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Preparation of oral Solid Dosage Form of TectonaGrandis(Sawdust) for Therapeutic Application As An Antidiabetic Agent

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Abstract: *Tectonagrandis(TG) Teak wood is one of the most highly valued timbers in the world and is famous for its dimensional stability, natural durability and which resists decay even when unprotected by paints and preservatives. TG is reported to have naphthoquinone derivatives and are known to possess antidiabetic activity. The sawdust which is by product of cutting, drilling or sanding of heartwood of Teak havebeenyet to be explored for antidiabetic activity. So, in current research is carried out on the alcoholic extract of sawdust of TG. The extract wassubjected to preliminary qualitative and quantitative phytochemical analysis. The HPTLC fingerprinting carried out on alcoholic extract to identify the component present in it. The preliminary OGTT study was carried out at different dose levels followed by Antidiabetic activity testing was done on STZ induced diabetic rat model. The blood glucose level, body weight, food and water intake were monitored on daily basis and other blood parameters such as Serum glucose, Serum insulin by Radio immuno assay(RIA), serum cholesterol, total protein, glycosylated haemoglobin, TBARS, liver glycogen etc., were evaluated after 7thand 14th day of treatment and scarification of animals. The phytochemical result showsthatalcoholic extract has presence of Steroids, Tannins, Phenolics, naphthoquinone and anthraquinone derivatives. The HPTLC fingerprinting able to be found lapachol, lupeol, beta-sitosterol present in it. TG extract (200mg/kg) possess good antidiabetic activity. All the blood parameters were also significantly ($p<0.05$) controlled by TG at dose of 200mg/kg. The animal dose was extrapolated to human dose and the semisolid extract filled in hard gelatinwas prepared and evaluated for stability for the period of three month. The result indicated that the waste product of TG wood (sawdust) possess good antidiabetic potential and oral solid dosage form can be made available for management of diabetes.*

Keywords: *Antidiabetic activity, HPTLC analysis, Liquid filled in hard gelatin capsule,Streptozotocin,Tectonagrandis (Sawdust),*

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I. Introduction

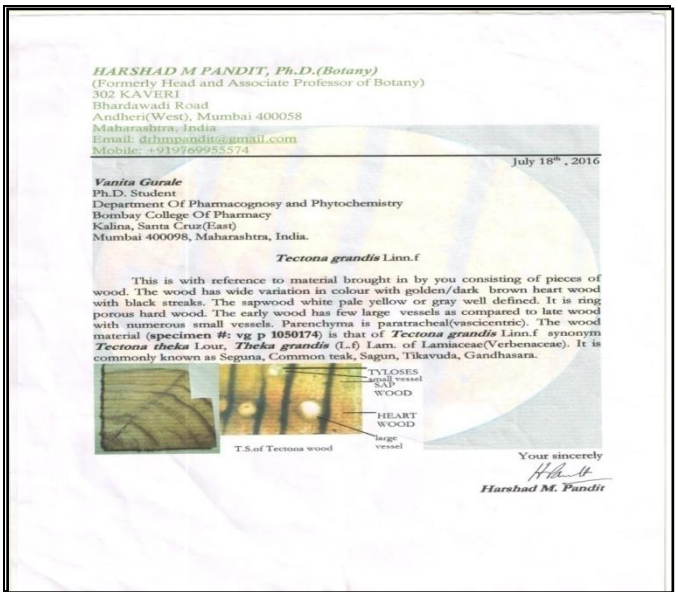
The teak plant i.e. *Tectona grandis* Linn. (Family - Verbenaceae) is locally known as sagon, sagwan[1]. It is one of the most important well known heart woods widely used all over the world. Timber value of teak has been very well recognized from decades[2, 3]. Traditionally, *Tectona grandis* (TG) is used as cooling agent, astringent, Laxative, sedative, bronchitis, diuretics and in the treatment of urinal discharge. Its also used in common cold, headache, hair problem and scabies, inflammation, ulcer, and bronchitis[3-5]. The other reported pharmacological activities of TG wood are antiinflammatory[6] Heapatoprotective[7], anticancer[8], Antipyretic[9] wound healing[10]etc.

The phytoconstituents like carbohydrate, tannins, alkaloids, saponins, sterols, quinones, phenolics and flavonoids are reported to be present in TG[11].Secondary metabolites such as tectoquinone, lapachol, 5-hydroxylapachol, tectol, betulinic acid,betulinic aldehyde, squalene are also reported to be present in the plant[12].All the diverse phytoconstituents may be responsible for its potent biological activity. The naphthoquinone derivatives such as lapachol, deoxylapachol, tectoquinone, saponins, tannins are reported to have good antidiabetic activities. The antidiabetic potential of sawdust has not been evaluated so far. Thecurrent research has been carried out on the alcoholic extract of sawdust of TG. The antidiabetic potential of TG has been evaluated using STZ induced diabetic rat model. The extract at dose of 200mg/kg showing promising antidiabetic potential,it has been formulated into capsule dosage form and capsules were evaluated for its stability for the period of three months.

II. Materials And Methods

2.1 Plant Material and Preparation of Extract

The wood powder (sawdust) of TGwas collected from the timber mill from Bhiwandi. The sample specimen of heart wood for authentication was obtained from same Timber mill and was authenticated by Dr. Harshad M. Pandit, Department of Botany, Guru Nanak Khalsa College, Matunga, Mumbai-400019 with the specimen number (Sp.#: vg p 1050174).



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Tectona grandis Linn.f

This is with reference to material brought in by you consisting of pieces of wood. The wood has wide variation in colour with golden/dark brown heart wood with black streaks. The sapwood white pale yellow or gray well defined. It is ring porous hard wood. The early wood has few large vessels as compared to late wood with numerous small vessels. Parenchyma is paratracheal(vascicentric). The wood material (**specimen #: vg p 1050174**) is that of *Tectona grandis* Linn.f synonym *Tectona theka* Lour, *Theka grandis* (L.f) Lam. of Lamiaceae(Verbenaceae). It is commonly known as Seguna, Common teak, Sagun, Tikavuda, Gandhasara.

TYLOSES
small vessel
SAP WOOD
HEART WOOD
large vessel
T.S.of Tectona wood

Your sincerely
Harshad M. Pandit

Fig. 1. Authentication certificate of wood of *Tectonagrandis*

Preparation of the extract: The extraction of Sawdust of TG was carried out by hot extraction method using soxhlet apparatus with ethanolas extracting solvent. The extraction conditions were optimized with respect to herb:menstrumratio, temperature, time, number of cycle of siphoning etc. The extraction carried out at 65°C using herb: menstruum ratio as 1:10 (marc: Solvent), siphoning was continued for 6-8 cycles and time required for complete extraction was 24hr. The extract was concentrated on water bath and the yield of brown colour solid mass was noted. The dried extract was stored in an airtight container until use in refrigerator.

The preliminary qualitative phytochemical analysis of extract was carried out which revealed the presence of different kind of phytoconstituent like carbohydrates, naphthoquinone, anthraquinones, saponins, alkaloids, steroids and phenolic compounds in it. The quantitative estimation of total phenol content equivalent to tannic acid, flavonoid content equivalent to standard rutin and total tannins content by titrimetric method has been carried out and reported.

The preliminary HPTLC fingerprinting of alcoholic extract was carried out to study the phytochemistry of the extract.

2.2 Antidiabetic activity:

2.2.**1Chemicals**

Streptozotocin, Glibenclamide, Dextrose, Tween-80, Auto analyzer (Analytical technological limited). Methyl paraben, Propyl paraben, All the other chemicals and reagents used were of analytical grade.

2.2.2Animals

Female Sprague–Dawley rats (200 ± 15 g) used in this study was obtained from Glenmark Pharmaceuticals, Mumbai. All animals were housed in standard polypropylene cages with wire mesh top and husk bedding and maintained under standard conditions of temperature 22 ± 3C and relative humidity 55 ± 10% with 12:12 hrs light: dark cycle. Animals were fed with commercially available standard rodent pellet diet (Amrut rat and mice feed manufactured by Nav Maharashtra Chakan Oil Mill Ltd). Water was provided to the animals (supplied by Municipal Corporation of Greater Mumbai) *ad libitum.*

The animal experimentation protocol was approved by the Institutional Animal Ethics Committee with registration number CPCSEA-BCP/2013-02/16; studies were performed in accordance to guideline of CPCSEA.

