of Fehling's A and 1 ml of Fehling B solution was mixed and boiled for one minute. Equal volume of extract was added and heated for 5-10 minute in boiling water bath and color was observed.

Benedict's test:**:**Equal amount of extract and Benedict's reagent were mixed in tst tube and heated in boiling water bath for 5 min, color was observed.

Test for proteins:

Million's test:**:**3 ml of extract was mixed with 5 ml Million's reagent. Then this mixture was warmed to observe the color change.

Xanthoprotein test:

3 ml of extract was mixed with 1ml conc. H_2SO_4 to check the formation of white precipitate and its color change.

Test for amino acids:

NinhydrinTable no:

5 Kits used for estimation of diabetic nephropathy test (General):**):-** 3 ml of extract and 3 drops of 5% Ninhydrin solution were heated in boiling water bath for 10 min and color change was observed.

Million's test:**:**3 ml of extract and 3 drops of Million reagents were mixed and heated. Colour change was observed.

Test for steroid:

Salkowskireaction:

To 2 ml of extract, 2ml chloroform and 2ml conc. H_2SO_4 were added andshaked well. Appearance of color change in chloroform layer ndin acid layer was observed.

Liebermann reaction:3ml of extract and 3 ml of acetic anhydride were mixed and heated. After colling, few drops of conc. H_2SO_4 were added and to observe color change.

Tests for glycosides:

Legal's test:

1 ml pyridine and 1 ml of sodium nitropruside was added to aqueous extract and pink to red colour change were observed.

Borntrager's test:

To 3 ml of extract, dil. H_2SO_4 was added, boiled and filtered. To cold filtrate, equal volume of benzene and chloroform were added and shaked well. Organic solvent was separated and ammonia was added. Change in colour of ammoniacal layer was noted. Keller–Killianitest:To 2 ml of extract, glacial acetic acid, one drop of 5% $Fecl_3$ and conc. H_2SO_4 were added and the appearance of colour at junction of two liquid layers was observed.

Tests for flavonoids:

Lead acetate test:

To small quantity of residue, lead acetate solution was added and formation of coloured precipitate was observed.

Sodium hydroxide test:

Sodium hydroxide was added in increasing amount in the extract and changes observed.

Extract was evaporated and to the residue, dil. HCl was added, shaked and filtered. With filtrate following tests were performed

Mayer's test:

To 2-3 ml of filtrate, few drops of Mayer's reagent were mixed and changes observed.

Murexide test:

To 2-3 ml extract, 2-3 drops of conc. HNO_3 were added and evaporate it to dryness. Cool and 2 drops of NH_4OH were added and color change was observed.

Test for Tannins and Phenolic compounds:

A. To 2-3 ml of extract 5% of $FeCl_3$ solution was added and color change was observed.

B. To 2-3 ml of extract few drops of dil. HNO_3 were added and color change was observed.

ANIMALS

Healthy adult Wistar albino rats (200-300g) of either sex between 6-8 months of age were used for investigation and were procured from Panacea Biotec Ltd, Lalru (140501), India. They were housed in group in the polypropylene cages were kept not more than 4 in each cage; maintained under standard conditions (12:12h light: dark cycle; 25±3ºC; 40-60% humidity) and fed with standard rat pellet diet (Shri Jagdambey feed industries, Moga, Punjab) and water *ad libitum.* The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) [Protocol no. **IAEC-CTIPS / 2017/ VIII / 0054 (PCL-M)]** of the Institute under the guideline of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment & Forests, New Delhi.

Hyperglycemia was induced by single dose alloxan. It was prepared freshly in water for injection and administered to rats 120 mg/kg, intraperitoneally. Glucose solution (5% w/v) was immediately administered orally to alloxan treated rats in order to prevent transient hypoglycemia.

