Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 2206-2212
Received: 14-03-2019
Accepted: 15-04-2019

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Evaluation of the anti-diabetic activity of ethanol extract of leaves of *Scurrula parasitica* in streptozotocin-induced diabetic rats

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Abstract
The current study investigated antihyperglycemic, hypolipidemic, GCMS analysis of ethanolic leaf extract of *Scurrula parasitica*. A total of 30 albino rats (5 groups, each having 6 rats) weighing around 150 to 200gm were selected injected with streptozotocin to increase the glucose level. After the induction of diabetes, rise in blood glucose level and elevation of biochemical parameters such as High-density lipoprotein (HDL), Low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), Total Cholesterol (TC), Triglyceride (TG), SGOT, SGPT, Alkaline phosphate and lowering of HDL was observed. The rats were treated with ethanol extract of *Scurrula parasitica* and compared with metformin, which is used as standard drug. The plant extract was given at 100 and 200mg/kg body weight. After administration with the plant extracts, significant lowering of blood glucose level and lipid serums with rise of HDL serum cholesterol was observed when compared to the diabetic control group after the 21st day. 5 anti-diabetic compounds were identified in the GC-MS analysis of the ethanolic extract of the leaves of *Scurrula parasitica*. This research illustrates that the parasitic plant *Scurrula parasitica* has antihyperglycemic and hypolopidemic properties and can further be subjected to drug formulation to isolate a compound, which can be used for the treatment of diabetes.

Keywords: *Scurrula parasitica,* streptozotocin, anti-hyperglycemic activity, GC-MS

Introduction
Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and lack of secretion or action of endogenous insulin. Although the cause of this disease is not clear, viral infection, autoimmune disease and environmental factors have been implicated (Sandler *et al.*,2000; Shewade *et al.*,2001) [24, 26]. Diabetes mellitus consist of two type's viz. Type I and Type II. Type II diabetes being the more frequent form, constituting over 90% of the diabetic population. Over the past two decades, the number of individuals having diabetes is believed to have been rising steadily with a high mortality rate in India. The rate of recurrence of this disease is ascending globally and is expected to hit 300 million by 2025 with India likely to be the highest prevailing country of diabetic occurrences. Although medical heritage is century's old, natives in rural areas are still relying on herbal medicines to gather their healthcare needs. For the tribal people, herbal medicines have always been favored instead of synthetic drugs as they have no side effects or adverse reactions and are readily accessible to collect while still being harmonious with the ecological system. Over the last century, verification of the lipid-lowering property of medicinal plants has been documented (Kritchevsky 1995) [17]. Researchers have verified the role of medicinal plants in the control of hyperlipidemia. Metabolic syndrome (Mets) consist of of endocrine/metabolic conflict characterized by type 2 diabetes mellitus (T2DM) due to insulin resistance and impaired glucose regulation, hypertension, obesity, and altered lipid profile consisting of elevated levels of triglyceride (TG) and low levels of high-density lipoprotein cholesterol (HDL-C) (Maurya *et al.*,2012) [19]. *Scurrula parasitica* is an herbaceous growing shrub of the family Loranthaceae. They are found growing on *Dendrophthoe falcata* and *Mangifera indica* (Bambaradeniya *et al.*, 2001; Weeraratna *et al.*,1960) [4, 30]. *Scurrula* consists of about 91 species. Majority of which are found in South East Asia, Malaysia and China along with small number in India and Australia. Loranthaceae consists of about 900 species and 75 genera, majority of which are found in the southern regions of India. Loranthaceae has spread into all kinds of woody habitat and several species have developed into exceptionally specialized parasites of a specific host, occasionally occurring only on other mistletoes (Kirkup *et al.*,2000) [16]. Traditionally it is used as a diuretic, tranquilizing and hypotensive drug. In south western regions of China, *Scurrula parasitica* are considered as important ethno-medicine and are mainly used as shock therapy for the treatment of schizophrenia (Soheil *et al.*,2014) [27].