2.2.3Preliminary OGTT study

The oral glucose tolerance test was performed in overnight fasted normal rats[13]. Rats divided into control group, standard drug treated group and extract treated groups (n=6), were administered drinking water, glibenclamide (5 mg/ kg body weight) and the crude extract respectively. Glucose was fed after the administration of extract. Blood was withdrawn from the retro orbital plexus, collected just prior to glucose administration (0 minute) and 30, 60, 90, 120 and 180 minute after glucose loading and the blood glucose levels were measured by glucose oxidase based assay kit. The results were expressed as mean ± S.E.M., the significant of various treatments were calculated using students t-test and were considered statistically significant for $p< 0.05$. Various dose levels of the extract were titrated to finalize the therapeutic dose for further studies. The alcoholic extract of TG was tested at 2 dose levels: 100 mg/kg body weight and 200 mg/kg body weight. After completion of OGTT study a 15days washing period was given to rats and then main study was started on same animals.

2.2.4STZ induced antidiabetic model

2.2.4.1 Diabetes induction in rats

The streptozotocin was used to induce diabetes in the animals. The rats were injected (intraperitonelly) with streptozotocin which freshly dissolved in 0.1 M citrate buffer (pH 4.5) at the dose of 50 mg/kg body weight within 2 minute of dissolution. Whereas normal control group was given citrate buffer only. The rats were observed for four days, after four days the rat blood glucose level was checked by glucose estimation kit. The rats were used for experiment when blood glucose level was found to be in the range of 250-350 mg/dl.

2.2.4.2 Experimental design:

The rats were divided into five groups (n=6) in each as follows:
Group I: Normal rats (Vehicle control);
Group II: Diabetic Control rats (Positive control);
Group III: Standard drug treated group (5mg/Kg);
Group IV: Low dose TG (100mg/Kg);
Group V: High dose TG (200mg/kg);
for the period of 21 days. The food and water intake of the animals was monitored for 21 days. The body weight, food and water consumption of the animals was measured daily for 21 days.

2.2.4.3 Estimation of biochemical parameter and histopathology:

On the 7^{th} and 14^{th} day of treatment, the animals were deprived of food overnight (12 h) and blood was collected from the retro orbital plexus. Serum glucose level was estimated on 7^{th} day and 14^{th} day of treatment by commercial glucose oxidase based assay[5]. Serum insulin levels were measured on 21^{st} day of treatment by Radioimmuno assay at BRIT, Vashi[14, 15].At the end of the experimental period all rats were sacrificed by cervical decapitation and blood was collected in 2 tubes, one with EDTA as the anticoagulant for evaluation of glycosylated haemoglobin[16, 17] and one without anticoagulant for serum separation. The blood without anticoagulant was centrifuged at 4000 rpm for 15 min to separate serum. The collected serum was subjected for various serum parameters like of Serum glucose, Serum insulin by RIA, Total protein, Cholesterol[18], HDL, Triglyceride[19], were performed. All serum biochemical parameters were measured using the Erba Diagnostics kits (Erba Diagnostics Mannheim GmbH.). Liver and Pancreas was immediately dissected, washed in ice cold saline, patted dry and weighed. The liver was used for analysing glycogen level[20, 21]. The pancreas were cut and divided in to two sections, one of the sections was stored at -70 C for SOD[22], Catalase[23] Reduced GSH [24]and TBARS [25]assay and the other section was fixed in 10 % formalin for histopathological observation. The samples were given for histopathological studies at Unique Biodiagnostics, Parel, Mumbai and studies done by veterinary pathologist.

2.2.4.4Statistical Analysis

All values are given as means±standard error of mean (S.E.M.) for each group. Data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni Multiple Comparisons Test using Graph pad prism software version 6. P values of less than 0.05 were considered to indicate statistically significant differences.

2.2.5Preparation of formulation:

The alcoholic extract of the *Tectonagrandis* was found to show good antidiabetic activity in STZ induced diabetic rats at the dose of 200mg/kg. The animal dose was extrapolated to get human dose for treatment of diabetes. The human dose calculation was done using formula given below[26].

$$\text{Human Equivalent Dose (mg/kg)} = \text{Animal Dose (mg/kg)} \times \frac{\text{Animal Km}}{\text{Human Km}}$$

The attempt was made to formulate oral solid unit dosage form for easy consumption. So, the extract was formulated into capsule dosage form. The extract was sticky reddish brown colour solid Hence,it was decided to incorporate the semisolid extract in special type of hard gelatin capsule. The formulation of choice was 'Liquid filled in hard gelatin capsule'[27][28].

2.2.5.1Procedure for Preparation of the capsule formulation

The required dose of the extract was triturated with castor oil till it was pourable by extrusion from syringe. The proportion of castor oil was adjusted to make it syringable into capsule. The capsules used to fill final preparation was special type of hard gelatin capsules of 00el size. The blend was filled into hard gelatin capsules and the capsules were sealed using sealing solution (15% gelatin solution). The capsule size was 00el having capacity of 1.02ml liquid to be filled.

Table 1: Optimized formula for TG capsules:

Sr. No.	Name	Qty for 10 capsules
1	TG Extract	5 gm
2	Castor oil	5 gm
3	Methyl paraben	0.1 %
4	Propyl paraben	0.05%

Fig. 2. Photo of filled TG capsules

2.2.6Evaluation of capsule formulation:

The capsules were evaluated for its physicochemical parameter like change in size, shape, appearance, leakage etc. The uniformity of weight, disintegration, content analysis and stability is done by HPTLC method of analysis. The HPTLC method was developed for analysis of capsule formulation and stability study for the period of 3 month.

2.2.6.1 The evaluation parameters of capsule: [29]

Uniformity of weight:

10 Capsules were weighed and opened. The content was carefully removed. The difference in weight represented the weight of the content. The average weight was determined.

Disintegration Test:

Disintegration test was performed using the IP disintegration test apparatus. Six capsules were selected randomly for the disintegration test. Capsules were placed into each tube inside the basket, disc was added into each tube and the assembly was suspended in the beaker containing distilled water. The temperature was maintained at 37°C. Time required to complete disintegration of capsules was noted.

Dissolution test:

The dissolution test was carried out on randomly selected capsules. The capsules were place in beaker containing 50ml distilled water and stirred frequently. The TG extract was poorly soluble in water. The 4% SLS added to water to improve solubility of capsule content in water. Time required for complete dissolution of

capsule was noted. The 1 ml aliquot was removed and diluted with methanol in 10ml volumetric flask. The diluted content of flask was applied on precoated TLC plate. The drug release in medium was analyzed by HPTLC method of analysis.

2.2.7Stability study of Capsules by HPTLC

The stability study of TG capsules was assayed by HPTLC method of analysis where all the spots were applied in triplicate to the plate as bands 6.00 mm wide and 15 mm from the bottom edge of the HPTLC plate (Silica gel $60F_{254}$) by using CAMAG LINOMAT V sample applicator equipped with 100 µl syringe. Ascending development to distance of 85 mm was performed at room temperature (25±2˚C) in the CAMAG twin trough chamber previously saturated for 15 minutes with mobile phase Toluene**:** Ethyl acetate: Glacial acetic acid (6:4:0.1 v/v/v). After development plates were scanned at 254 and 366nm in Scanner 3.

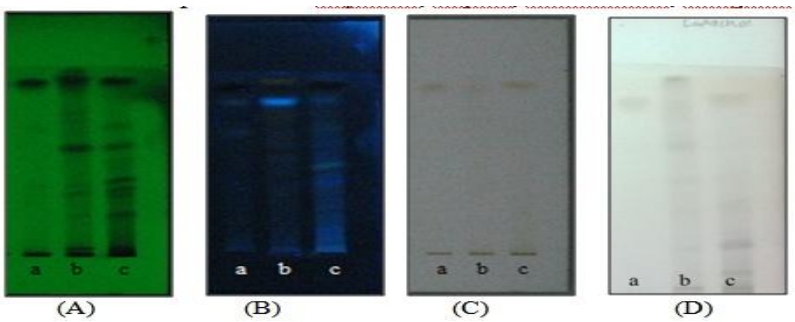
Preparation of stock solution of formulations:

The capsules were selected at random and emptied into small flask. Methanol was added and sonicated for 10 minutes. The contents of flask were filtered through whatman filter paper. The filtered solution was evaporated and reconstituted to 1ml with methanol. This solution was used for the HPTLC analysis. The same procedure was followed for preparation of stock solution of extract, standard Lapachol and excipient used in formulation.

