ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Assessment of Anti-Hyperglycemic and Anti-Oxidant Activities of *Tinospora Cordifolia* & *Juglans Regia* Composite Extract in STZ Induced Diabetes in Wistar Rats

Kapil Kumar[1], Praveen K Dixit[*1], Harshit Takru[1], Nagarajan K[2]

[1]Department of Pharmacology, KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad-U.P 201206, India
[2]Department of Pharmaceutical Chemistry, KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad-U.P 201206, India

Article History:

Received on: 17 Sep 2020
Revised on: 17 Oct 2020
Accepted on: 22 Oct 2020

Keywords:

Streptozotocin, Nicotinamide, Diabetes mellitus, Tinospora cordifolia, Juglans regia

ABSTRACT

The present study explored the assessment of the antidiabetic potential of *Tinospora cordifolia* & *Juglans regia* composite extract in STZ induced diabetes in wistar rats. As streptozotocin -associated infiltrations of increase glucose level has been reported to be responsible for diabetes. We evaluated the glucose lowering potential of *Tinospora cordifolia* & *Juglans regia* on the basis of its anti-diabetic property. Rats were administered streptozotocin (55 mg/kg i.p., once) with nicotinamide (120mg/kg) to induce experimental toxicity. The development of diabetes was assessed biochemically as well as histologically 72 hours after induction of diabetes. Body weight and blood glucose levels were determined in (0, 7th, 14th, 21st, 28th) days. Serum lipid profile and enzyme estimated, (kidney, liver, pancreas) tissue was measured at the end of the experimental period. Treatment with composite extracts TCAE high dose (350 gm/kg b.w.) & JRAE high dose (800 mg/kg b.w.) and TCHE high dose (350 gm/kg b.w.) & JRHE high dose (800 mg/kg b.w.) were noted to be more effective against the streptozotocin- induced toxicity as compared to Glibenclamide (5 mg/kg b.w.). it may be concluded that streptozotocin-induced glucose may be accountable for the induction of diabetes toxicity in rats. Interestingly, improvement in body weight, glucose level, lipid profiles, biochemical parameters and histopathological changes in kidney, liver and pancreas was observed following herbal treatment in STZ induced diabetic rats. Furthermore, composite extract of TCAE (350mg/kg b.w.) & JRAE (800mg/kg b.w.) was found to be efficacious than the composite extract of TCHE (350mg/kg b.w.) & JRHE (800mg/kg b.w.).

*Corresponding Author

Name: Praveen K Dixit
Phone: 91 8192026467
Email: praveendixit87@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11iSPL4.4356

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

Diabetes mellitus (DM) is a global public health problem with an escalating incidence and prevalence, particularly in developing and newly industrialized countries. DM is a disease which decreases the body capacity to make or use insulin. Insulin is a hormone conveyed in the pancreas (beta- cells) that facilitates shipping (glucose) from the dissemination framework into the cells so they can isolate it and use it for body fuel. People cannot survive without insulin (Riaz, 2009). We currently watch

high rates of DM-associated removals, cerebrovascular contamination, coronary heart-related problems, and kidney disease in masses that became now not as of late appreciate for those trying out medicinal issues (Stratton *et al.*, 2000). Diabetes includes more treatment in different ways, like "broken red coral, oil of roses, unrefined quinces and slop, dates, sweet almonds and new blossoms of outwardly hindered weeds (Lakhtakia, 2018). Glibenclamide is an II -generation sulfonylurea that decrease blood glucose level by increasing insulin secretion from pancreatic beta cells. Glibenclamide-induced hypoglycemia more likely in the elderly in patients with irregular eating habits, and in renal impairment (Naidoo *et al.*, 2014). *Tinospora cordifolia* is very important herb in ayurvedic system of medicine. *Tinospora cordifolia* are used in various somatic, psychosomatic and lifestyle disorder of human being since times immune Systemic and proper use of Giloy can cure various life-threatening disorder like diabetes, arthritis, fever, malignancy etc (Biswas *et al.*, 2015). *Juglans regia* is important herb in ayurvedic system of medicine. These are used in various diseases like diabetes, inflammation, cancer etc. On the basis of ability of *Tinospora cordifolia* & *Juglans regia* to decrease the glucose level. The present study has been designed to investigate the anti-diabetic potential of a composite extract of *Tinospora cordifolia* and *Juglans regia* against Glibenclamide-induced diabetic toxicity.

MATERIALS AND METHODS

Plant material and extraction

The stems of *Tinospora cordifolia* & Kernel of *Juglans regia* were purchased from Delhi, India and identified by National Institute of Science Communication and Information Resources (NISCAIR), Delhi, India with Reference No. NISCARE/RHMD/Consult2018/3264-65-1 of *Tinospora cordifolia* and Reference No. NISCARE/RHMD/Consult2016/3264-65-2 of *Juglans regia*. Dried *Tinospora cordifolia* Stem & *Juglans regia* kernel were powdered mechanically through mesh sieve. The drug powder of *Tinospora cordifolia & Juglans regia* was extracted with alcohol (Ethanol 90%) and Hydroalcoholic (Ethanol 50% and 50% water) solution using the Soxhlet method (Mishra *et al.*, 2014).

Animal

Wistar rats of either sex (6-7-week-old) weighing 200-250 gm were issued from the Animal House Facility, KIET School of Pharmacy, Ghaziabad. The protocol was permitted via the Institutional Animal Ethics Committee (IAEC) of KIET School of Pharmacy (Registration number 1099/ PO/ Re/ S/ 07/ CPCSEA, 27/07/2007) Ghaziabad.

Streptozotocin Induced of Diabetes

Diabetes was induced in overnight fasting Wistar rats. For the ambition, STZ and Nicotinamide were diffused in citrate buffer (pH 4.5), Nicotinamide was induced (120 mg/kg, i.p) 15 min (Mohammadi *et al.*, 2012). Before the induction of Streptozotocin (55 mg/kg, i.p) (Puranik *et al.*, 2010). After 72 h.

Experimental groups

All the experimental animal was divided into 13 groups with each group consisting of 6 animals as follows: **Group 1-** Control: This group was used for studying the baseline values of the parameters studied. **Group 2-** Positive control: This group consisted of streptozotocin (55mg/kg/b.w.) + Nicotinamide (120mg/kg/b.w.) induced diabetic rats. **Group 3-** Diabetic rats treated with (5 mg/kg b.w.) Glibenclamide. **Group 4-** Diabetic rats treated with (350 mg/kg b.w.) high dose of alcoholic extract of TC. **Group 5-** Diabetic rats treated with (100 mg/kg. b.w.) low dose of alcoholic extract of TC. **Group 6-** Diabetic rats treated with (350 mg/kg. b.w.) high dose of hydroalcoholic extract of TC. **Group 7-** Diabetic rats treated with (100 mg/kg. b.w.) low dose of hydroalcoholic extract of TC. **Group 8-** Diabetic rats treated with (800 mg/kg. b.w.) high dose of alcoholic extract of JR. **Group 9-** Diabetic rats treated with (200 mg/kg. b.w.) low dose of alcoholic extract of JR. **Group 10-** Diabetic rats treated with (800 mg/kg. b.w.) high dose of hydroalcoholic extract of JR. **Group 11-** Diabetic rats treated with (200 mg/kg. b.w.) low dose of hydroalcoholic extract of JR. **Group 12-** Diabetic rats treated with composite (350 mg/kg. b.w. + 800 mg/kg. b.w.) high dose of alcoholic extracts of *Tinospora cordifolia & Juglans regia.* **Group 13-** Diabetic rats treated with composite (350 mg/kg. b.w. + 800 mg/kg. b.w.) high dose of hydroalcoholic extracts of *Tinospora cordifolia & Juglans regia.*

Drug treatment

The dosing of all the groups were given from 0 to 28th once daily orally. Parameter i.e. Body Weight, Glucose have been evaluated on day 0, 7th, 14th, 21th, 28th, Animals were senseless with diethyl ether and blood was compiled by retro orbital puncture. The serum evaluated for serum Blood Glucose on day 0, 7th, 14th, 21th, 28th and HDL, LDL, VLDL and were assed 28th day AST, ALT, ALP, ALB, Bilirubin.