The plant also displays anticancer, antidiabetic, antihepatotoxic, antioxidant, immunomodulatory and cytotoxic activity (Mahajan *et al.,* 2013) [18]. Previous investigation have confirmed the presence of significant phytochemicals like lectins, 4-O-acetylquercitrin, viscotoxins, alkaloids, flavonoids, amines, terpenoids, icariside, aviculin, oleanolic acid, lupeol, quercetin, catechin, rutin etc. from *Scurrula parasitica* (Mahajan *et al.,* 2013; Nilesh *et al.,* 2013) [18, 21]. Mass spectrometry, together with chromatographic separations like Gas chromatography (GC/MS) is generally used for direct investigation of components present in ethno-medicinal plants and traditional drugs. Important constituents like fatty acids, nonpolar components, volatile essential oil and lipids in plants are analyzed by using GC-MS technique (Jie and Choi, 1991; Betz *et al.,* 1997) [14, 5]. Over the years, numerous publications have been reviewed; focusing on herbal products and ethno-medicinal plants with anti-diabetic action (Hays *et al.,* 2008) [12]. The current investigation was aimed to carry out the in vivo anti-diabetic activities of *Scurrula parasitica* in streptozotocin-induced diabetic rats and GC-MS analysis to categorize the compounds which are effective in lowering of blood glucose level, which may be useful in production of anti-diabetic drugs and for its therapeutic values. The effects of Scurrula parasitica on body weight, blood glucose level, the lipid profile of rats were considered for the in vivo anti-diabetic research.

Materials and methods

Preparation of ethanol extract of *Scurrula parastica* (ESP)

Scurrula parasitica was collectd from Kolasib district (24^0 13' 52"N $92^0$40'34"E/ 24.23111^{0}N 92.67611^{0}E), in March 2017. The identification of plant was done at Botanical Survey of India, Shillong (No: BSI/ ERC/ Tech/ 2017/ 43) and the herbarium was deposited and authenticated as voucher number MZU 742 in the Department of Environmental science, Mizoram University.

The leaves of the plant were shade dried and prepared into coarse powder and stored in a beaker. Approximately, 200gm of the plant powder was weighed and subjected to continuous hot extraction using Soxhlet apparatus. The extraction was carried out successively using petroleum ether, chloroform, and ethanol. Subsequently, the extracts were evaporated under pressure using rotary evaporator until all the solvents have evaporated to give pure crude extracts. The ethanol extract will be used to carry out the experiment. The percentage yield of ethanol extract was 3.8% w/w per one extraction.

Animals

Male Albino Wistar rats of body weight 190 to 200g were selected for this research. The animals were kept in an animal house at the Department of Pharmacy (RIPANS), (IAEC approval. No. IAEC/RIPANS/18, dated 14th November 2017) with a12 hours dark: 12hours light cycle. The animal was fed a pellet diet (Pranav Agro-industries, Vadodara, Gujarat), water and *ad libitum* were also provided (Upwar *et al.,* 2010) [29].

Phytochemical screening

The following preliminary tests were performed for identifying different chemical groups as reported by Trease and Evans 1983 [28].

Induction of streptozotocin on experimental diabetes

STZ-induced diabetes has been illustrated as a constructive investigational model to study the action of hypoglycemic activity (Junod *et al.,* 1969) [15]. The induction was done using the technique given by Upwar *et al*. (2011) [29]; with slight alteration. Following an overnight fasting (deprived of food for 16 hours had been allowed free access to water). The induction of diabetes in rats was completed by intraperitoneal injection of STZ dissolved in 0.1M sodium citrate buffer pH 4.5 at a dose of 40mg/kg body weight. Then, after 72 hours, rats with moderate diabetes (fasting blood glucose >250 mg/dl) were employed for the study. Testing by urine test strips (One touch select, Bayer diagnostics Ltd, India) were considered as diabetic.