II. Results And Discussion

The alcoholic extraction of Sawdust of TG was carried out by soxhlet apparatus and extraction conditions were optimized as herb to solvent ratio was taken 1:10, number of cycle of siphoning was 6-8 cycles and time required for complete extraction was 24hr. The yield of extract was found to be 7.23±0.39 % w/w and the extract was brown colour solid sticky mass. The preliminary phytochemical analysis showed presence of carbohydrates, naphthoquinone, anthraquinones, saponins, alkaloids, steroids and phenolic compounds in it. The literature reported that alcoholic extract of TG contains Tannins, Terpenoids, Saponins, Naphthoquinone derivatives [30].

The quantitative estimation of total phenol content equivalent to tannic acid was found to be 25.86±1.38 %w/w. The total tannin content by titrimetric method was found to be 19.16±2.98 %w/w and the flavonoid content equivalent to standard rutin and quercetin was found to be 11.86±4.38 and11.33±1.08 % w/w respectively.The preliminary HPTLC fingerprinting was carried out on alcoholic extract of TG where, the extract showed presence of Lapachol, Lupeol, bitasitosterol, diosgenin etc. (Fig. 3 and 4).



Track details: a) Standard lapachol, b) TG extract, c) TG fraction

Fig. 3: Photo of HPTLC plate containing standarddlapachol, TG extract and TG fraction (A=at 254nm, B=366nm, C=at visible light, D=after derivatization with anisaldhyde H_2SO_4 reagent)

Track details: a) standard β-sitosterol, b) standard lupeol, c) TG extract and d)TG fraction

Fig. 4: Plate photo showing band of β-sitosterol and lupeol in TG ext and fraction (After derivatization withAnisaldehyde reagent)

The preliminary OGTT study shows that in control group percentage increase in serum glucose level was found to be about 100% at 60 minute after glucose loading (Table no.2). while as the glibenclamide treated, group did not show a significant increase in serum glucose level as compared to that of the control group. The mean percent increase in serum glucose level was found to be around 4% at 60 min in glibenclamide treated group (Table no.2). The alcoholic extract of TG at the dose level of 100 and 200 mg/kg body weight showed 54 and 44% increase in serum glucose level at the time point of 60 min (Table no.3). There was a significant difference in serum glucose level when dose increased from 100 to 200 mg/kg/b.w.

So, the preliminary OGTT study revealed that alcoholic extract of TG at dose level of 100 and 200 mg/kg body weight was showing promising results when compare with control group. Hence, the alcoholic extract of TG at dose level of 100 and 200 mg/kg body weight was selected for Streptozotocin induce diabetic animal model study.

Table 2: Serum Glucose Levels and Percentage Increase in Serum Glucose Level for the Control Group and Glibenclamide Treated Group.

Time (minute)	Control Group Mean Serum Glucose Level		Glibenclamide Treated Group Mean Serum Glucose Level	
	(mg/dl) ± SEM	% Increase ± SEM	(mg/dl) ± SEM	% Increase ± SEM
0	100.13 ± 1.09	-	95.53 ± 2.74	-
30	157.21 ± 1.87	57.03 ± 3.17	99.83 ± 3.69	4.50 ± 0.20
60	213.65 ± 2.54	113.40 ± 2.06	100.02 ± 2.81	4.70 ± 0.13
90	183.29 ± 2.91	83.10 ± 2.69	102.84 ± 2.65	7.70 ± 1.17
120	161.72 ± 3.97	61.50 ± 2.48	101.21 ± 1.69	5.9 ± 1.19
180	135.06 ± 1.29	34.90 ± 2.27	98.35 ± 3.78	3.03 ± 1.20

Table 3: Mean Serum Glucose Levels and Mean percent increase in serum Glucose levels for Alcoholic Extract of TG Treated Groups

Time (minute)	Mean Serum Glucose Level (mg/dl) ± SEM		Mean % Increase in Serum Glucose Level ± SEM	
	Alc (100mg/kg b.w.)	Alc (200mg/kg b.w.)	Alc (100mg/kg b.w.)	Alc (200mg/kg b.w.)
0	99.46 ± 1.34	96.45 ± 1.32	-	-
30	131.54 ± 5.12	121.75 ± 3.54	32.38 ± 5.13	26.62 ± 1.57
60	153.56 ± 3.23	139.62 ± 2.50	54.46 ± 6.13	44.80 ± 1.32
90	144.16 ± 3.43	141.18 ± 2.27	44.91 ± 2.99	46.40 ± 2.08
120	132.32 ± 5.95	136.49 ± 4.79	33.04 ± 3.56	41.56 ± 1.36
180	126.42 ± 5.12	121.27 ± 2.91	27.14 ± 3.38	25.70 ± 2.49

The antidiabetic potential of the extracts at the optimized dose levels was evaluated using STZ induced diabetic rat model usingglibenclamide as a reference standard (5 mg/kg). The parameter like body weight, food and water intake was examined on daily basis. The results were found that the control group animals showed an increase in body weight during the study. The body weight of the rest all groups was reduced by about 22 - 25 % on the 7th day post STZ induction. The weight of the animals of the diabetic control group showed a gradual decline in the body weight till the end of the study. The diabetic animals treated with the standard drug, glibenclamide showed an increase in body weight maximum upto 22% on the 21st day of the treatment (Table no.4). Among both the both the dose level of TG, maximum increase in body weight after 21day treatment was shown at the dose of TG 200 mg/kg (about 22%).

Table 4: Effect of alcoholic extract of TG on time course of body weight in STZ induced rats

Group	Treatment	Average body weight (g) ± (S.E.M.)				
		0 Day	STZ 7	T-7	T-14	T-21
I	Vehicle control(NC)	231.4±7.5	243.1±9.4	269.1±15.3	290±13.2	309.5±5.4
II	Diabetic control(DC)	237.2±13.3	184.7±12.1	195.7±8.7	201.7±6.7	201.4±2.3
III	Glibenclamide (5mg/kg)	231.1±10.6	180.6±13.9	193.6±8.5	206.8±7.8	226.9±7.0
IV	TG(100mg/kg)	238.4±14.3	178.4±9.2$^{\#}$	189.7±7.2$^{\#}$	199.2±5.2	207.5±4.5
V	TG(200mg/kg)	229.7±9.5	194.4±6.3	206.8±6.3	222.4±3.83$^{\#}$	237.5±3.5*

All Values are means ± S.E.M. (n=6). Significance was determined by One-Way ANOVA followed by Bonferroni Multiple Comparisons Test *: $p < 0.05$ when compared with positive control group. #: $p < 0.05$ when compared with vehicle control group.

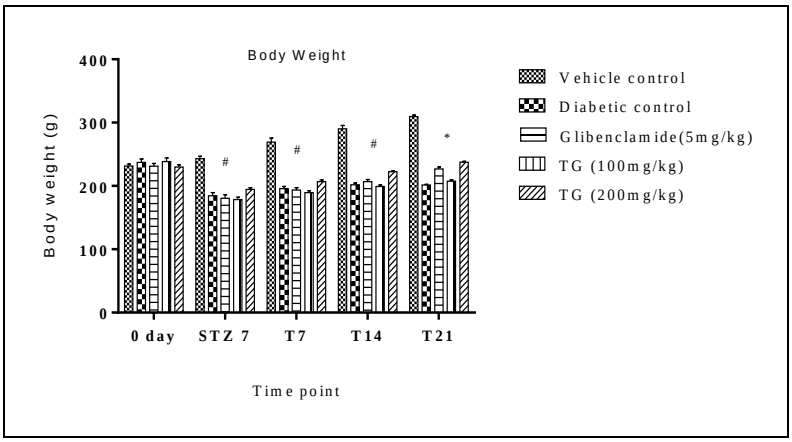


Fig 5: Effect of alcoholic extract of TG on time course of body weight in STZ induced rats

The food intake in control group showed a minor increase in food intake during the study. The food intake had increased about 90% on the 7th day of post STZ induction in all STZ induced groups. The food intake of the animals of the diabetic control group showed a gradual increase in the food intake till the end of the study. The increased in food intake was decreased in treated groups; glibenclamide shows decrease in food intake up to 34%. The extract treated group TG (200mg/kg) shows 23% (Table 5).