Body weights and blood glucose levels

Body weight and glucose level of rats were measured by using a weighing balance (Ekambaram

et al., 2010) and Blood sample was collected from retro-orbital of rat and measured glucose level by Dr. Morepen glucometer was bought from Morepen Laboratories constrain (Chougale *et al.*, 2009).

Estimation of Biochemical Parameters

High density lipoprotein (HDL)

Measuring of High-density Lipoprotein (HDL) Was done by Phosphotungstic acid Method with the procedure from Germany kit. The HDL level was measured by using the formula given below

$$HDL\ cholesterol\ \left(\frac{mg}{dl}\right) = Abs.of\ \frac{\square}{test\ standard} \cdot \times$$

$$Concentration\ of\ standard\ \left(\frac{mg}{dl}\right) \times$$

$$Dilution\ factor$$

Total Cholesterol

Estimating of TC was done by CHOD/PAP method with the procedure from Germany kit.

The Total cholesterol level was estimated with the formula

$$Cholesterol\ \frac{mg}{dl} = \frac{Absorbance\ of\ test}{Absorbance\ of\ standard}$$

$$concentration\ of\ the\ standard\ (mg/dl)$$

Triglyceride

Estimating of Triglyceride was done by CHOD/PAP method with the procedure from Germany kit (Diagnostics Mannheim GmbH).

The Total cholesterol level was estimated with the formula

$$Cholesterol\ \frac{mg}{dl} = \frac{Absorbance\ of\ test}{Absorbance\ of\ standard}$$

$$concentration\ of\ the\ standard\ (mg/dl)$$

LDL

LDL cholesterol was evaluated by using Fried Wald's (1972) formula as follows

$$LDL\ in\ mg\ \% = \frac{Total\ cholesterol - HDL - Triglyceride}{5}$$

VLDL

VLDL cholesterol was evaluated by calculating the following formula.

$$VLDL\ in\ mg = \frac{Triglyceride}{5}$$

Enzymatic Estimation

Bilirubin

Measuring of Bilirubin was done Diazo Method with the procedure from Germany kit (Diagnostic System International) cat no. 10 135 021. The Bilirubin level was measured with the formula

$$Total\ Bilirubin = Abs.of\ test \times Factor\ \left(23\frac{mg}{dl}\right)$$

Alkaline Phosphate (ALP) level measurement

Measured of Alkaline Phosphatase (ALP) was done by Kinetic method recommended by international federation of clinical chemistry (IFCC) method. The ALP level was measured with the formula

$$ALP\ \ activity\ \left(\frac{IU}{L}\right) = \triangle Abs./min. \times Factor\ (2764)$$

Albumin (ALB) level measurement

Measured of Albumin (ALB) was done by Bromocresol green (BCG) Dye Method with the procedure from Germany kit.

$$Albumin\ \left(\frac{g}{dl}\right) = \frac{Absorbance\ of\ test}{Absorbance\ of\ standard} \times$$

$$Concentration\ of\ standard\ (g/dl)$$

AST

Measured of Serum glutamic-oxaloacetic transaminase (SGOT/AST) was done by international federation of clinical chemistry (IFCC) method with the procedure from Germany kit. The SGOT level was measured with the formula

$$\frac{IU}{L} = \frac{\left(\frac{\triangle A}{min}\right) \times T.V \times 10}{S.V. \times Absorptivity \times P}$$

ALT

Measured of Serum glutamic pyruvic transaminase (SGPT/ALT) was done by international federation of clinical chemistry (IFCC) method with the procedure from Germany kit (Diagnostic System International) cat no.10 135 021. The ALT level was measured with the formula

$$\frac{IU}{L} = \frac{\left(\frac{\triangle A}{min} \cdot\right) \times T.V \times 10}{S.V. \times Absorptivity \times P}$$

Statistical analysis

All values were expressed as mean ± SEM. One-way ANOVA, followed by Tukey's multiple comparison test, was applied for statistical analysis of the data obtained from different groups. For statistically significance, p-value $\leq$0.05 was considered.

RESULTS

Effect of Alcoholic and Hydro-alcoholic extracts on Body weight in wistar rats.

The body weight of rats was significantly ($P < 0.05$) reduced as compared to control and standard groups. Oral administration of TCAE at

Table 1: Effect of all doses with combinations on body weight along with Control, Positive Control, Standard

Group Description	Days				
	0th	7th	14th	21st	28th
Control	193.333±4.409	208.333±3.333	195.833±3.745	217.500±7.719	215.833±3.270
Positive control	212.500 ±7.500###	190.000 ±8.266###	160.833 ±5.974###	140.000 ±6.454###	125.833 ±6.112###
Standard	195.000±6.191	209.166±7.120	225.000±6.582	233.333±5.426	252.166±5.833
TCAE (HD)	188.333±3.333	205.166±5.833	244.166±5.230	259.000±5.416	277.000±6.333
TCAE (LD)	210.000±5.773	223.33±4.013***	225.166±5.688	239.166±4.728	250.833±6.881
TCHE (HD)	209.166±8.407	217.500±6.422**	234.166±7.573	251.666±9.279	270.000±6.055
TCHE (LD)	200.000±6.708	224.166±4.549***	241.666±4.772	232.500±10.626	257.500±7.041
JRAE (HD)	206.666±4.772	217.500±3.095**	220.833±3.961	255.166±4.969	275.833±4.728
JRAE (LD)	215.833±5.833	225.000±4.082***	229.166±6.247	255.000±7.071	271.666±9.279
JRHE (HD)	211.666±3.333	232.500±3.818	248.500±4.752	266.000±4.008	281.500±4.856
JRHE (LD)	200.833±5.540	212.166±4.693*	218.500±4.821	243.000±6.531	247.333±8.452
TCAE (HD) + JRAE (HD)	202.500±6.291	226.666±4.216***	239.166±3.515	253.333±3.073	291.666±6.540
TCHE (HD) + JRHE (HD)	195.500±8.539	215.000±8.266*	239.666±7.423	257.000±5.053	280.833±5.387

350mg/kg/b.w. and 100mg/kg/b.w. significantly ($P < 0.05$) improved as comparision with positive control. TCHE at 350mg/kg/b.w. significantly ($P < 0.05$) improved body weight at 14th, 21st and 28th-day time point while the improvement was seen to be on 14th and 28th days respectively at 100 mg/kg b.w. which was approximately equal to standard diabetic group. JRAE at 800mg/kg b.w. and 200mg/kg b.w. significantly ($P < 0.05$) improved body weight on 14th, 21st and 28th days, respectively. JRHE at 800mg/kg/b.w. significantly ($P < 0.05$) improved which was almost better to standard diabetic group. Combination dose of alcoholic TCAE (H.D) + JRAE (H.D) significantly ($P < 0.005$) increased body weight which was better as compared to standard diabetic group. Combination dose of hydroalcoholic TCHE (H.D) + JRHE (H.D) significantly ($P < 0.05$) increased the body weight observed on 7th,14th, 21st and 28th days respectively. Body weight changes among different groups are shown in Table 1 and Figure 1.