Selection of Doses

Acute toxicity studies were carried out following the guidelines of OECD by different doses of leaves of *Scurrula parisitica* extract which showed no toxic effects up to 500mg /kg body weight. For the evaluation of hypoglycemic activity, two dose levels were chosen in such a way that, one dose was just about one-tenth of the maximum dose during acute toxicity studies and a high dose, which was twice that of one-tenth dose (100mg/kg and 200mg/kg).

Experimental design

In the experiment, the rats were divided into 5 groups with six animals in each group.

Group I: Normal control rats.

Group II: Diabetic control rats

Group III: Diabetic rats given Metformin (250 mg/kg b.w./Rat/day) for 21 days.

Group IV: Diabetic rats given MLS (200 mg/kg b.w./Rat/day) for 21 days.

Group V: Diabetic rats given MLS (100 mg/kg b.w./Rat/day) for 21 days.

Blood samples were collected at 0 hr (prior to the administration of the extract) on the 5th, 10th, 15th and 20th day, for estimation of blood glucose level with the help of Glucometer (One Touch Select) and readings were tabulated. The weight and serum biochemical parameters were monitored on the first and final day of the treatment. After the 21st day, the rats were sacrificed under mild anesthesia. The biochemical parameters were monitored using Auto-analyzer (EM 200).

Blood collection and serum separation

The tip of the tail was cut and few droplets of blood was collected for estimation of glucose level (Maurya *et al.,* 2012) [19] and for serum biochemical parameters estimation, the blood samples were collected from 8hours fasted animals from the retro-orbital plexus in capillary tubes (Micro Hemocrit capillary, Mucaps) and serum was separated within 30 minutes after collection using centrifuge at 2000 rpm for 2 min Upwar *et al.,* (2011) [29].

GC - MS Analysis

Gas Chromatography-Mass Spectrometry using JEOL GCmate™ II GC/MS Double-Focusing Mass Spectrometer. An HP-5 MS capillary column (28 m × 0.25 mm × 0.25 μm) with helium as carrier gas (1.0 ml/min) was used for the gas chromatographic separation. The injection mode was split (split ratio: 20:1), injection volume was 2 μL, and the temperature of the vaporization chamber ranged from 50°-250°C. The column was eluted with ethanol at a flow rate of 0.3ml/min with an increase of 10°C every min. The mass detector was EI-Detection (70 eV). The mass spectra of the

compounds from compared with the NIST08 GC-MS database library with 346,757 Kovats retention index values for 70,835 compounds (38,648 in the EI library), covering both polar data.

Statistical analysis

Results were expressed as Means±SD and the variations between the groups was tested by two-way analysis of variance (ANOVA) followed by the Tukey multiple mean comparison test using the software "GraphPad Prism v6". The $p<0.05$ were considered as statistically significant.

Results

The streptozotocin-induced rat revealed the increased in the level of blood sugar. All the treatment groups showed the declined in the level of blood glucose from 5th day till 21st day when compared to streptozotocin alone. All the treatment groups showed no statistical difference at day 10 and15 and 21st ($p<0.001$) post-treatment.

Table 1: Effect of ESP on blood glucose levels in STZ-induced diabetic rats

Time (Day)	Group I (Normal rat)	Group II (Diabetic rat)	Group III (Metformin)	Group IV (High dose)	Group V (Low dose)
0	88.3 ± 2.55	311.8 ± 2.42	312.8 ± 2.30	318.1±2.66	320.0±2.88
5	85.0 ± 4.48	354.5 ± 2.96	237.0± 1.57[b]	262.5±2.33[b]	338.3±4.42[a]
10	85.5 ± 1.86	393.7 ± 3.53	214.8± 2.14[b]	176.0±3.56[b]	290.0±4.30[b]
15	83.7 ± 2.69	392.0 ± 3.92	179.0 ± 3.53[b]	171.8±3.71[b]	190.6±2.40[b]
21	88.7 ± 2.93	395.5 ± 2.63	124.8 ± 2.39[b]	159.8±2.77[b]	169.8±2.91[b]

All values are Mean ± SEM; N=6. [a]$P<0.01$ when compared with diabetic control. [b]$P<0.001$ when compared with diabetic control. [1]$P<0.05$ when treatment group III & IV compared with standard (metformin).