Table no.5: Effect of alcoholic extract of TG on time course of Food Intake in STZ induced rats

Group	Treatment	Average Food intake (g/day/animal) ± (SEM)				
		0 Day	STZ 7	T-7	T-14	T-21
I	Vehicle control(NC)	14.05±1.9	18.55±3.0	24.23±4.6	26.90±2.7	29.20±2.8
II	Diabetic control(DC)	14.70±4.0	41.88±5.0	52.13±7.2#	54.93±9.9#	55.85±15.5#
III	Glibenclamide (5mg/kg)	15.75±2.61	47.08±4.8	38.90±2.4	38.05±6.4	31.90±4.4*
IV	TG(100mg/kg)	16.15±2.0	43.63±9.1	46.00±3.2	43.36±2.1	39.39±0.8
V	TG(200mg/kg)	14.78±3.5	39.38±6.2	38.75±3.7	34.18±3.3	30.55±1.6*

All Values are means ± S.E.M. (n=6). Significance was determined by One-Way ANOVA followed by Bonferroni Multiple Comparisons Test *: $p < 0.05$ when compared with positive control group. #: $p < 0.05$ when compared with vehicle control group

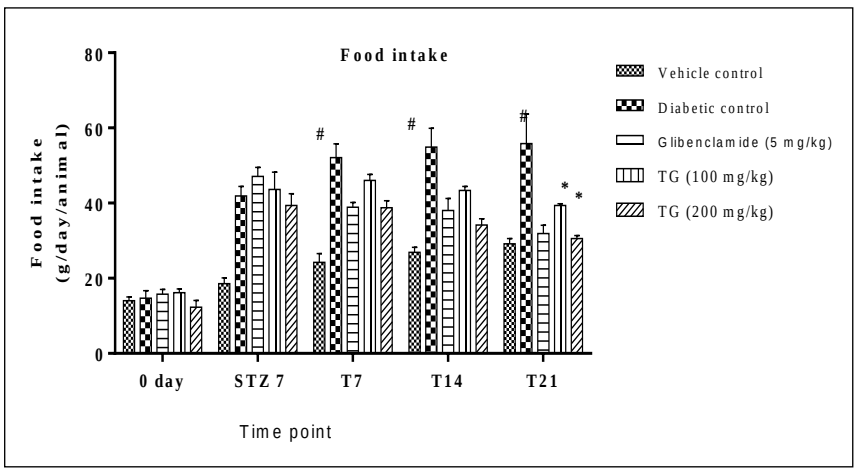


Fig. 6: Effect of alcoholic extract of TG on time course of Food Intake in STZ induced rats

The water intake of animals has increased by 400-600% on 7th day of post STZ induction and has keep increasing till the end of study. The water intake by treated groups shows reduction upto 80% by glibenclamide. Amongst the extract treated groups the maximum reduction in the water intake was shown by TG (200mg/kg) up to 93%.

Table 6: Effect of alcoholic extract of TG on time course of Water Intake in STZ induced rats

Group	Treatment	Average Water Intake (ml/day/animal) ± (SEM)				
		0 Day	STZ 7	T-7	T-14	T-21
I	Vehicle control(NC)	23.63±2.3	24.55±6.8	35.58±4.2	29.20±4.6	39.00±1.1
II	Diabetic control(DC)	20.80±2.9	138.2±8.5	151.8±6.7#	143.8±10.3#	150.1±4.8#
III	Glibenclamide (5mg/kg)	24.78±4.6	134.3±5.1	132.2±8.5	122.7±7.7	106.4±5.5
IV	TG(100mg/kg)	26.50±4.8	129.6±5.3	137.3±6.0	127.5±1.3	121.0±0.6
V	TG(200mg/kg)	23.83±2.4	143.1±6.9	136.4±5.6	124.2±7.4*	113.6±3.1

All Values are means ± S.E.M. (n=6). Significance was determined by One-Way ANOVA followed by Bonferroni Multiple Comparisons Test *: $p < 0.05$ when compared with positive control group. #: $p < 0.05$ when compared with vehicle control group

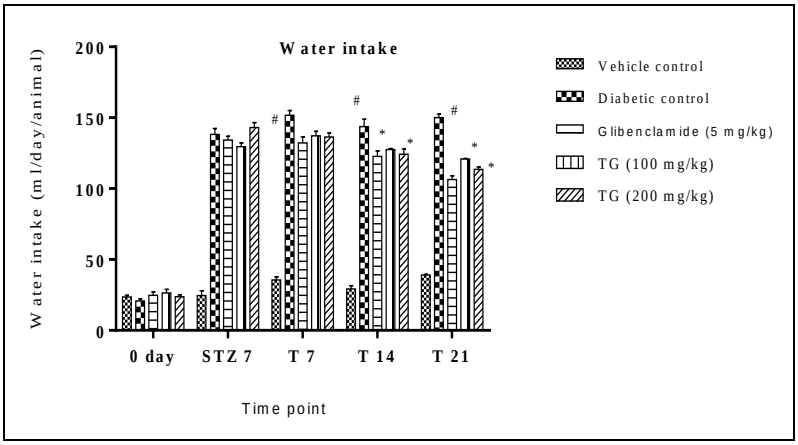


Fig 7: Effect of alcoholic extract of TG on time course of Water Intake in STZ induced rats

Serum glucose levels of the animals were estimated before STZ induction (0 day), on 7th day of STZ induction and on the 7th, 14th and 21st day of the treatment. The Mean Serum Glucose levels were calculated for each group on Day 0, STZ 7, T 7, T 14 and T 21.There was no major change in the serum glucose levels of the normal control rats. The level of glucose was significantly increased in diabetic control rats as compared to normal control. The serum glucose level was found to be decreased in the treated groups significantly as compared to that of diabetic control group on the 21st day of the treatment.

The group treated with Glibenclamide and TG extract (200mg/kg) showed lowest level of serum glucose as compared to other treated group on the 21st day of the treatment. Among both dose levels of extract treated groups, the high dose of TG 200mg/kg showed decrease in serum glucose level up to 50-55 % when compared with that of diabetic control group and shows equivalent effect when compared with standard treated group.

Table 7: Effect of alcoholic extract of TG on serum glucose level in STZ induced rats

Group	Treatment	Mean Serum Glucose level (mg/dl) ± (SEM)				
		0 Day	STZ 7	T-7	T-14	T-21
I	Vehicle control(NC)	112.1±6.2	112.3±7.2	106.4±6.7	104.6±5.08	110.3±7.4
II	Diabetic control(DC)	107.0±6.8	334.9±7.6	336.9±7.9#	339.7±8.58#	339.7±8.5#
III	Glibenclamide (5mg/kg)	115.5±12.5	336.4±11.4	258.1±12.0	220.8±4.3	161.0±4.9
IV	TG(100mg/kg)	120.6±7.1	341.7±7.0	261.6±17.14	225.3±18.0	202.7±4.0
V	TG(200mg/kg)	107.1±5.7	322.7±12.4	239.8±26.0	203.8±5.5*	160.0±2.63*

All Values are means ± S.E.M. (n=6). Significance was determined by One-Way ANOVA followed by Bonferroni Multiple Comparisons Test *: $p < 0.05$ when compared with positive control group. #: $p < 0.05$ when compared with vehicle control group

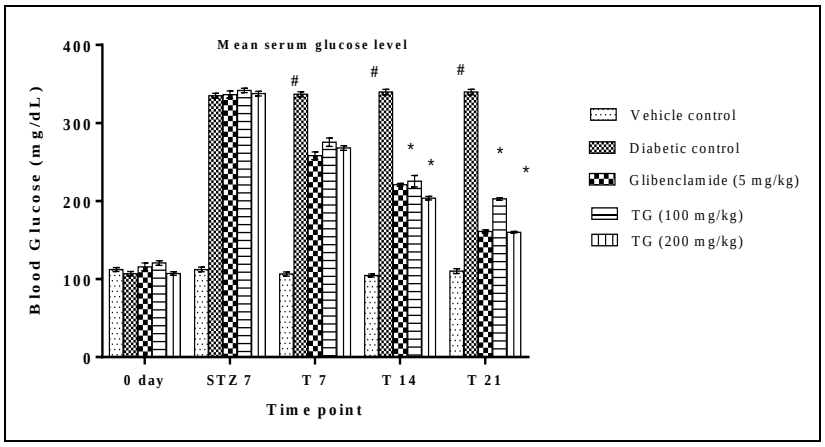


Fig 8: Effect of alcoholic extract of TG on serum glucose level in STZ induced rats

The serum insulin level was measured by RIA method at BRIT, Vashi for all groups where. there was no major change was found in the serum insulin levels of the normal control rats. The level of insulin was significantly reduced in diabetic control rats (10.82±0.80) as compared to normal control (23.61±1.6). The serum insulin level was found to be increased in the treated groups. The group treated with glibenclamide showed highest level of insulin followed by the high dose of extract (200 mg/kg) that is 18.53±0.79 and 16.96±1.16 respectively.

