

## **ATTENUATION OF BIOCHEMICAL PARAMETERS OF SHOREA ROBUSTA LEAVES IN STREPTOZOTOCIN-INDUCED DIABETIC RATS**

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Article Received on  
22 May 2015,

Revised on 15 June 2015,  
Accepted on 08 July 2015

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### **ABSTRACT**

*Shorea robusta* belongs to family Dipterocarpaceae, traditionally is used to treat wounds, ulcers, leprosy, cough, gonorrhea, earache and headache and many more. Diabetes is associated with profound alterations in plasma lipid and lipoprotein profile and with an increased risk of coronary heart disease. The presence of phytochemicals such as tannins, alkaloids, proteins, flavonoids, triterpenes and saponins in the ethanolic extract/fractions was revealed by the preliminary phytochemical screening, keeping in view of medicinal importance the present study is an effort to investigate the biochemical parameters of *m shorea robusta* leaves in streptozotocin induced diabetic rats for 21

days.<sup>[1]</sup> The acute toxicity studies of oral doses of ethanolic leaves extract/fractions in rats revealed that it has a high safety profile, as the extract/fractions was well tolerated by the animals. The test drug was administered for 21 days at a four different dose level 100, 200 mg/kg for ethanolic extract and 100, 100mg/kg each of two successive fractions (chloroform and n-butanol) made in aqueous and given by orally. Body weight, urine sugar was analyzed before and after treatment of extract/fractions while serum glucose was analyzed every week and lipid and lipoprotein profile from serum was analyzed after 21 days. The ethanolic extract/fractions of *Shorea robusta* leaves significantly prevented loss of body weight and reduce urine sugar. The results indicated that the ethanolic extract/fractions produced significant ( $p < 0.001$ ) in biochemical parameter.

**KEY WORDS:** *Shorea robusta*, streptozotocin (STZ), total cholesterol, total glycerides.

## 1. INTRODUCTION

Type II diabetes is the commonest form of diabetes constituting 90% of the diabetic population. The countries with the largest number of diabetic patients in the year 2025 will be India, China and United States.<sup>[1]</sup> Therefore, it has become necessary to look for novel oral therapeutically effective treatment especially for usage in the developing as well as under-developed countries. India is a country with a vast reserve of natural resources and a rich history of traditional medicine. Ethno pharmacological surveys indicate that more than 1200 plants are used in traditional medicine for their alleged hypoglycemic activity.<sup>[2]</sup>

*Shorea robusta* belongs to Dipterocarpaceae family is ,traditionally is used for disorders of gastrointestinal, cardiovascular, biliary, diabetes, hepatic, urinary and respiratory origin and many more.<sup>[2]</sup> *Shorea robusta* species are used in folk medicine such as disorders of gastrointestinal, cardiovascular, biliary, diabetes, hepatic, urinary and respiratory origin.<sup>[2,3]</sup> However, to the best of our knowledge and based on the citations of the use, the purpose of present study was to evaluate biochemical parameter of the ethanolic leaves extract/fractions of *shorea robusta* in streptozotocin induced diabetic rats.

## 2. MATERIALS AND METHODS

### 2.1 Plant material

The fresh leaves of *shorea robusta* were collected during Nov 2014, from the villages of Madhya pradesh. The plant species was identified and authenticated by taxonomist.

### 2.2 Laboratory Animals

Healthy, adult Wistar rats of both sexes (180-220g) were selected for present study. Animals were obtained from school of pharmaceutical sciences, Animal House, chennai, India, maintained under standard laboratory condition and the experimental protocol was approved from Institutional Animal Ethical Committee (IAEC).

### 2.3 Preparation of extract

The collected fresh plant materials were dried in shade for two days and then dried in a hot air oven at 25<sup>0</sup>C for three days and they were made in to coarse powder with the use of mixer grinder. The power of leaves of *shorea robusta* obtained were weighed separately and transferred to a round bottomed flask and then went with soxhlet extraction using 95% ethanol for 24 hour. Then the extract of ethanol was concentrated and then the marc was stored for fractionation.<sup>[4]</sup>

## 2.4 Preliminary Phytochemical studies

The preliminary phytochemical screening of extract/fractions was performed to identify the presence of alkaloids, proteins, triterpenoids, saponins, flavonoids and tannins.