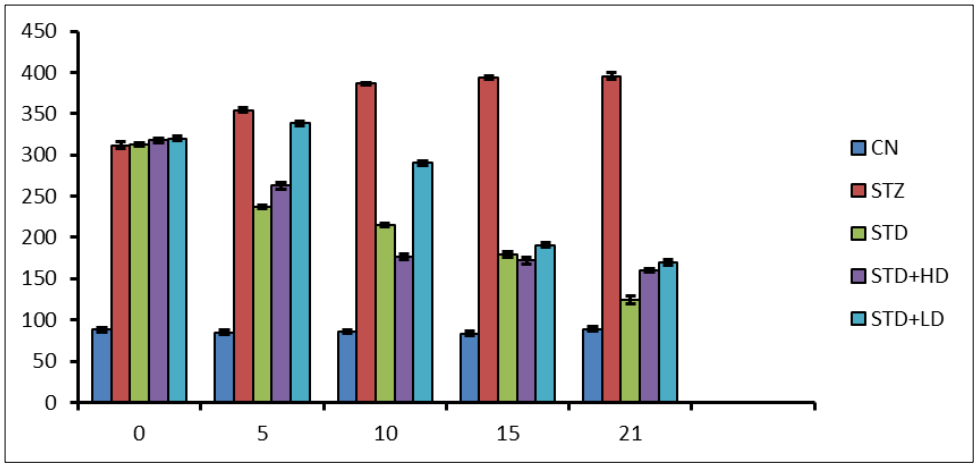


Fig 1: Effect of ESP on blood glucose levels in STZ-induced diabetic rats

Table 2: Effect of ESP extract on changes in body weight in normal and experimental rats

Time (Day)	Group I	Group II	Group III	Group IV	Group V
0 Day	199.33 ±6.21	193.83 ± 3.25	195.33 ± 5.00	192.50±4.18	198.16 ±3.31
21st Day	207.16 ± 4.42	183.50 ± 4.84	204.16 ± 3.86	197.66±4.50	201.83 ±3.65

All values are Mean ± SEM; N=6. There is no statistical difference in the body weight between 0 days and a 21st day or among groups.

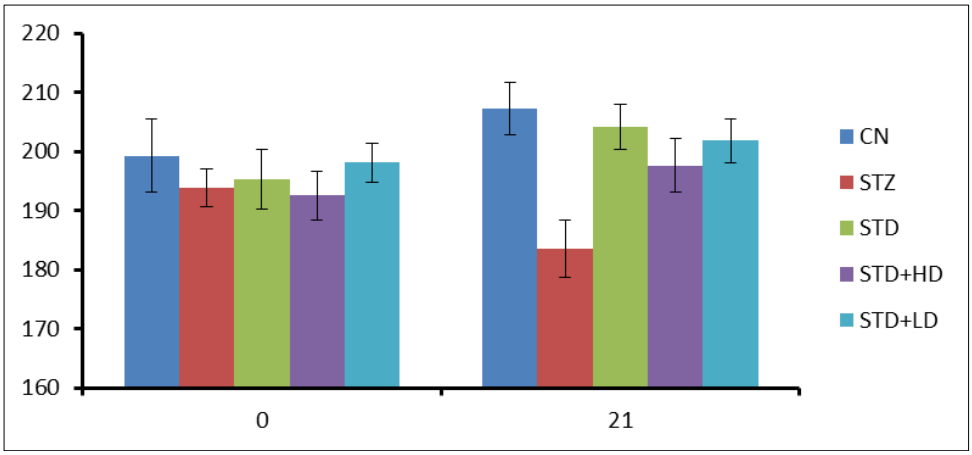


Fig 2: Effect of ESP on body weights on STZ-induced diabetic rats

Table 3: Effect of ESP on Triglyceride, Cholesterol, HDL-Cholesterol, and LDL-Cholesterol