Table 8: Determination of serum insulin by radioimmuno assay (RIA):

Group	Treatment	Mean Serum Insulin level (µU/ml) ± (SEM)				
		0 Day	STZ 7	T-7	T-14	T-21
I	Vehicle control(NC)	23.75±0.90	23.75±0.90	23.30±1.31	23.97±1.30	23.61±1.6
II	Diabetic control(DC)	23.73±0.81	11.39±1.10	11.39±1.10	10.82±0.80	10.82±0.80
III	Glibenclamide (5mg/kg)	23.94±0.98	10.70±1.32	15.58±0.81	17.27±0.86	18.53±0.79
IV	TG(100mg/kg)	24.16±0.56	10.96±1.41	10.96±1.41	12.16±0.90	12.78±0.64
V	TG(200mg/kg)	22.64±1.01	11.77±1.42	13.17±0.94	15.23±0.98	16.96±1.16

All Values are means ± S.E.M. (n=6). Significance was determined by One-Way ANOVA followed by Bonferroni Multiple Comparisons Test *: $p < 0.05$ when compared with positive control group. #: $p < 0.05$ when compared with vehicle control group

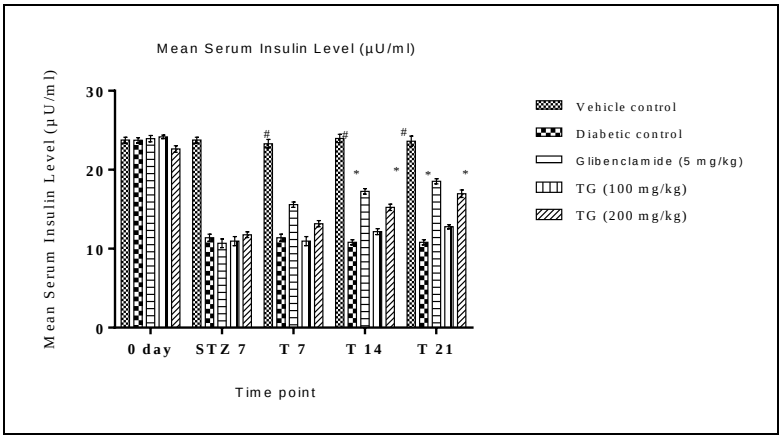


Fig 9: Effect of alcoholic extract of TG on serum Insulin level in STZ induced rats

The level of glycosylated haemoglobin was significantly increased in diabetic control rats as compared to normal control. The level of glycosylated haemoglobin decreased in the treated groups. The group treated with glibenclamide showed lowest level of glycosylated haemoglobin (8.20 ± 0.23) as compared to all other treated groups. The TG extract at dose of 200mg/kg showed lowest level of glycosylated haemoglobin among both the dose level of extract shown in Table 9.

Table 9: Glycosylated haemoglobin

Group	Glycosylated Haemoglobin (% GHb)
I (Normal)	6.43 ± 0.26
II (Diabetic)	17.15 ± 0.83
III (Glibenclamide)	8.20 ± 0.23
IV (TG 100mg/kg)	11.86 ± 0.91
V (TG 200mg/kg)	9.86 ± 0.86

The level of total cholesterol was significantly increased in diabetic control rats as compared to normal control. The level of cholesterol was found to be decreased in the treated groups. The group treated with glibenclamide and TG extract at dose of 200mg/kg showed lowest level of cholesterol. (Table 10)

Table no. 10: Effect of alcoholic extract of TG on serum Cholesterol level in STZ induced rats

Group	Treatment	Mean Serum total cholesterol (mg/dl) ± (SEM)				
		0 Day	STZ 7	T-7	T-14	T-21
I	Vehicle control(NC)	56.59± 2.03	58.80±1.70	58.44±2.56	62.70±3.4	67.4±0.80
II	Diabetic control(DC)	56.09 ±1.61	86.86±3.72	112.3±7.9	172.6±12.4	197.1±5.90
III	Glibenclamide (5mg/kg)	57.23 ±2.09	85.33±3.54	101.5±5.01	107.7±6.86	111.2±5.79
IV	TG(100mg/kg)	55.20 ±2.80	92.04±5.4	107.3±5.41	136.1±3.70	154.0±4.64
V	TG(200mg/kg)	54.96±2.01	87.34±3.26	106.4±6.24	114.9±3.10	125.7±3.16

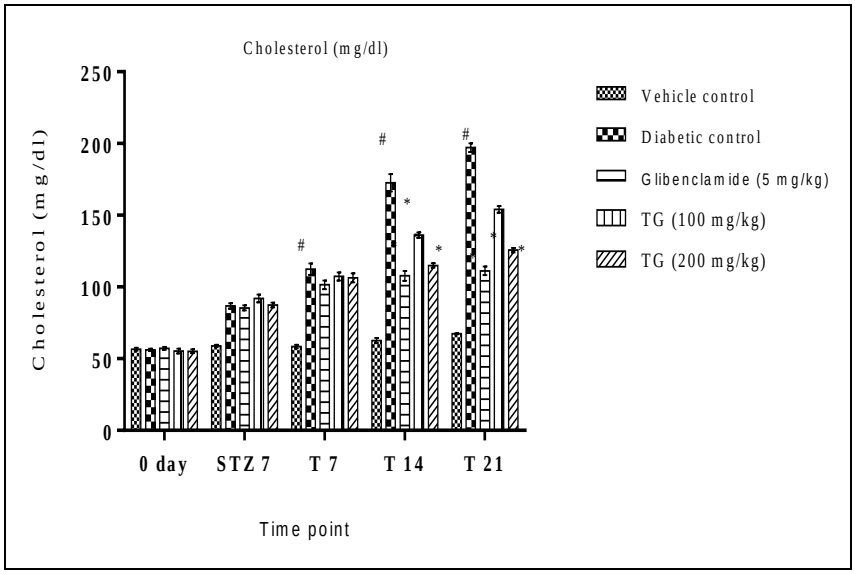


Fig. 10: Effect of alcoholic extract of TG on serum Cholesterol level in STZ induced rats

There wasasignificant increaseobservedintheserumtriglyceridelevelsinthepositivecontrolgroup (292.0±4.99mg/dl,p<0.05) ascomparedtothevehiclecontrolgroup(163.7± 1.86mg/dl).A significantinhibitionin the rise oftriglyceride levels was observed aftertreatment with Glibenclamide and TG at dose of 5 and 200mg/kg respectively.

Table 11: Mean serum Triglyceride

Group	Treatment	Mean Serum Triglyceride (mg/dl) ± (SEM)				
		0 Day	STZ 7	T-7	T-14	T-21
I	Vehicle control(NC)	146.8±3.12	150.3±3.62	153.2±2.17	156.5±3.31	163.3±1.86
II	Diabetic control(DC)	145.3±4.8	181.8±2.77	205.0±4.33	245.4±3.08	292.0±4.99
III	Glibenclamide (5mg/kg)	147.1±6.65	190.1±7.53	215.3±3.23	220.3±3.45	224.1±4.96
IV	TG(100mg/kg)	151.3±2.80	196.8±5.69	215.1±7.01	223.6±6.08	227.4±5.86
V	TG(200mg/kg)	150.0±3.37	191.3±6.72	213.6±10.7	229.3±4.12	207.5±4.34

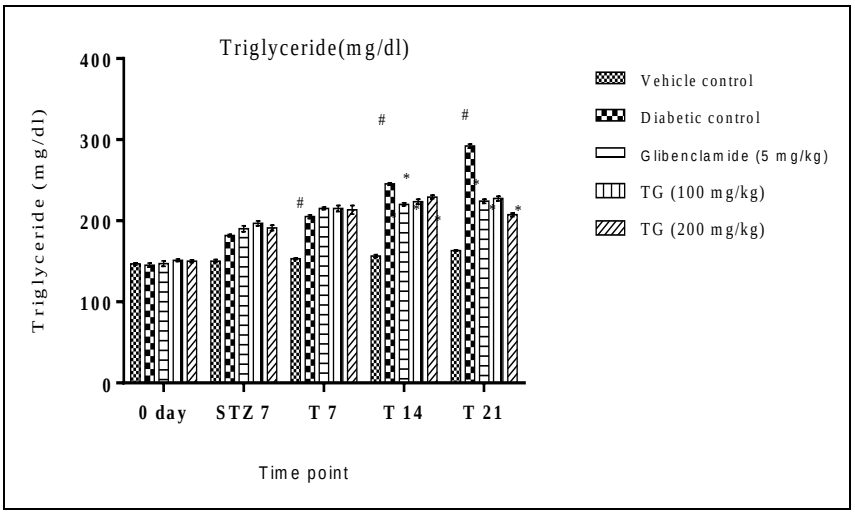


Fig. 11: Effect of alcoholic extract of TG on serum Triglyceride level in STZ induced rats