GROUPING OF ANIMAL

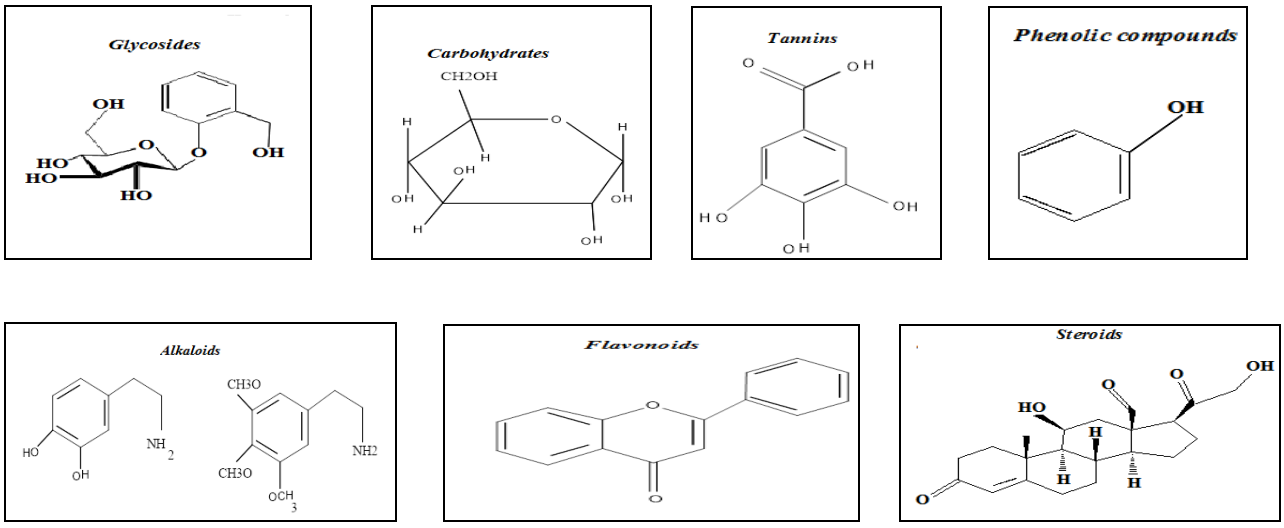
Table No: 6 Grouping of Animal

Treatments Groups	Group Name	(n=5 for each group)
Group I	Normal Control	Normal Control rats received standard pellet diet & water *ad libitum*
Group II	Negative Control	Negative control has been receive single I.P injection of Alloxan 120 mg/kg
Group III	Standard	Alloxan induced Diabetic rats has been treated with reference standard Glibenclamide at a dose of 10 mg/Kg/ for 28 days
Group IV	CSB 125	Alloxan induced Diabetic rats has been treated with CSBat a dose of 125 mg/kg, P.O for 28 days
Group V	CSB 250	Alloxan induced Diabetic rats has been treated with CSB dose of 250 mg/kg, P.O for 28 days
Group VI	CSB 500	Alloxan induced Diabetic rats has been treated with CSB dose of 500 mg/kg, P.O for 28 days

RESULTS & DISCUSSION

Phytochemical screening of *Chloroxylon swietenia* DC

The following active constituents of methanolic extract bark of *Chloroxylon swietenia* DC *are* ***Glycosides, Carbohydrates, Alkaloids, Tannins, Flavonoids, Phenolic compounds, Steroids.***



Animals study:-

Physiological parameters

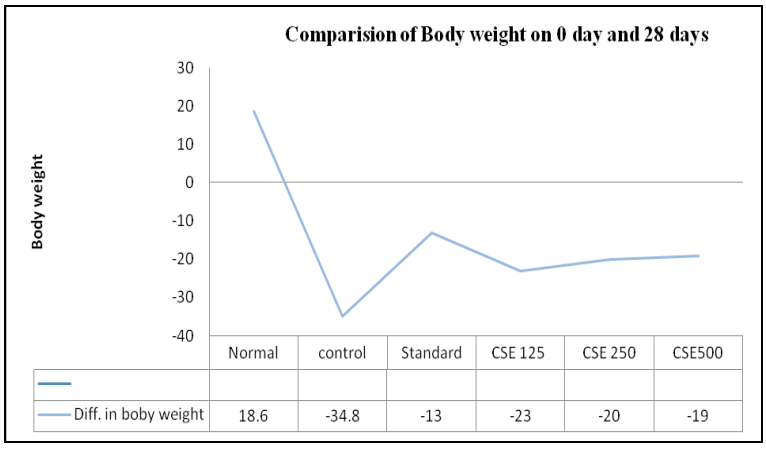


Figure 3: Effect of Methanolic bark extract of *Chloroxylon swietenia* DC on body weight