Group	Triglyceride (mg/dl)		Cholesterol (mg/dl)		HDL-Cholesterol (mg/dl)		LDL-Cholesterol(mg/dl)	
	0 Day	21st Day	0 Day	21st Day	0 Day	21st Day	0 Day	21st Day
Group I	90.9±2.67	95.7±0.49	76±3.77	84.2±3.25	30.4±1.04	33.4±1.44	11.4± 2.57	10.3±2.80
GroupII	152.6±0.59	187.2±4.12	75.8±4.08	123.6±4.06	36.4±3.72	37.8±1.73	22.2±1.08	25.3±3.54
Group III	104.8±1.16	76.9±2.42[a]	95.5±3.76	73.8±4.58[a]	62.7±2.26	65.7±1.83	16.9±3.71	13.6 ±2.41[c]
Group IV	120.2±2.28	98.3±2.34[a]	118.6±2.81	102.3±3.34[a]	63.5±2.83	62.9±2.91	15.8±2.21	14.7±1.54[c]
Group V	140.5±4.70	122.8±3.83[a]	128.7±3.43	118.4±4.26[a]	61.4±3.32	64.4±2.94	16.0±1.14	15.1±2.73[c]

All values are Mean ± SEM. [a]*P*<0.001significantly decrease when compared with control and compared with 0 day and 21st day among all treatment groups. [b]*P*<0.01 compared with 0 day and 21st day among all treatment groups. There was no statistically significant change among groups in HDL-Cholesterol level but there was significantly declined in the LDL-Cholesterol among groups ([c]*P*<0.05), N=6.