The analysis of other biochemical parameters like ReducedGlutathione(GSH),Catalase(CAT),Superoxide dismutase(SOD), lipid peroxidation (thiobarbituric acid reactivesubstances, TBARS) were carried out on homogenates obtained from rat pancreas.
Thedecrease in valueofreducedglutathione (GSH),superoxidedismutase(SOD) andcatalase(CAT) wereobservedindiabeticcontrolgroupinwhencomparedwiththevehiclecontrolgroup. The TG extract treatment preventeddecreaseinCAT and SOD levelsatdose of 200 mg/kg. TheactivitiesofGSHwerefoundtobenormalatdose of 200 mg/kg of TG extract.

Table 12: Readings of GSH, SOD and Catalase

Groups (n=6)	Treatment	ReducedGSH(µg/mg tissue protein)	SOD (UA/mg tissue protein)	Catalase (UA/mg tissue protein)
I	Vehicle control (NC)	16.37±1.81	1.09±0.08	49.63±4.34
II	Diabetic control (DC)	4.47±0.39	0.16±0.05	12.79±1.28
III	Glibenclamide (5mg/kg)	15.24±0.88	0.73±0.06	41.74±1.53
IV	TG(100mg/kg)	8.39±1.21	0.39±0.14	32.30±2.92
V	TG(200mg/kg)	11.98±0.50	0.84±0.02	36.23±3.09

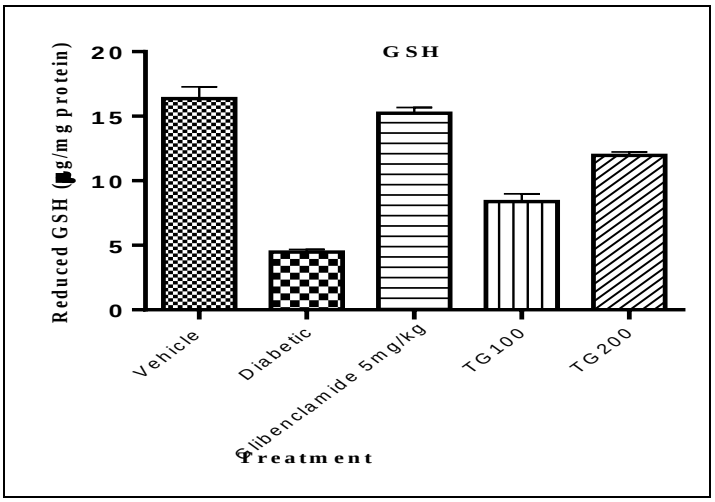


Fig 12: Effect of alcoholic extract of TG on GSH level in STZ induced rats

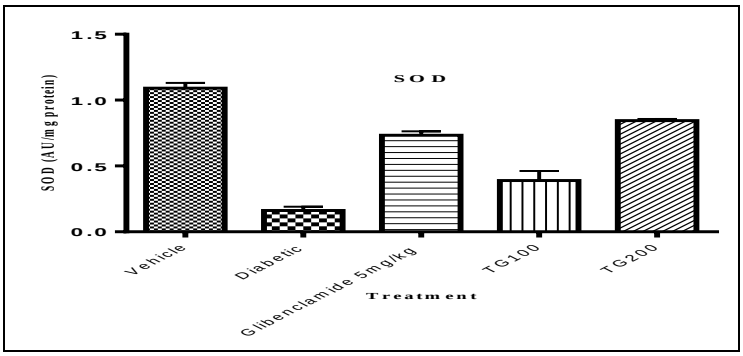


Fig 13: Effect of alcoholic extract of TG on SOD level in STZ induced rats

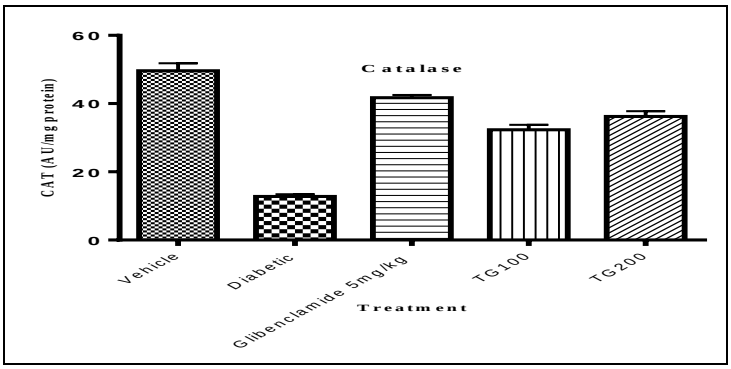


Fig 14: Effect of alcoholic extract of TG on Catalase level in STZ induced

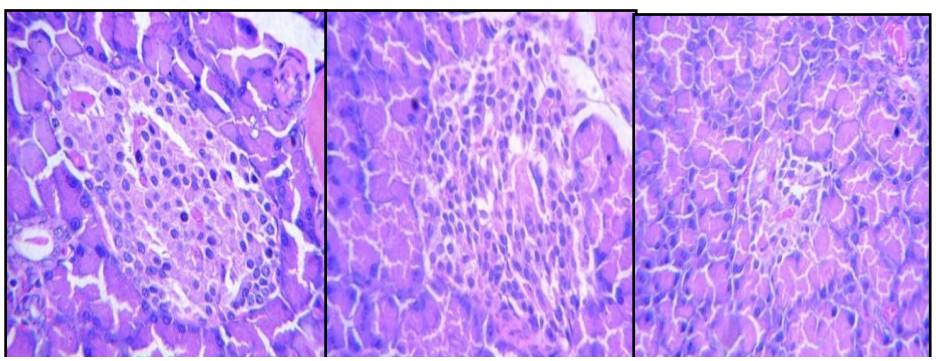
Table 13: TBARS Level

Group	Mean TBARS Level (nMoles/mg tissue) ± SD
I (Normal)	2.92 ± 0.38
II (Diabetic)	5.45 ± 0.67
III (Glibenclamide)	2.87 ± 016
IV (TG 100mg/kg)	4.55 ± 0.23
V (TG 200mg/kg)	3.35 ± 0.33

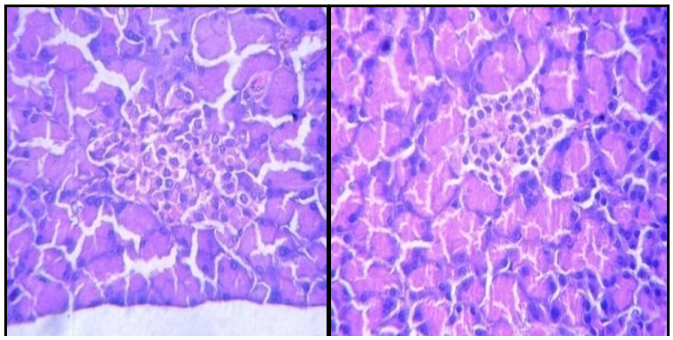
The level of pancreatic TBARS was significantly increased in diabetic control rats as compared to that of the normal control. The level of TBARS decreased significantly in the treated groups as compared to the diabetic control group. The group treated with TG at dose of 200 mg/kg showed lowest level of TBARS as compared to all other treated groups.

Histopathological studies results:

Pancreatic sections stained with hematoxylin and eosin (fig. 15) showed that streptozotocin caused severe necrotic changes of pancreatic islets. Relative reduction of size and number of islets and severe reduction of beta cells were seen. Study of pancreas of treated diabetic groups showed increased size of islets. The pancreas of glibenclamide treated diabetic group showed no detectable abnormality. The high dose of TG extract (200mg/kg) treated diabetic groups showed minimal detectable abnormality and shows mild depletion of islets of langerhans.