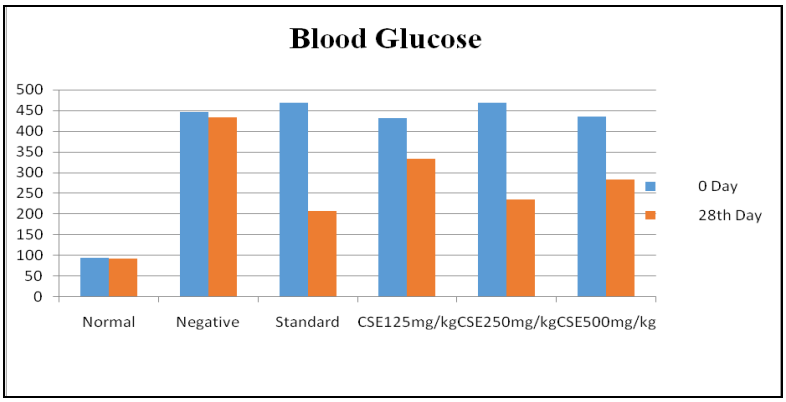


Figure 5: Effect of methanolic bark extract of *Chloroxylon swietenia* DCon blood Glucose

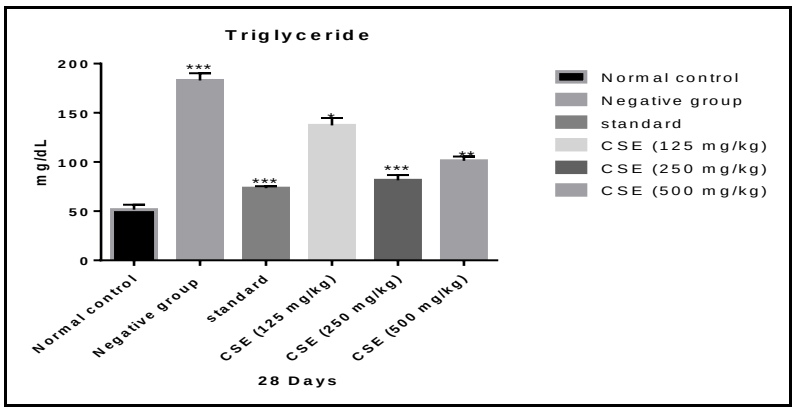


Figure 6: Effect of methanolic bark extract of *Chloroxylon swietenia* DCon triglycerides

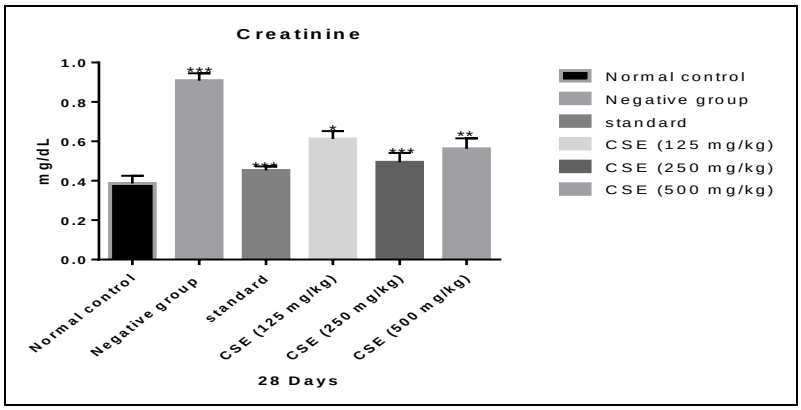


Figure 7: Effect of methanolic bark extract of *Chloroxylon swietenia* DCon creatinine