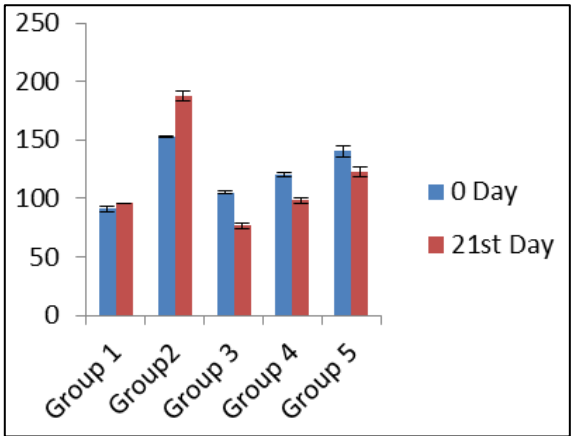


Fig 3: Effect of ESP on serum Triglyceride

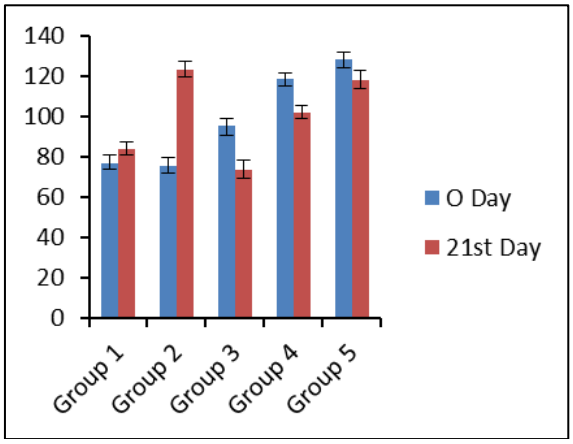


Fig 4: Effect of ESP on Serum Cholesterol

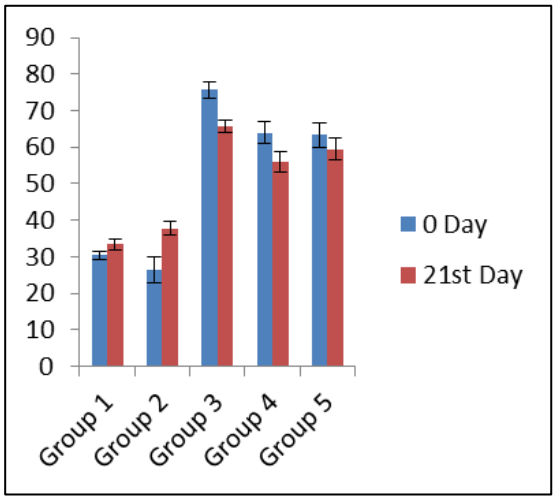


Fig 5: Effect of ESP on serum HDL

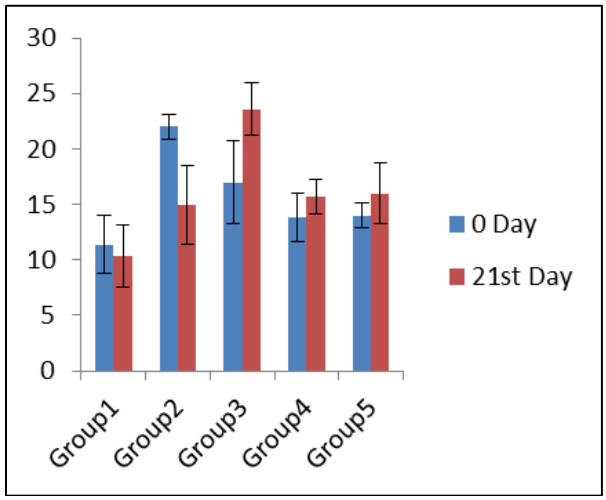


Fig 6: Effect of ESP on serum LDL

Table 4: Effect of ESP on serum SGPT, SGOT and ALP

Group	SGPT(U/L)		SGOT(U/L)		ALP(U/L)	
	0 Day	21st Day	0 Day	21st Day	0 Day	21st Day
Group I	55.9 ± 2.33	48.1 ± 2.79	95.7 ± 1.75	93.8 ± 2.14	91.8 ± 4.09	89.4 ± 1.25
Group II	54.1 ± 3.68	99.3 ± 1.33	103.3 ± 2.38	123.5 ± 4.05	84.7 ± 3.34	117.3 ± 4.65
Group III	57.5 ± 1.74	31.6 ± 1.23[a]	101.8 ± 0.95	95.4 ± 1.31[b]	98.4 ± 1.52	93.7 ± 1.17[a]
Group IV	71.48±4.7	70.7±4.46[b]	105.9±4.43	97.3 ±2.75[b]	95.0 ±4.38	85.6±3.75[b]
Group V	68.73±1.73	65.17±1.38[b]	107.5 ± 3.30	99.4 ± 1.41[b]	91.7 ± 4.73	84.8 ± 3.96[b]

All values are Mean ± SEM. [a]*P*<0.001 significantly decrease when compared with control and compared with 0 day and 21st day among all treatment groups. [b]*P*<0.01 compared with 0 day and 21st day among all treatment groups. [c]*P*<0.05,N=6.

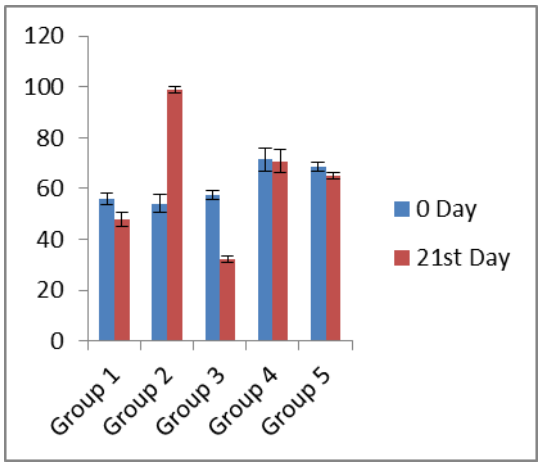


Fig 7: Effect of ESP on serum SGPT(UL)