## 2.5 Acute toxicity study

The study was carried out according to the OECD guidelines 423.

Female Wistar rats of weight (180-220g) were taken for the study and kept for overnight fasting. Next day, body weight was taken and standardized shorea robusta leaf extract and fractions were administered orally at a dose of 2000mg/kg in distill water. Then the animals were observed for mortality and morbidity at 0, ½, 1, 2, 4, 6, 8, 12 and 24 hr. Feed was given to the animals after 4 hr of the dosing and the body weight was checked at 6 hr after dosing. Morbidity like convulsions, tremors, grip strength and pupil dilatation were observed. The animals were observed twice daily for 14 days and body weight was taken. The same experiment will be repeated once again on 3 rats (preferably female) if there is no observable clinical toxicity for the animals on the acute toxicity study.<sup>[6,7]</sup>

## 2.6 Preparation and induction of type 2 diabetes in rats by Streptozotocin

The Streptozotocin was made at a final concentration of 50mg/kg body weight by dissolving in citrate buffer (pH 4.5) the solution was then kept refrigerated overnight to facilitate its dissolution. Non-Insulin dependent diabetes mellitus (NIDDM) was induced in overnight fasted rats by a single intraperitoneal injection (i.p.) of 50mg/kg streptozotocin. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72hr. The rats with permanent Non insulin dependent diabetes mellitus (NIDDM) (250-350 mg/dL) were used for the study.<sup>[8,12,13]</sup>

## 2.7 Experimental design

The Wistar rats weighing 180-220 gm of either sex were used for the experimental study. The animals were divided into seven groups of six animals each.

### GROUPING OF THE ANIMALS

GROUP I – Untreated Control

GROUP II- Diabetic control

GROUP III- Positive control (glibenclamide 10mg/kg b.w i.p)

GROUP IV- EtOH extract of shorea robusta (100mg/kg, orally)

GROUP V- EtOH extract of shorea robusta (200mg/kg, orally)

GROUP VI- ChF of shorea robusta (100mg/kg, orally)

GROUP VII- BtF of shorea robusta (100mg/kg, orally)

The extract/fractions was administered for 21 days at a four different dose level 100, 200 mg/kg for ethanolic extract and 100, 100mg/kg each of two successive fractions (chloroform and n-butanol) made in aqueous and given by orally. The blood was collected by sinous orbital under light diethyl ether anesthesia. The blood was centrifuged at 3000 rpm for 10 minutes. Body weight, urine sugar was analyzed before and after treatment of extract/fractions while serum glucose was analyzed every week and lipid and lipoprotein profile from serum was analyzed after 21 days.

### 3. ANALYTICAL METHODS

#### 3.1 Determination of body weight and urine analysis

Body weight, urine sugar was analyzed before and after treatment of extract/fractions. Urine sugar was estimated by using Reagent strips from Diastix (2802B).

#### 3.2 Estimation of serum glucose

Glucose estimation in serum was assayed by using Ecoline diagnostic kit.

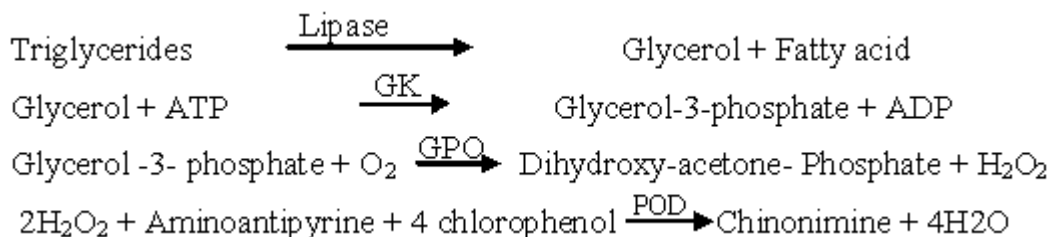
Glucose content is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator. Measured the absorbance of the sample and standard against the reagent blank value at 500nm. Glucose level in serum was expressed as mg/dL.