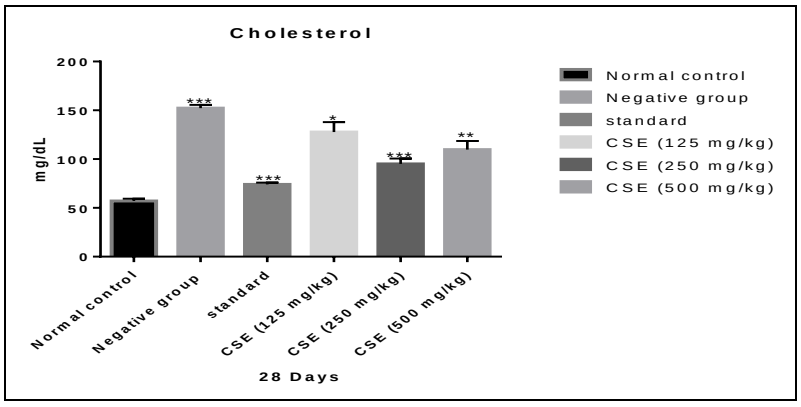


Figure 8: Effect of Methanolic bark extract of *Chloroxylon swietenia* DCon cholesterol

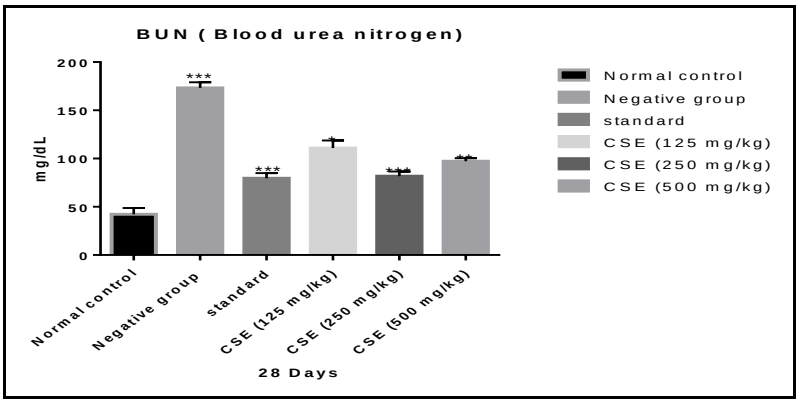


Figure 9: Effect of Methanolic bark extract of *Chloroxylon swietenia* DCon BUN (Blood urea nitrogen)

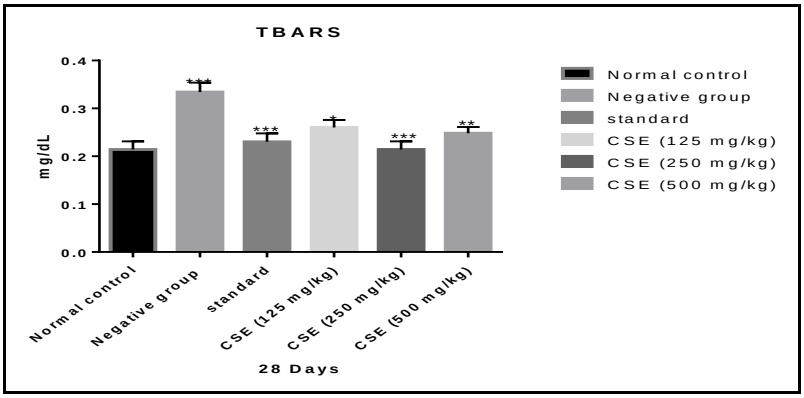


Figure 10: Effect of Methanolic bark extract of *Chloroxylon swietenia* DCon serum TBARS

Alloxan monohydrate at a dose of 120 mg/kg dissolved in 0.9 %w/v saline was induced intraperitoneally toovernight fasted rats. The dose of alloxan was standardized by previous pilot studies conducted in the same laboratory which produce fatal hyperglycaemia in rats at this dose. Seventy-two hours after injection, fasting blood glucose levels were measured from the tail vein using blood glucose monitoring system. Animals having blood glucose levels greater than 250 mg/dl were selected for further studies. Since alloxan produces fatal hypoglycaemia 6 h after the injection, the rats were treated with 20 % glucose solution in small water feeding bottles of 50 ml for the next 24h. Body weight and blood glucose level was measured on 0 day, 3rd day, 7th day, 14th day, 21st day and 28th day of the experiment. Biochemical parameters such as creatinine, triglycerides, cholesterol, Blood urea nitrogen (BUN) were observed on the 28th days of the study.