(a) Group I (b) Group II (c) Group III

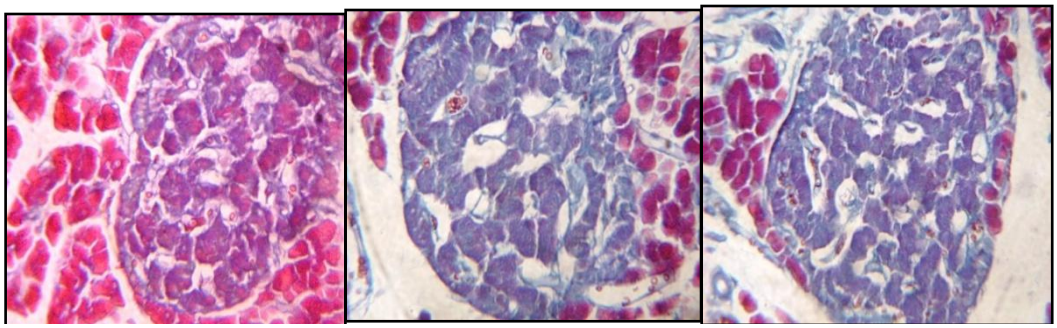


(d)Group IV (e) Group V

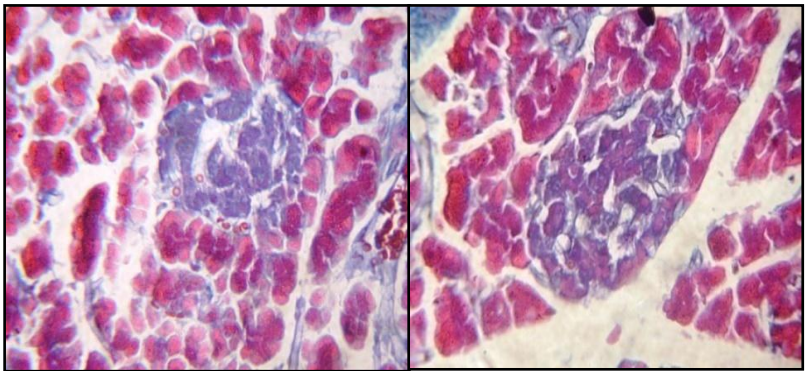
a) Group I: NAD, Pancreas of Normal (Vehicle) control rat
b) Group II: +++ Severe depletion of islets of langerhans, Pancreas of Diabetic control rat Diabetic rat shows Moderate degree vacuolar degeneration
c) Group III: +Mild depletion of cells, Diabetic rat treated with Standard Glibenclamide (5mg/kg) Minimal degree vacuolar degeneration
d) Group IV: ++ Moderate depletion of islets of langerhans Diabetic rat treated with TG extract 100mg/kg Mild degree vacuolar degeneration
e) Group V: +Mild Depletion of islets of langerhans Diabetic rat treated with TG extract 200mg/kg, Minimal degree vacuolar degeneration

Fig 15: Histopathological images of Pancreas of rat (stained with H & E)

The rat pancreas when stained with Massion trichrome, in normal group shows presence of α, β and δ cells and in diabetic group there were minimum α and β cells. The group which was treated with standard glibenclamide and TG extract at dose of 200mg/kg shows mild to modereate presence of α, β and δ cells that substantiate the protective effect of TG extract to rat pancreas(Fig 16).



(a)Group I: NAD (b) Group II (c) Group III



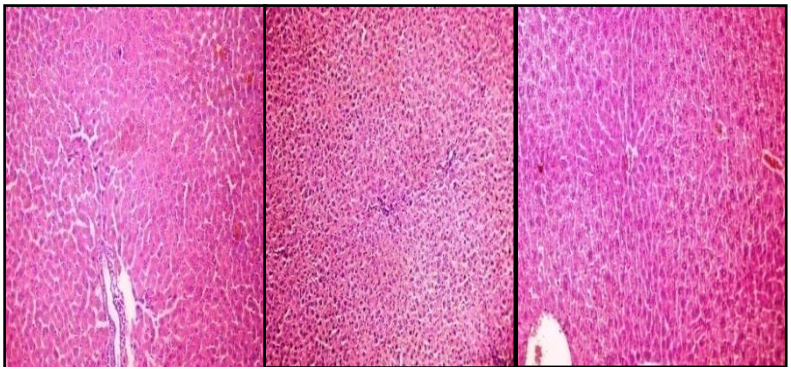
(d) Group IV (e) Group V

a) Group I: NAD, Pancreas of Normal (Vehicle) control rat, Marked presence of α, β and δ cells
b) Group II: Pancreas of Diabetic rat, Minimal presence of α and β cells and moderate presence of δ cells
c) Group III: Pancreas of diabetic rat treated with standard glibenclamide (5 mg/kg), Moderate presence of α, β and δ cells

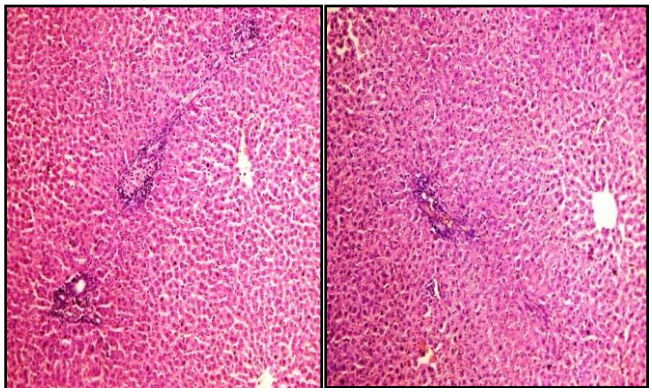
d) Group IV: Pancreas of diabetic rat treated with TG extract 100mg/kg, Minimal to mild presence of α, β and δ cells
e) Group V: Pancreas of diabetic rat treated with TG extract 200mg/kg, Mild to moderate presence of α, β and δ cells

Fig 16: Histopathological images of rat pancreas stained with Massion trichrome

The rat Liver section when stained with H & E it was found that diabetic group rats shows moderate degree of diffuse macular degeneration while the group treated with glibenclamide and TG extract 200mg/kg shows minimal degree of swelling (Fig.17).



(a) Group I (b) Group II (c) Group III



(d) Group IV (e) Group V

a) Group I: Normal (Vehicle) control rat liver, NAD
b) Group II: Diabetic control rat, Moderate degree of diffuse granular degeneration
c) Group III: Diabetic rat treated with standard Glibenclamide, Minimal degree cloudy swelling
d) Group IV: Diabetic rat treated with TG 100mg/kg, Mild degree diffuse granular degeneration
e) Group V: Diabetic rat treated with TG extract 200mg/kg, Minimal degree cloudy swelling

Fig 17: Histopathological images of rat Liver (stained with H &E)

The literature shows the TG extract contains 1, 4 naphthoquinone derivatives, phenolic compounds, tannins and flavanoids etc. The presence of naphthoquinone derivatives and its analogues with other allied component it shows protective activity towards liver and pancreatic tissue. Based on the literature the mode of antidiabetic activity appearsto be regeneration of beta cells of pancreas leading to increase in insulin level.

The antidiabetic potential of alcoholic extract of TG was tested at two different doses i.e. 100, 200 mg/kg. The result of all biochemical parameter reveals that the higher dose of TG extracts that is 200mg/kg was remarkably decreasing blood glucose level up to 50-55% when compared with that of diabetic control group. The other blood parameters such as serum insulin, cholesterol, Triglyceride, Liver glycogen, TBARs level etc, were also controlled. The tissue parameter such as reduced glutathione, SOD, Catalase were also in range after completion of treatment with alcoholic extract of TG at 200mg/kg.

The TG causes increase in glycosylated haemoglobin level which thereby increases the total haemoglobin and improves glycemic controlled. TG also shows inhibition of endogenous synthesis of cholesterol and enhancement of degradation of formed cholesterol by excretion through intestinal tract.The serum level of SOD, Catalase and GSH are controlled by TG. The decrease in TBAR level in tissues shows

antioxidant potential of TG. Streptozotocin induces diabetes and thereby liberates free radical which causes lipid peroxidation which mediate pancreatic injury. The histopathological study of TG treated rat pancreatic tissue shows minimal depletion which confirms that TG facilitate reconstruction of pancreatic cells to produce more insulin which ultimately produces antidiabetic effect. The increase in level of lipid peroxidation is indicative of decrease in enzymatic antioxidant defence mechanism. Several studies indicated that O_2 free radicals generated in diabetic beta cells and causes over expression of SOD and Catalase which plays key role in protecting cells from oxidative damage. In the present study TG shows increase in SOD and catalase activities in liver tissues of diabetic rats this indicates that TG extract could inhibit oxidative stress in diabetes.
The TG extract contains lapachol, deoxylapachol, tectoquinone, steroidal compounds like β-sitosterol, lupeol, diosgenin etc.