#### 3.3 Estimation of Lipids and lipoprotein profile

**Total Cholesterol (TC):** Cholesterol in serum was estimated by using an Ecoline Diagnostic Kit. Cholesterol and its esters were released from lipoproteins by detergents. Cholesterol esterase hydrolysis the esters. In the subsequent enzymatic oxidation by cholesterol oxidase, H<sub>2</sub>O<sub>2</sub> was formed. This was converted into a colored quinoneimine in a reaction with 4-aminoantipyrine and phenol catalyzed by peroxidase. The absorbance of the sample and of the standard was measured against the reagent blank value at 546nm. Cholesterol level in serum was expressed as mg/dL.

**Triglycerides (TG)**

Triglyceride level in serum was estimated using an Ecoline Diagnostic Kit.



The absorbance of the sample and of the standard was measured against the reagent blank value at 546 nm. Triglyceride level in serum was expressed as mg/dL.

**HDL (High density lipoprotein) Cholesterol**

The HDL cholesterol was separated from serum after precipitation of LDL and VLDL cholesterol by phosphotungstic acid precipitating reagent. The supernatant after centrifugation was estimated using Ecoline diagnostic kit. The absorbance of the sample and of the standard was measured against the reagent blank value at 546nm. HDL Cholesterol level in serum was expressed as mg/dL.

**LDL (Low density lipoprotein) Cholesterol**

LDL Cholesterol was calculated by using the formula

$$\text{LDL Cholesterol} = \text{Total Cholesterol} - \left[ \text{HDL Cholesterol} - \frac{\text{Triglycerides}}{5} \right]$$

LDL Cholesterol level in serum was expressed as mg/dL.

**VLDL (Very low density lipoprotein) Cholesterol**

VLDL Cholesterol was calculated by the formula

$$\text{VLDL Cholesterol} = (\text{Triglycerides})/5$$

VLDL Cholesterol level in serum was expressed as mg/dL.

**4. STATISTICAL ANALYSIS**

Evaluation of statistical significance results by computer aided program and systemic documentation. Value were presented as mean  $\pm$  SEM. Data were analyzed using analysis of variance (ANOVA) and group means were compared with Bonferroni Multiple Comparisons Test using Instat graph pad and prism software.

## 5. RESULTS

### 5.1 Effect on body weight and fluid intake

Gradual increase in body weight in untreated control while the diabetic control continue to loose the weight. However treated diabetic group gained 16%, 11%, 16%, 15% as compared to diabetic control and body weight of diabetic treated towards normal range ( $P < 0.001$ ). On the other hand the administration of ethanolic extract/fractions. Ethanol (EtOH) (100mg/kg and 200mg/kg b.w.), Chloroform (ChF) and n-butanol fraction (BtF) (100mg/kg b.w.) of *shorea robusta* decrease urine sugar level respectively. In diabetic control animal urine sugar remaining that +4 level but there was decrease of 2% to 3%, in urine sugar in case of treated diabetic rats. The change in body weight and urine sugar in all group of animals were given in table 5.1

### 5.2 Effect of *shorea robusta* on Serum glucose

The initial blood glucose levels of the diabetic rats selected for the study were in the range of 240-300mg/dL. In the untreated control (diabetic) rats the blood glucose level increase to 379 mg/dL on the 7<sup>th</sup> day the glucose levels on the 14<sup>th</sup> and 21<sup>th</sup> day of the animals which survived were 410 mg/dL respectively. In the *shorea robusta* treated rats the blood glucose level suddenly decreased ( $P < 0.001$ ,  $P < 0.01$ ) thus the *shorea robusta* treatment restore the serum glucose levels almost nearer to normal value and comparable to that of positive control ( $P < 0.001$ ). The changes in Serum glucose estimation in all groups of animal were given in table no.5.2

### 5.3 Serum lipid profile and lipoprotein profile

Effect of extract/fraction of *shorea robusta* on the control and experimental animals. STZ diabetic rats group were found to have significantly increased HDL, LDL, VLDL, TG, TC, levels as compared to control group ( $P < 0.001$ ,  $P < 0.01$ ) HDL cholesterol was also reduced significantly in diabetic rats after treatment of ethanolic extract/fraction of RS. Positive control was significantly preventing the increasing the serum TC, TG, HDL, LDL, VLDL, as compared to diabetic group. Diabetic treated group was significantly increased in HDL cholesterol level as compared to diabetic group. ( $P < 0.001$ ,  $P < 0.01$ ). Thus the *shorea robusta* treatment restores all these changes near to normal value. The change in serum lipid and lipoprotein profile were tabulated in table no 5.3.