Physiological Information:

- Body weight

Biochemical description:

- Blood glucose level
- serum creatinine
- Blood urea nitrogen urea
- Triglycerides level
- Total Cholesterol

HISTOLOGY OF KIDNEYS

Immediately after the withdrawal of blood, rats treated with normal saline, Et TT, one rat from each group were sacrificed by cervical dislocation at the end of

experiment. Kidneys were removed, washed with cold saline and preserved in 10% formalin in buffered form and subsequently dehydrated in graded alcohol. After embedding in paraffin 4-6 µM sections were cut and mounted on aminosilane coated glass slides. After drying overnight, paraffin sections were warmed for 1 h at 15ºC. Sections were then deparaffinized in four changes xylene, 5 minute each, followed by one change on 100% ethanol for five minute. The slides were rehydrated by one min changes each of 100%, 95%, 75%, and 50% ethanol then held in distilled water. After staining with hematoxylene for 1-2 minute, slides were rinsed in distilled water for 20-30 minute. Following a one min wash in 70% ethanol, slides were stained with eosin for one min and then dehydrated and put in Xylene for 3 or 4 times. Coverslips are mounted with DPX. The tissues were examined by light microscopy.

Statistical analysis:

The data were expressed as mean ± SEM (standard error of mean). Statistical differences between groups were analysed using two-way Analysis of Variance (ANOVA) followed by Bonferroni post hoc test using GraphPad Prism 5.0 software.

Discussion:

Diabetic nephropathy is one of the major micro-vascular complication of diabetes, leading to renal failure.

Diabetic nephropathy is characterized by glomerular hypertrophy, reduction in glomerular space, tubular dilation, leukocyte infiltration, necrosis of proximal.In the given study the methanolic extract of *Chloroxylon swietenia* DC was used to study and evaluate the protective effect on alloxan induced diabetic nephropathy in rats. Alloxan is widely used in experimental lab due to its ability to cause cytotoxic effects. Treatment with methanolic extract of *Chloroxylon swietenia* DC at doses 125mg/kg, 250mg/kg, 500mg/kg tends to lower the increase in blood glucose. Glucose level showing its antihyperglycemic activity which was well sported with previous findings. The selective dose at 250mg/kg showed highly effective When compared with negative control.Alloxan causes reduction in the body weight due to degeneration of adipocytes and protein muscles. All drug treated groups showed near equal effect on body weight when compare with control group. Standard group showed highly improvement in body weight when compared with control group. Insulin inhibits the hormone lipase, which causes the breakdown of fats from its deposit. Lack of insulin stimulates this hormone, resulting in high levels of serum lipid in diabetic nephropathy.Creatinine and blood urea nitrogen (BUN) level are generally taken as a marker for renal impairment. In the present study the oral treatment of methanolic extract of *Chloroxylon swietenia* DC at a doses 125mg/kg, 250mg/kg, 500mg/kg showed significantly decrease altered levels of blood urea nitrogen (BUN) and creatinine, when compared with control group. The control group showed significantly increased the level of creatinine and BUN on 28th day of the study when compared with normal group.

CONCLUSION

The given study explain nature of methanolic extract of *Chloroxylon swietenia* DC on alloxan induced experimental of diabetes by restoring the normal architecture of the kidney tissue. It also improves the physiological and biochemical description. The treatment with methanolic extract for a period of 4 weeks halts the hyperglycemia mediated oxidative stress and declines in pro-inflammatory cytokines. Among all the doses 250mg/kg proved fruitful.The methanolic extract of *Chloroxylon swietenia* DC may be used to treat the renal dysfunction in diabetic nephropathy for future aspects.

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