Effect of Alcoholic and Hydro-alcoholic extracts on glucose levels.

Glucose level of STZ induced positive control group rats (after 28 days) was significantly ($P < 0.05$) elevated as comparison with control and standard group. Oral administration of TCAE at 100 mg/kg/b.w. significantly ($P < 0.05$) decreased glucose level as comparison with positive control and at 350 mg/kg/b.w. glucose level reduction was observed on 14th, 21st and 28th days respectively. TCHE at 350mg/kg/b.w. significantly ($P < 0.05$) evaluated which was almost equal as compared to standard diabetic group. TCHE at 100 mg/kg/b.w. significantly ($P<0.05$) decreased glucose level as compared with positive control. JRAE at 800mg/kg/b.w significantly ($P<0.05$) reduced glucose level as compared with positive control and at 200 mg/kg/b.w. significantly ($P<0.02$) reduced glucose level when compared with positive control group. JRHE at 200 mg/kg/b.w. significantly ($P<0.02$) decrease glucose level observed on 14th and 28th day only and at 800mg/kg/b.w. significantly ($P<0.05$) reduced glucose level which was better as compared to standard diabetic group. Combination dose of alcoholic TCAE (H.D) + JRAE (H.D) significantly ($P < 0.05$) decreased glucose level which was better as compared to standard diabetic group. Combination dose of Hydroalcoholic TCHE

Table 2: Effect of all doses with combinations on glucose level along with Control, PositiveControl, Standard

Group Description	Days				
	0th	7th	14th	21st	28th
Control	96.166±4.028	104.833±5.108	107.666±7.017	87.833±3.114	99.333±7.311
Positive control	140.833 ±13.088###	175.667 ±13.894###	178.50 ±11.854###	185.833 ±16.760###	190.833 ±12.197###
Standard	177.833±17.732	81.166±9.867	204.333±7.370	120.333±3.246	84±7.929
TCAE (HD)	206±26.622	170.166±26.342	124±3.864 ***	110.833±6.457	97.5±7.907
TCAE (LD)	165.666±21.428	149.000±6.491	139.883±10.731*	100.166±5.935	113.833±2.845
TCHE (HD)	179.667±16.709	117.166±6.819*	108.334±8.756	100.333±3.801	96.231±6.445
TCHE (LD)	191.167±18.335	178.833±10.316	155.833±3.719	161.500±4.193	136.500±8.543
JRAE (HD)	172.167±14.916	144.833±12.986	124.333±10.573 ***	113.500±7.796	111.333±13.110
JRAE (LD)	161.253±5.978	153.333±15.628	156.253±4.524	136.033±1.949	122.333±1.944
JRHE (HD)	156.667±12.980	105.833±14.349 **	98.666±10.761	87.333±2.123	78.667±3.583
JRHE (LD)	152.667±15.700	124.167±15.054	132.500±14.609 **	120.833±3.134	116.333±2.551
TCAE (HD) + JRAE (HD)	154.333±7.535	128.667±11.627	96.667±5.667	114.167±4.936	72.333±5.323
TCHE (HD) + JRHE (HD)	139.333±11.661	115.5±6.796*	99.333±7.060	98.666±6.491	82.667±1.994

Table 3: Effect of all doses with combinations on Lipid Profile of streptozotocin induced diabetic rats along with Control, Positive Control, Standard after 28 days

Group Description	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Control	85.667±1.687	105.5±1.408	53±1.033	41.473±.0179	21.055±0.602
Positive control	188.333 ±3.0148###	157 ±2.65###	31.167 ±1.302###	125.635 ±0.167###	31.331 ±1.012###
Standard	94.5±1.176	120.333±0.453	39.5±0.847**	30.781±0.172	24.166±.0703 ***
TCAE (HD)	180±5.112	129.833±1.195	43.833±1.014	89.999±0.355	25.00±1.211 ***
TCAE (LD)	173.166±0.946 ***	145.333±1.626	58.166±0.601	106.338±0.213	30.833±1.815
TCHE (HD)	177±1.366*	148.833±3.591**	69.666±3.252	76.669±0.209	32.366±0.932
TCHE (LD)	192±1.155	165.5±1.384*	65.333±1.085	93.596±0.174	33.666±.0615
JRAE (HD)	129.333±2.092	125.5±1.784	53.833±0.946	51.659±1.199	25.18±0.338**
JRAE (LD)	154.5±2.617	142.166±1.138	45.666±1.054	81.599±0.619	29.85±0.573
JRHE (HD)	166.166±2.056	175.833±1.046	38.50±0.619**	99.667±0.528	35.00±0.816
JRHE (LD)	183.666±2.445	181.166±0.946	36.50±1.335	110.529±0.201	35.333±1.256
TCAE (HD) + JRAE (HD)	91.666±1.706	117.166±0.946	54.166±1.493	16.910±1.025	23.00±0.577
TCHE (HD) + JRHE (HD)	129±2.380	161.333±1.909	50.333±1.926	48.881±0.399	30.866±1.902

Table 4: Effect of all doses with combinations on Liver function of streptozotocin induced diabetic rats along with Control, Positive Control, Standard after 28 days

Group Description	AST (U/L)	ALT (U/L)	ALP (U/L)	Albumin (g/dL)	Bilirubin (mg/dL)
Control	33.333±0.881	29.166±1.400	96.833±1.956	4.283±0.598	0.816±0.083
Positive control	83.833 ±2.442###	62.666 ±3.073###	164.166 ±1.537###	4.431 ±0.014###	2.350 ±0.950###
Standard	18.258±0.004	29.571±5.154	104.285±1.539	3.207±0.098***	0.95±0.118**
TCAE (HD)	36.333±0.760	43.428±2.983	112.142±1.033	4.981±0.005	1.166±0.288*
TCAE (LD)	65.666±0.955	52.285±4.465*	131.285±16.493***	2.712±0.031	2.033±0.145
TCHE (HD)	43.000±0.966	46.285±3.564***	122.714±2.504	3.555±0.002	1.216±0.048
TCHE (LD)	56.142±0.911	54.571±1.525	134.857±4.345**	4.786±0.016	1.820±0.163
JRAE (HD)	13.256±0.006	14.147±0.005	125.571±1.088	5.628±0.010	1.288±0.003
JRAE (LD)	37.124±0.003	45.666±0.955***	140.500±3.566*	5.628±0.010	1.828±0.043
JRHE (HD)	33.510±0.041	10.636±0.018	87.714±1.475	4.328±0.265	2.141±0.002
JRHE (LD)	28.357±0.095	5.219±0.010	105.666±0.558	6.285±0.376*	1.430±0.003
TCAE (HD) + JRAE (HD)	22.764±0.200	15.943±0.026	54.333±1.054	2.866±0.220**	1.791±0.003
TCHE (HD) + JRHE (HD)	13.166±0.703	7.857±0.508	76.500±0.885	3.400±0.111**	1.836±0.042