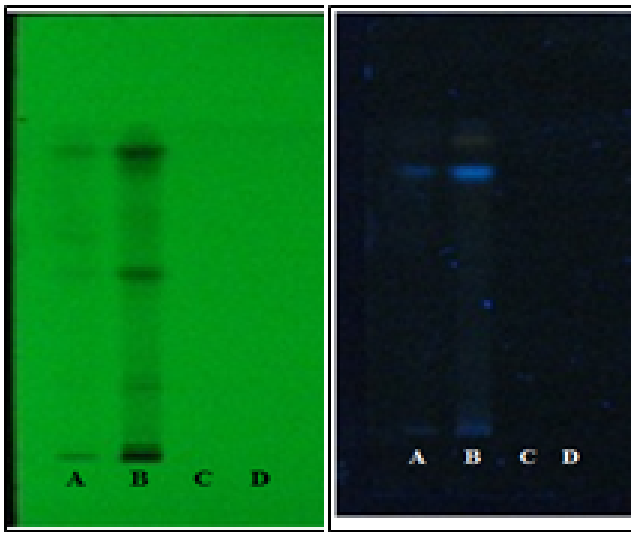
Hence, taken together all this finding conclude that the 1, 4 naphthoquinone derivatives and other allied component altogether responsible for antidiabetic activity.Hence, in conclusion the alcoholic extract of TG at dose of 200mg/kg shows significant antidiabetic activity as compared to the diabetic control group. Thus, the higher dose of extract i.e. 200mg/kg of TG was showing prominent activity so, the TG extract at dose of 200mg/kg was selected for preparation of solid oral formulation. The human dose was extrapolated, and suitable solid oral dosage form was formulated using the extract.

The Human Equivalent Dose for alcoholic extract of TG was found to be (1.98) ~ 2 gm in divided dose i.e. 500mg/kg each dose. Hence, decided formulation of choice was 'Liquid filled in hard gelatin capsule' The capsule size was chosen 00el to accommodate the high dose of extract with oil. The amount of oil required was optimized and that was in the ratio of 1:1 (extract: oil) to make it pourable. The extract triturated in castor oil and other ingredients was filled in 00el capsules. The final capsules were sealed appropriately using sealing solution.The capsules are prepared and evaluated for their physicochemical parameter like change in size, shape, appearance, leakage etc. The uniformity of weight, disintegration time and dissolution time was estimated. It was found that the TG capsules were passes all the tests. (Table no.15)

Table 15: Evaluation of Capsules

SN	Parameter	TG capsules
1.	Weight variation (mg)	961.44±15.77%
2.	Disintegration time	6 min ±10 sec
3.	Dissolution time	30 ±5 min

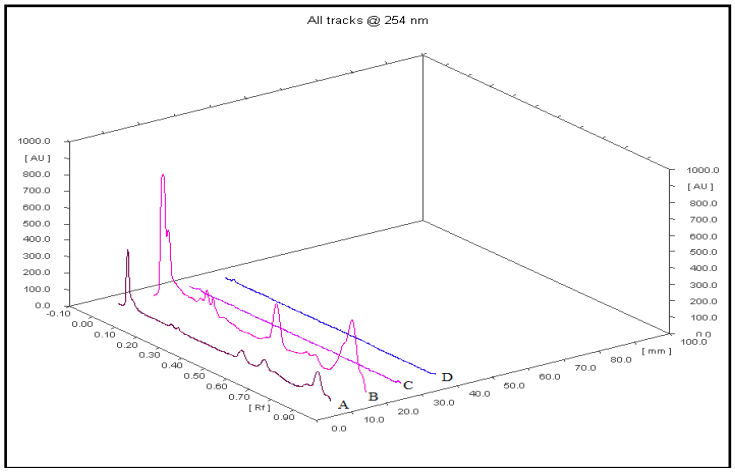
The HPTLC method was developed for the dissolution assay of capsules where release of extract in medium was observed (Track A of fig 18). The spectra of track A was matching with track B (Fig 18). Hence, the release of extract from capsule was achieved within 30 min and hence it passes this test also.



(a) (b)

Track details: A-TG capsule dissolution aliquote, B- TG extract, C-Empthy capsule solution, D-SLS solution (4%),

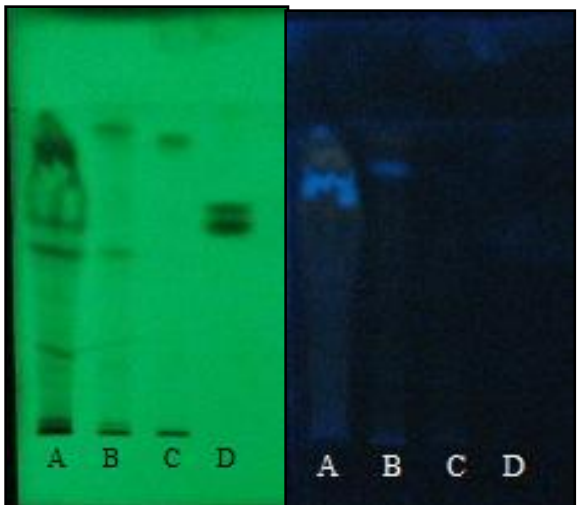
Fig. 18: HPTLC plate photo of TG (a) At 254 nm and (b) At 366 nm



(a)

Track details: A-TG capsule dissolution aliquote, B- TG extract, C-Empthy capsule solution, D-SLS solution (4%),

Fig.19: 3D graph of HPTLC plate of TG capsule showing the drug release in mediumStability study of capsules was also carried out by HPTLC method of analysis. The HPTLC method was developed for capsule formulation and was applied on HPTLC plate. The content of capsule, TG extract, standard Lapachol and excipient solution were spotted on HPTLC plate and scanned (Fig.20,21). It was found that the capsules were stable for the period of three months when analysed by HPTLC method of analysis.



(a) (b)

Track details: A – TG capsule, B – TG extract, C – Standard Lapachol, D – Excipient solution

Fig. 20: HPTLC Plate photo of TG capsule stability (a) at 254 nm and (b) at 366nm

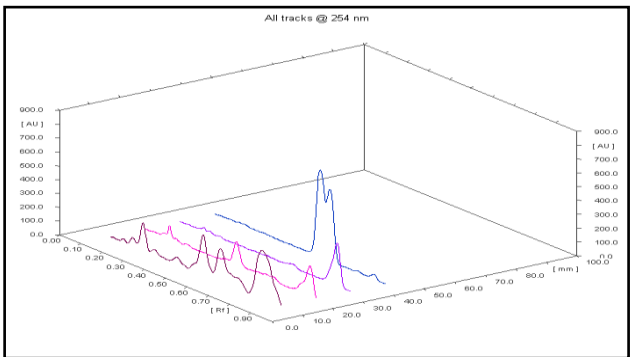


Fig. 21: 3D view of HPTLC chromatogram of TG capsules, TG extract, STD Lapachol, excipient solution

III. Conclusion

The current research explores medicinal potential of the byproduct of Timber mill that is sawdust of *Tectonagrandis* as a antidiabetic agent. The detailed study has been carried out for the standardization of the herb and optimization of extraction method was done successfully for getting maximum yield of extract. The extract has been studied for phytochemical analysis and preliminary HPTLC fingerprinting. The antidiabetic potential of TG extract has beenstudied in detailed using STZ induced diabetic rat model and results were compared with that of standard glibenclamide. There was increase in liver glycogen level and a decrease in pancreatic TBARS level. The decrease in the pancreatic TBARS may be attributed to the antioxidant effect of the extract, thereby preventing the damage of pancreatic islets and increasing the serum insulin levels.

The high dose of TG extract (200mg/kg) showed decrease in serum glucose level up to 50-55 % when compared with diabetic control group and shows equivalent effect when compared with standard glibenclamide treated group. The other blood parameters such as seruminsulin, cholesterol, Triglyceride, Liver glycogen, TBARs level etc, were also controlled by TG extract.The dose of TG extract showing promising activity was selected further for preparation stable oral formulation and formulated into capsule dosage form. The formulations were found to be stable over a period of 6 months when evaluated with developed analytical parameter.Hence, the final oral formulation can be made available of TG extract for management of diabetes.

Acknowledgement

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