### 5.1 Effect of administration of feeding the Ethanolic extract/fractions of *shorea robusta* leaves on body weight and urine sugar analysis in normal and diabetic rats for 21 days.

No	GROUP	Body Weight (g)		Urine sugar	
		Before treatment	After treatment	Before treatment	After treatment
1.	Untreated control	194±1.88	220.5±1.839	Nil	Nil
2.	Diabetic control	202.66±2.33	168.5±2.513 <sup>###</sup>	+4	+4
3.	Diabetic+Glibenclamide (10mg/kg)	206.66±1.745	222.33±1.96***	+4	+1
4.	Diabetic+EtOH(100mg/kg)	209±1.932	220.16±1.078***	+4	+2
5.	Diabetic+EtOH(200mg/kg)	197±2.176	213.6±1.476***	+4	+1
6.	Diabetic+ChF(100mg/kg)	205.83±2.182	220.83±2.182***	+4	+1
7.	Diabetic+BtF(100mg/kg)	202.16±2.056	217.16±2.056***	+4	+1

**Table5.2: Effect of administration of feeding the Ethanolic extract/fractions of shorea robusta leaves on serum glucose estimation in normal and diabetic rats**

S.No	GROUP	Serum glucose (mg/dL)			
		0 day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>th</sup> day
1.	Untreated control	84.83±5.41	85.33±5.87	84.66±5.77	84.83±5.09
2.	Diabetic control	298.16±17.20	367.33±4.7 <sup>##</sup>	413.83±16.61 <sup>###</sup>	410±2.045 <sup>###</sup>
3.	Diabetic+Glibenclamide (10mg/kg)	280.33±2.44	205.33±1.145**	165±1.29***	114.83±1.302***
4.	Diabetic+EtOH (100mg/kg)	277±7.69	223±4.195**	164.66±1.406***	116.5±1.232***
5.	Diabetic+EtOH (200mg/kg)	282.66±4.49	223.33±1.94**	164.16±1.406***	115±0.966***
6.	Diabetic+ChF (100mg/kg)	290.83±5.46	221.5±3.51**	167±1.065***	115.83±0.945***
7.	Diabetic+BtF (100mg/kg)	285±533	219±2.63**	166.83±1.77***	115.33±0.881***

All value are expressed as mean ± SEM (n=6). \*\*\*P<0.001, \*\*P<0.01 as compared to diabetic control. ##P<0.01, ###P<0.001 as compared to untreated control. One-way ANOVA followed by Bonferroni multiple comparison tests.

**Table 5.3: Effect of ethanolic extract/fractions on serum lipid and lipoprotein profile in streptozotocin induced rats**

No	GROUP	TC(mg/dL)	TG(mg/dL)	HDL(mg/dL)	LDL(mg/dL)	VLDL(mg/dL)
1.	Untreated control	86.5±0.76	54.83±1.138	54.80±0.693	18.38±0.40	8.793±0.40
2.	Diabetic control	156.68±0.72 <sup>###</sup>	183.5±11.59 <sup>###</sup>	21.33±0.44 <sup>###</sup>	37.73±6.75 <sup>###</sup>	37.10±2.85 <sup>##</sup>
3.	Diabetic+Glibenclamide (10mg/kg)	69.33±2.81***	116.5±5.21***	32.63±2.30***	16.27±1.39***	23.96±0.19**
4.	Diabetic + EtOH(100mg/kg)	126.88±0.25***	64.16±1.53***	35.45±3.66***	52.38±1.51***	13.82±0.22**
5.	Diabetic + EtOH (200mg/kg)	126.95±0.22***	69.66±4.63***	38.4±2.725***	53.07±0.79***	13.98±0.78**
6.	Diabetic + ChF(100mg/kg)	124.45±1.07***	68.33±1.12***	39.48±1.94***	59.51±3.05***	16.33±0.91**
7.	Diabetic + BtF(100mg/kg)	124.58±1.12***	64.33±0.80***	47.06±3.44***	53.06±4.03***	14.49±0.32**