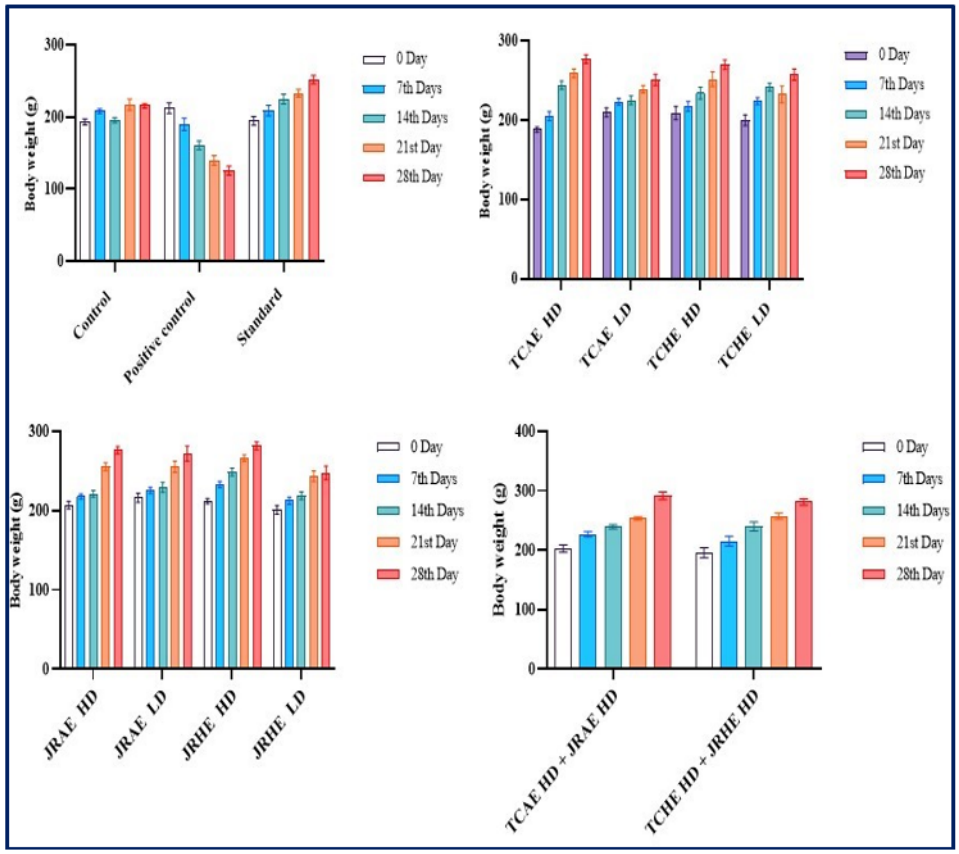

Figure 1: Bar diagram representing changes in body weight among different treatment group along with control, positive control and standard

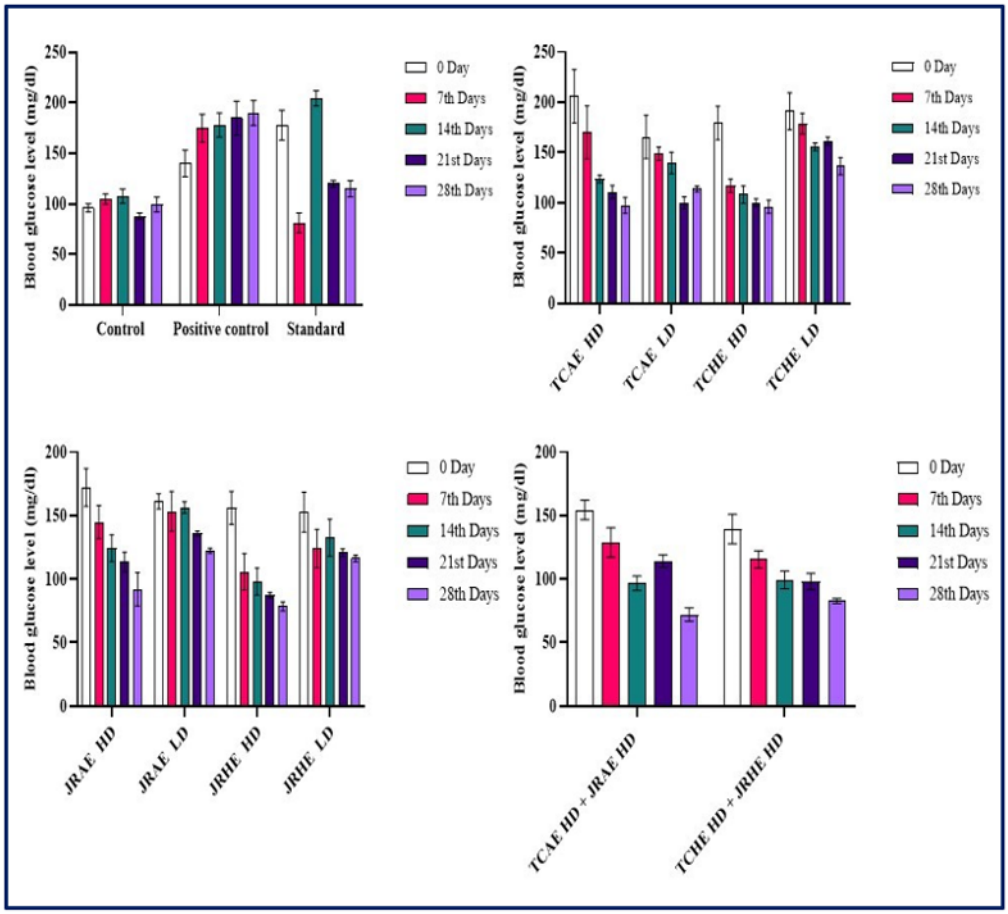

Figure 2: Bar diagram representing changes in blood glucose level among different treatment group along with control, positive control and standard

(H.D) + JRHE (H.D) significantly (P < 0.005) reduced glucose level which was better as compared to standard diabetes group. Changes in blood glucose level is shown in Table 2 and Figure 2 among different groups.

Biochemical profile of alcoholic and hydro-alcoholic extracts

Effect of alcoholic and hydro-alcoholic extracts on lipid profile and enzymatic parameters are mentioned below.

Lipid profile

STZ induced positive control group significantly (P < 0.05) increased TC, TG, LDL, VLDL while decreased HDL level as comparison to control group. Oral admin of TCAE (350mg/kg & 100mg/kg) significantly (P < 0.05) reduced the level of TG, TC, LDL, VLDL and increment the level of HDL as compared with positive control.

However, TCHE (350mg/kg) significantly (P<0.05) reduced the level of LDL, TC VLDL, TG and increased the level of HDL as compared with positive control. And TCHE (100 mg/kg) significantly (P < 0.05) decreased of LDL and increased TC, TG, HDL, VLDL as compared with positive control. JRAE (350 mg/kg, 100mg/kg) did not show significant changes on lipid profile. JRHE (350mg/kg & 100 mg/kg) significantly (P<0.05) reduced the level of TC, LDL and increased the level of TG, HDL, VLDL as compared with positive control.

Composite dose of alcoholic TCAE (H.D) (350 mg/kg) and JRAE (H.D) (800 mg/kg) significantly (P < 0.05) reduced the level of TG, TC, LDL, VLDL and improved the level of HDL as compared to standard diabetic group, while composite dose of hydro-alcoholic TCHE (H.D) (350mg/kg) and JRHE (H.D) (800mg/kg) did not produce significant effect on lipid profile as shown in Table 3 and Figure 3.

Enzymatic parameters

STZ induced positive control group significantly (P<0.05) evaluated the levels of AST, ALT, ALP, albumin, bilirubin as comparison with positive & control group.

Oral admin of TCAE (350mg/kg), TCHE (100mg/kg) dose significantly (P< 0.05) improved the level of ALT, AST, ALP, bilirubin, albumin compared control group.

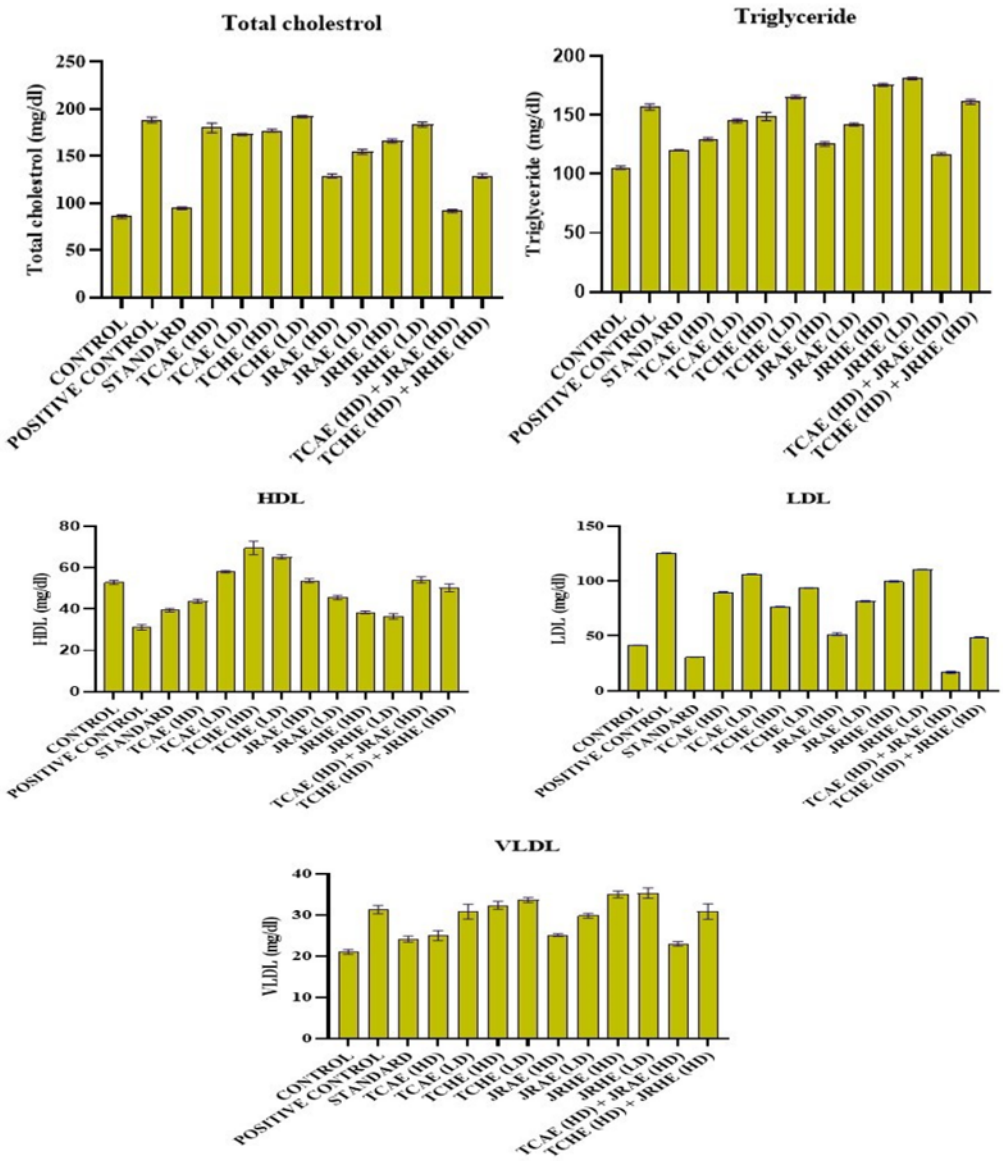

Figure 3: Bar diagram representing changes in lipid profile (among different treatment group along with control, positive control and standard.

TCAE (100mg/kg), TCHE (350mg/kg) dose significantly (P<0.05) improved the level AST, ALT, ALP, bilirubin but albumin was not improved compared to control group. JRAE (800 mg/kg), JRHE (200 mg/kg) dose significantly (P < 0.05) improved the level ALP, albumin, bilirubin and JRAE (800mg/kg, 200mg/kg), JRHE (800 mg/kg, 200mg/kg) significantly (P<0.005) reduced the level of ALP, ALT, AST, bilirubin but albumin level increased. When compared with positive control group. Combination dose of alcoholic TCAE (H.D) (350 mg/kg) + JRAE (H.D) (800 mg/kg), Hydroalcoholic TCHE (H.D) (350 mg/kg) + JRHE (H.D) (800mg/kg) significantly (P<0.05) reduced the level of albumin, bilirubin, ALP, ALT and AST when compared with positive control group as shown in Table 4 and Figure 4.

Histopathology of Liver, Kidney and Pancreas.

Liver

Histopathological profile of liver necrosis and fibrotic changes with conspicuous evidence of fatty deposits in the diabetic liver when comparison with normal control group presenting the central vein with radiating cords of hepatocytes. Glibenclamide group shows normal portal tract (PT). TCAE high dose 350 mg/kg group also shows evidence of swelling and deposits of fats. TCAE Low dose 100 mg/kg group shows minor necrosis and fibrotic changes. TCHE High dose 350mg/kg group shows also minor necrosis and fibrotic changes. TCHE low dose 100mg/kg group shows evidence of hypertrophy and disarrangement of hepatic parenchyma h. JRAE high dose 800 mg/kg group shows the

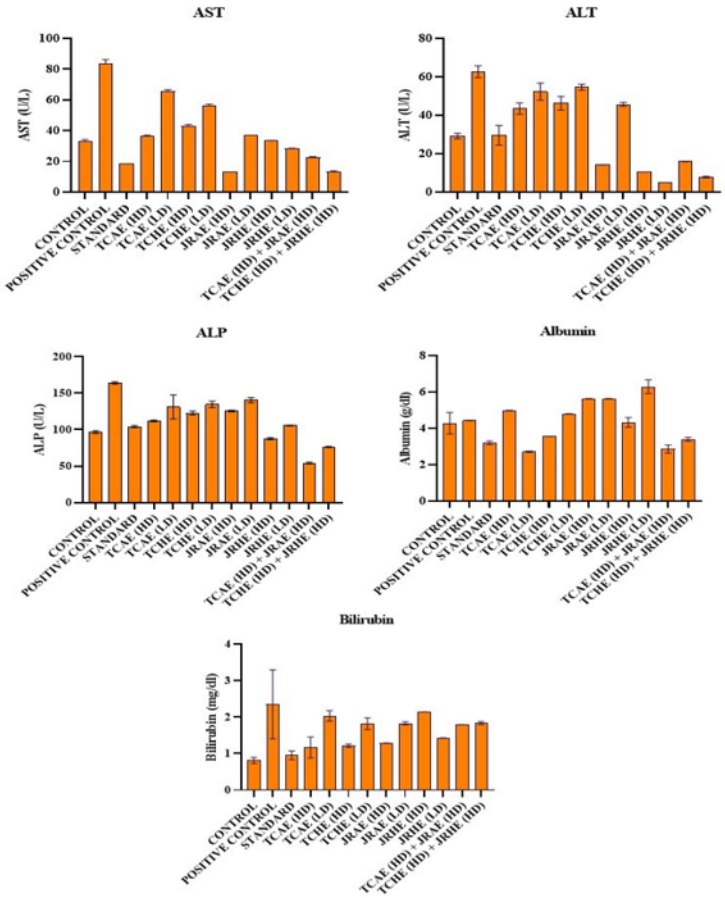

Figure 4: Bar diagram representing changes in liver enzymatic activity among different treatment group along with control, positive control and standard