All value are expressed as mean ± SEM (n=6). \*\*\*P<0.001, \*\*P<0.01, as compared to diabetic control. ###P<0.001, ##P<0.01, as compared to untreated control. One-way ANOVA followed by Bonferroni multiple comparison tests.



## 6. DISCUSSION

Traditional Chinese medicines have been used by over one-fifth of the world population since ancient times and is a potential source of pharmaceutical remedies. Now a major challenge to curing liver and kidney injuries are to find novel chemical entities with less toxicity and greater effectiveness than those used in current chemotherapy. Diabetes is associated with profound alterations in plasma lipid and lipoprotein profile and with an increased risk of coronary heart disease.

Insulin and sulphonyl urea drugs (glibenclamide) causes hypoglycemia when taken in excessive doses and overt hypoglycemia is the most worrisome effect of these drug .However Biguanides donot cause hypoglycemia even when taken in excessive doses.<sup>[1,15]</sup> *Shorea robusta* also did not cause hypoglycemic effect. One of the Biguanide, Metformin does not stimulate insulin secretion and act by reducing hepatic glucose production through inhibition of gluconeogenesis and to a lesser extent by enhancing insulin sensitivity in the tissues.<sup>[16]</sup> In the present study *Shorea robusta* extract/fractions did not influence serum levels of insulin in diabetic as well as normo-glycemic rats. Further the extract/fractions reduced serum lipid.<sup>[16]</sup>

Other parameters of diabetes such as body weight and urine sugar were also affected on treatment with *Shorea robusta*. Treatment with *Shorea robusta* inhibits the reduction in body weight and urine sugar by diabetes as the treatment altered the body weight and urine sugar of diabetic animals towards normal.<sup>[17]</sup>

Hyperlipidemia is a recognized complication of DM characterized by elevated levels of cholesterol, triglycerides and changes in lipoprotein composition.<sup>[18]</sup> The results of our present study clearly show that *Shorea robusta* leaves has a lipid lowering effect on serum TG, TC, VLDL, LDL, There is a substantial evidence that lowering the total cholesterol (TC), particularly LDL level will lead to a reduction in the incidence of coronary heart disease (CHD), which is still a leading cause of death in diabetic patients.<sup>[18,19]</sup>

Since lipid abnormalities accompanying with premature atherosclerosis is the major cause of cardiovascular disease (CVS) in diabetic patient therefore ideal treatment for diabetes in addition to glycemic control, should have a favorable effect on lipid profile.

The dose of ethanolic extract EtOH (100,200mg/kg), Chf, BtF (100mg/kg) not only lowered the TC, TG, VLDL and LDL levels but also enhanced the cardio protective lipid HDL in



normal and diabetic rats after 21 days of treatment. In present study the extract/fractions not only decrease the TC level but also enhance the HDL cholesterol significantly.<sup>[19]</sup> High level of triglycerides and more importantly LDL cholesterol is major cause of coronary risk factors. Administration of leaves of extract/fractions to diabetic rats for 21 days lowered TC and LDL cholesterol level respectively,<sup>[19,20]</sup> This is the important finding of this study as diabetes is associated with coronary complications.

Conclusion drawn from the present study on *Shorea robusta* leaves demonstrated that the ethanolic extract/fractions of *Shorea robusta* the decreased blood glucose, improve lipid metabolism, maintain bodyweight and urine sugar in STZ induced diabetic rats.

### ACKNOWLEDGEMENT

The authors are grateful to the management, School of pharmaceutical sciences, chennai, India for providing the necessary infrastructure to carry out this research work in successful manner.

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