evidence of cell swelling and congestion. JRAE low dose 200 mg/kg group shows portal veins with mild hemorrhage. JRHE high dose 800 mg/kg group shows normal features. JRHE low dose 200 mg/kg group shows also minor necrosis and fibrotic changes. Composite of TCAE 350mg/kg + JRAE 800mg/kg group shows normal cells. Composite of TCHE 350mg/kg + JRHE 800mg/kg group shows mild swelling on hepatic cells with evidence of hypertrophy. Show in Figure 6

Kidney

we found that the positive control group had necrosis and damage epithelium cells and proteinuria when compared with control group showing proximal convoluted tubules and glomeruli. Standard group showed degeneration in tubular epithelium cells and deterioration of the glomerular structure. Impact of TCAE, TCHE, JRAE, JRHE, Glibenclamide on obsessive variation in glomeruli. The segments 3-5 μM in fatness were recoloured & made with eosin & hematoxylin to survey the neurotic variation of glomeruli utilizing by light microscopy (400 X). Control group shows proximal convoluted tubules and glomeruli. Positive control group shows necrosis and damage epithelium cells and proteinuria. Glibenclamide 5 mg/kg shows degeneration in tubular epithelium cells and deterioration of the glomerular structure. Treatment TCAE high dose 350mg/kg shows decreased the pathological variation in glomeruli by increasing glomerular size comparison to treatment TCAE low dose 100 mg/kg. Treatment TCHE 350 mg/kg show also degeneration and deterioration of glomerular structure compared to the positive control group. Treatment TCHE 100mg/kg shows mild necrosis and damage epithelium cells compared to TCAE 100 mg/kg. Treatment JRAE 800 mg/kg decreased the pathological changes in glomeruli atrophy. Treatment JRAE 200 mg/kg shows degeneration and deterioration of glomerular structure. Treatment JRHE 800 mg/kg shows the pathological variation of glomeruli. Treatment JRHE 200 mg/kg shows thickening of renal vesicles and fibrotic changes of the glomeruli. Treatment TCAE 350 mg/kg + JRAE 800mg/kg shows decreased the pathological variation in glomeruli by increasing the glomerular capillary size. Treatment TCHE 350 mg/kg + JRHE 800

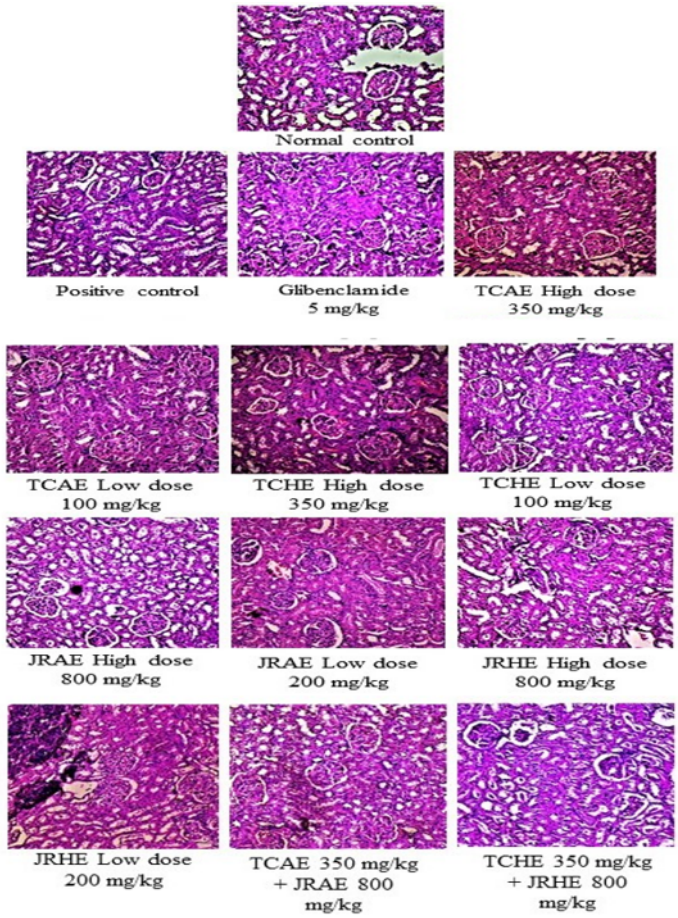

Figure 5: Representative images showing changes in rat kidney tissue of control, positive control, standard and all treatment groups.

mg/kg shows glomerulus have suffused RBC's with mild necrosis of epithelium cells. Show in Figure 5

Pancreas

Histological changes in islets of Langerhans in pancreas after treatment with streptozotocin. Control bunch demonstrating ordinary engineering of the pancreas. The exocrine segment types of pancreas firmly pressed by acinar cells and masterminded into little lobules. The islets showed up softly recoloured than the encompassing acinar cells. Positive benchmark group uncovered neurotic changes of both exocrine and endocrine parts.

The acinar cells were swollen and little vacuoles were seen in practically all acinar cells. Islet β-cells are primarily lost in STZ-rewarded rodents. Glibenclamide indicating bending of the overall design. Most exocrine acini uncovered acinar harm spoke to by cytoplasmic vacuolation and cell decay. Wider interlobular and intralobular duct were observed. Treatment TCAE 800 mg/kg demonstrated an ordinary structure of islets of Langerhans. Atrophic difference in the acinar cells was less serious and the fringe among exocrine and endocrine parts turned out to be progressively unmistakable.

Treatment TCAE 100 mg/kg showed the small vacuoles in the basal area of acinar cells small. Treatment TCHE 350 mg/kg showed improved in size of islets of Langerhans. Treatment TCHE 100 mg/kg showed normal pancreatic islets. Treatment JRAE 800mg/kg presented mild improved in the size of islets of Langerhans. Treatment JRAE 200 mg/kg showed mild fibrosis and inflammatory cell infiltration into the islets of Langerhans. Treatment JRHE 800 mg/kg showed decreased islets size and decreased β- cells number.

Treatment JRHE 200 mg/kg showed irregular islets shape with degenerated entering connective tissue sheet. Treatment TCAE 350 mg/kg + JRAE 800 mg/kg showed marked improvement with restored size of islets of Langerhans & decrease in a number of β-cells. TCHE 350 mg/kg + JRHE 800 mg/kg showed regular islets cells with increased number and abundant eosinophilic cytoplasm and central small nuclei & decreased a number of β-cells. Show in Figure 7

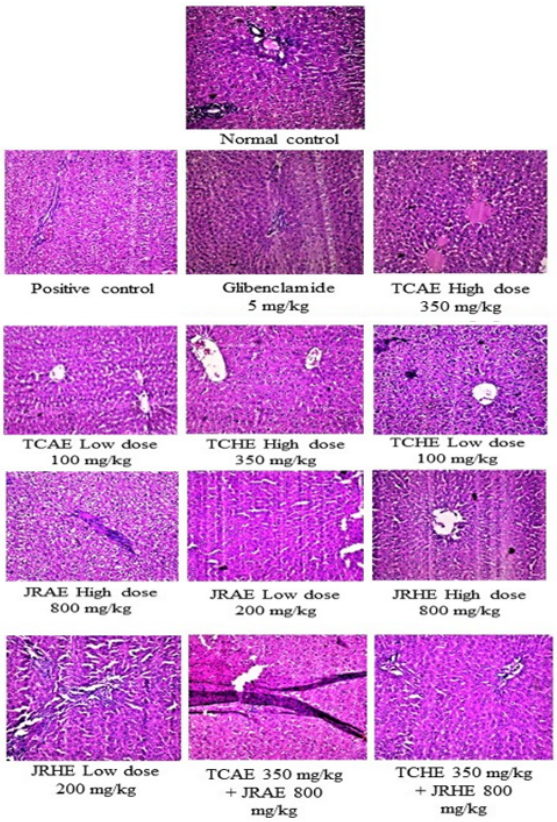

Figure 6: Representative images showing changes in rat liver tissue of control, positive control, standard and all treatment groups

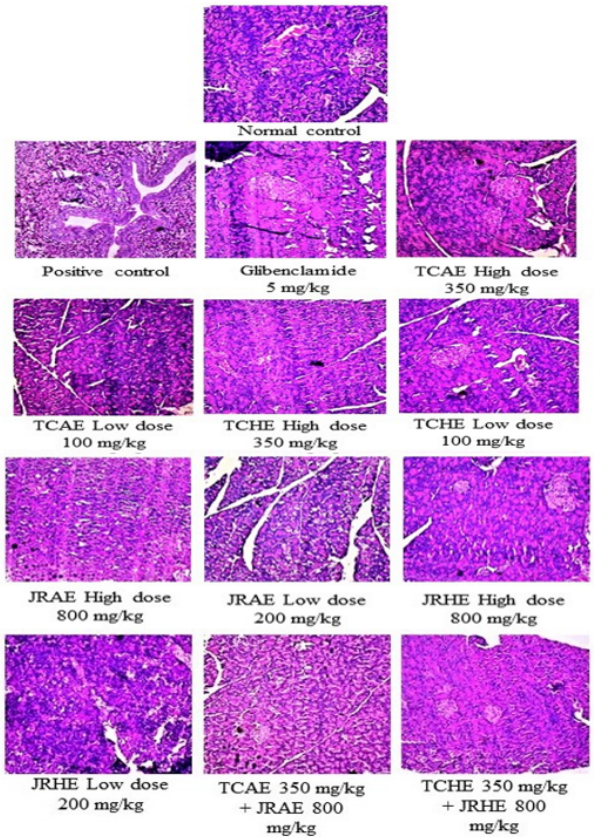

Figure 7: Representative images showing changes in rat pancreas tissue of control, positive control, standard and all treatment groups

DISCUSSION

Diabetes mellitus is a metabolic disorder that influences individuals of all age gatherings and from varying backgrounds. The executives of diabetes with no symptoms is as yet a test in the clinical field, as by and by accessible medications for diabetes have at least one antagonistic impact. Since the current medications for the treatment of diabetes mellitus do not satisfy our need totally, the quest new medications continue (Shobha, 2015). Other than commendable improvement in the field of allopathic drugs, the herbal drug additionally has their radiant importance as they have no side effect not at all like allopathic medications. Therefore, an efficient approach has been done to find out the utility of *Tinospora cordifolia* and *Juglans regia* in composite against diabetes. Review of literature *Tinospora cordifolia* revealed that this plant various pharmacological activities like - diabetes, microbial, obesity, inflammatory, pain-relieving, depressant, oxidant, cancer etc. (Tripathi *et al.*, 2011). and *Juglans regia* revealed that this plant possesses pharmacological activities like diarrhea, stomachache, arthritis, asthma, eczema, skin disorders, and various endocrine diseases such as diabetes mellitus, thyroid dysfunctions, anorexia, cancer and infectious diseases etc. The Present study was title to evaluated the antidiabetic activity of alcoholic and hydroalcoholic extracts of stem of *Tinospora cordifolia* and kernel of *Juglans regia* in STZ induced diabetes in wistar rats for 28 days.

Its Lipid profile, enzymatic activity was investigated on 28 days. And histopathological study was examined in the kidney, liver, pancreas.

Data analysis of this present study revealed that the following groups such as all treated groups improved body weight but body weight increased the most in the combination group, normalized the blood glucose level and restored the lipid profile as well as liver biochemical parameters towards the normal range. Our finding revealed that all treated group had a positive effect in lowering the blood glucose and body weight on 28th day as compared to the initial observation on 0th day. A previous study had shown that the methanolic extract of stem *Tinospora cordifolia* 250mg/kg.b.w. Decreased the blood glucose level and activities of glucose-6-phosphatase, fructose-1,6disphosphatase (Rajalakshmi *et al.*, 2009). Previous study showed that

Tinospora cordifolia has significant (P < 0.05) antidiabetic activity in diabetic animals and has viability of 40% to 80% compared to insulin. *Tinospora cordifolia* administration in diabetic animals' recovery of pancreatic β cells but showed increased hepatic glycogen synthase and decreased glycogen phosphorylase activity (Ekambaram *et al.*, 2010). Alcoholic extract of *Juglans regia* has protective effect on streptozotocin-nicotinamide induced diabetic rats. Streptozotocin-nicotinamide injection can give rise diabetes mellitus, and ruin the β-cells in the islets of Langerhans. The increase of blood glucose could damage β cells and lead to reduction in consumption of glucose by muscle tissues. Alcoholic extract of *Juglans regia* (400mg/kg/b.w.) decreased the blood glucose level and regenerate of β cells (Puranik *et al.*, 2010).

In our study alteration in lipid profile such as HDL, LDL, VLDL, triglycerides and total Cholesterol was observed on 28th days in diabetic rats when compared to treatment and standard group. Elevated cholesterol, LDL, VLDL and HDL were normalized in diabetic rats after the treatment of some treated groups did not produce significant effect on lipid profile. The previous study of (Kannadhasan and Venkataraman, 2012) had shown that the ethanolic extract of *Tinospora cordifolia* (1000 mg/kg) reduced the total cholesterol, triglyceride level near to the normal and increased in HDL and more significantly decrease of LDL compared with diabetes control (Kannadhasan and Venkataraman, 2012). Ethanolic extract of *Tinospora cordifolia* (200mg/kg/b.w.) The levels of total cholesterol, triglycerides LDL-C and VLDL-C were increased in diabetic rats whereas the level of HDL-C were reduced in diabetic rats when compared to the control normal rats. Administration of Ethanolic extract of *Tinospora cardifolia* to alloxon induced diabetic rats restored all these changes to near normal levels by reduction of the level of total cholesterol, triglycerides, LDLC and VLDLC of diabetic rats and significant increase in the level of HDL-C (Ravikiran *et al.*, 2015).

Extracts of stem *Tinospora cordifolia* and kernel *Juglans regia* not only decrease the level of triglyceride and total cholesterol but also the restore the function of the hepatic enzyme by inhibition of oxidative stress. Study by (Bostani *et al.*, 2014) had shown that the ethanolic extract of leaf *Juglans regia* (200mg/kg) decreased the serum AST and ALT level compared to diabetes control group. Elevation of renal enzymes such as AST, ALT, ALP, Albumin, and Bilirubin was also evaluated in diabetic rats pointed to trained liver function due to hepatic damage (Khedekar, 2016). Treatment with herbal formulation at the dose of 200 and 400 mg/kg/day along with alcohol showed reduced levels of SGPT, SGOT, albumin, creatinine and increased the levels of total protein as compared with alcohol treated group (Sreshta *et al.*, 2018). 28 days treatment with TCAE and TCHE at (350mg/kg/b.w.) and (100 mg/kg/b.w.), JRAE and JRHE at (800mg/kg/b.w.) and (200mg/kg/b.w.) and composite of TCAE (HD) + JRAE (HD) and TCHE (HD) + JRHE (HD) restored al the biochemical parameters toward the normal levels.

Histopathology, kidney section of STZ induced diabetes mice showed damage epithelium cells, proteinuria and necrosis, while the extract of *Tinospora cordifolia* (50, 100, 200 mg/kg/b.w) normal tubules with congested glomerulus, and showed congested glomerulus and tubules with vacuolated epithelial cells (Gupta and Sharma, 2011). Which was similar to the extracts of *Tinosora cordifolia*, *Juglans regia* and their combination. Liver section of STZ induced diabetic rats showed necrosis and fibrotic changes with conspicuous evidence of fatty deposits, which got restored by extract of *Tinospora cordifolia*, *Juglans regia* and their composite treatment. The extract of *Tinospora cordifolia* produced protective effects in the kidney against lead toxicity (Sharma and Pandey, 2010). The extract of *Tinospora cordifolia* (100mg/kg) changed in the centrilobular region and vacuolar congestion and graded 1-2 by hepatoprotective system (Gurav *et al.*, 2017). Pancreas section of STZ induced diabetes rats showed damaged pancreatic islets cells and the extract of *Tinospora cordifolia* comparatively less degeneration of Islets of Langerhans and degranulation (Sreshta *et al.*, 2018) which was also similar to our finding.

CONCLUSIONS

Taken together, the present study for the first time reported the effect of *Tinospora cordifolia* and *Juglans regia* (Alcoholic and Hydroalcoholic) and their composite extract in STZ induced diabetes rats. Interestingly, improvement in body weight, glucose level, lipid profiles, biochemical parameters and histopathological changes in kidney, liver and pancreas was observed following herbal treatment in STZ induced diabetic rats. Furthermore, composite extract of TCAE (350mg/kg b.w.) and JRAE (800mg/kg b.w.) was found to be more efficacious than the composite extract of TCHE (350mg/kg b.w.) and JRHE (800mg/kg b.w.).

ACKNOWLEDGEMENT

Authors are thankful to KIET Group of Institutions, Delhi-NCR, Ghaziabad and KIET School of Phar-

macy for providing all the necessary requirements to carry out the research work.

Conflict of interest

The authors declare that they have no conflict of interest for this study.

Funding support

The authors declare that they have no funding support for this study.

REFERENCES

Biswas, P., *et al.* 2015. The antistress activity of Tinospora Cordifolia with Application of Yoga. *International Journal of Ayurvedic Medicine*, 6(3):220–224.

Bostani, M., Aqababa, H., *et al.* 2014. A Study on the effects of walnut oil on plasma levels of testosterone pre and post-puberty in male rats. *Am J Ethnomedicine*, 1(4):266–275.

Chougale, A. D., *et al.* 2009. Alpha glucosidase inhibition by stem extract of Tinospora cordifolia. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 24(4):998–1001.

Ekambaram, S., *et al.* 2010. Evaluation of antiarthritic activity of Strychnos potatorum Linn seeds in Freund's adjuvant induced arthritic rat model. *BMC Complementary and Alternative Medicine*, 10(1).

Gupta, R., Sharma, V. 2011. Ameliorative effects of Tinospora cordifolia root extract on histopathological and biochemical changes induced by aflatoxin-B1 in mice kidney. *Toxicology International*, 18(2):94–98.

Gurav, A., *et al.* 2017. Evaluation of hepatoprotective and antidiarrhoeal activity of guduchi, Tinospora cordifolia (Willd.) Miers ex Hook. f. & Thoms. in experimental rats. *Annals of Phytomedicine: An International Journal*, 6(2):156–161.

Kannadhasan, R., Venkataraman, R. 2012. Antidiabetic and Antihyperlipidaemic activity of sedimental extract of Tinospora cordifolia in Streptozotocin-induced Type 2 diabetes. *Int J Pharm Pharm Sci*, 4(3):520–527.

Khedekar, S. B. 2016. Anti-diabetic activity of dried extract of Tinospora cordifolia (Guduchi Ghana) and honey in streptozotocin-induced streptozotocin-diabetic rats. *International Journal of Green Pharmacy (IJGP)*, 9(4).

Lakhtakia, R. 2018. The History of Diabetes Mellitus. *Sultan Qaboos University Medical Journal*, 13:368–370.

Mishra, P., *et al.* 2014. Phytochemical analysis and assessment of in vitro antibacterial activity of Tinospora cordifolia. *International Journal of Current Microbiology and Applied Sciences*, 3(3):224–234.

Mohammadi, J., *et al.* 2012. The effect of hydroalcoholic extract of Juglans regia leaves in streptozotocin-nicotinamide induced diabetic rats. *Pak J Pharm Sci*, 25(2):407–411.

Naidoo, P., *et al.* 2014. Glibenclamide in patients with poorly controlled type 2 diabetes: a 12-week, prospective, single-center, open-label, dose-escalation study. *Clinical Pharmacology: Advances and Applications*, 6:63–63.

Puranik, N., *et al.* 2010. Anti-diabetic activity of Tinospora cordifolia (Willd.) in Streptozotocin diabetic rats; does it act like sulfonylureas? *Turkish Journal of Medical Sciences*, 40(2):265–270.

Rajalakshmi, M., *et al.* 2009. Anti-diabetic properties of Tinospora cordifolia stem extracts on streptozotocin-induced diabetic rats. *African journal of pharmacy and pharmacology*, 3(5):171–180.

Ravikiran, T. N., *et al.* 2015. Evaluation of anti-hyperglycemic and hypolipidemic activities of ethanolic extract of Tinospora cardifolia whole plant in alloxan-induced diabetic rats. *Der Pharmacia Lettre*, 7(3):108–113.

Riaz, S. 2009. Diabetes mellitus. *Scientific Research and Essay*, 4(5):367–373.

Sharma, V., Pandey, D. 2010. Protective role of Tinospora cordifolia against lead-induced hepatotoxicity. *Toxicology International*, 17(1):12–12.

Shobha, K. 2015. G Patil Antidiabetic Activity of Tinospora Cordifolia (FAM: Menispermaceae) in Alloxan Treated Albino Rats. *Applied Research Journal*, 1(5):316–319.

Sreshta, B., *et al.* 2018. Evaluation of Hepatoprotective activity of polyherbal Formulation Against Ethanol Induced Hepatotoxicity in rats. *World Journal of Pharmacy and Pharmaceutical Sciences*, 7(5):1390–1399.

Stratton, I. M., *et al.* 2000. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): a prospective observational study. *Bmj*, 321(7258):405–412.

Tripathi, P., *et al.* 2011. Alternative therapies useful in the management of diabetes: A systematic review. *Journal of Pharmacy and Bioallied Sciences*, 3(4):504–504.