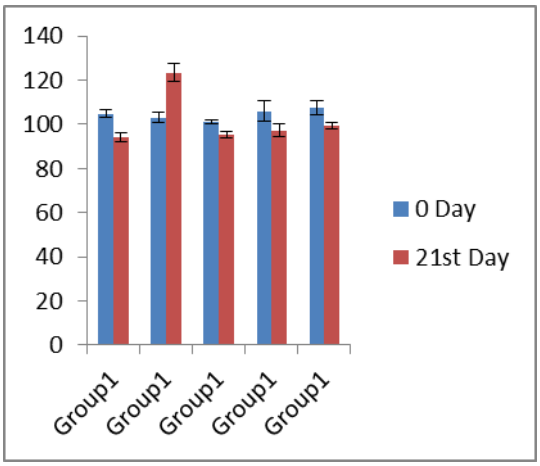


Fig 8: Effect of ESP on serum SGOT(UL)

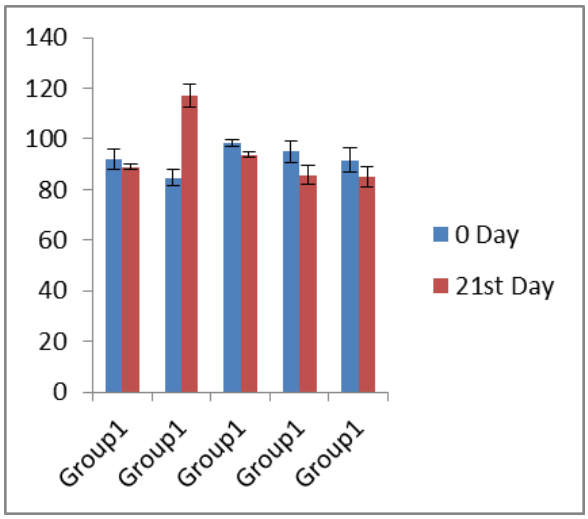
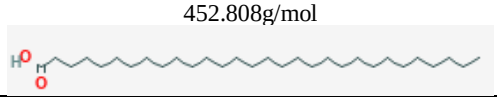
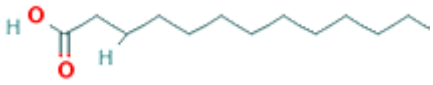
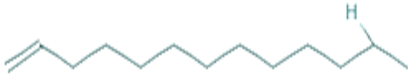
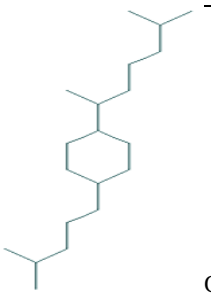


Fig 8: Effect of ESP on serum ALP(UL)

Table 5: Detection of anti-diabetic compounds in *Scurrula parasitica* by GC-MS.

S. No	Compound	RT	Area %	Structure & MW	Ref/journal
1	Triacontanoic acid	18.855	14.87	$C_{30}H_{60}O_2$ 452.808g/mol 	Eddouks *et al.*, 2005 [10]
2	Hexadecanoic acid	19.296	52.91	$C_{16}H_{32}O_2$ 256.43g/mol 	Ahmad *et al.*, 2012 [2]
3	1-Tridecene	20.606	11.44	$C_{13}H_{26}$ 182.351 g/mol 	FAOU, 1997 [11]. Chien *et al.*, 2009 [8]
4	Cyclohexane, 1-(1,5-Dimethylhexyl) -4-(4-Methylpentyl)	21.146	12.24	 $C_{20}H_{40}$, 280.54g/mol	Mohammad Nadeem Akhtar and Gayathri, 2015 [20]

5	Heneicosanoic acid	21.562	8.54	$C_{21}H_{42}O_2$ 326.565 g/mol	D` Souza *et al*., 2014 [9]

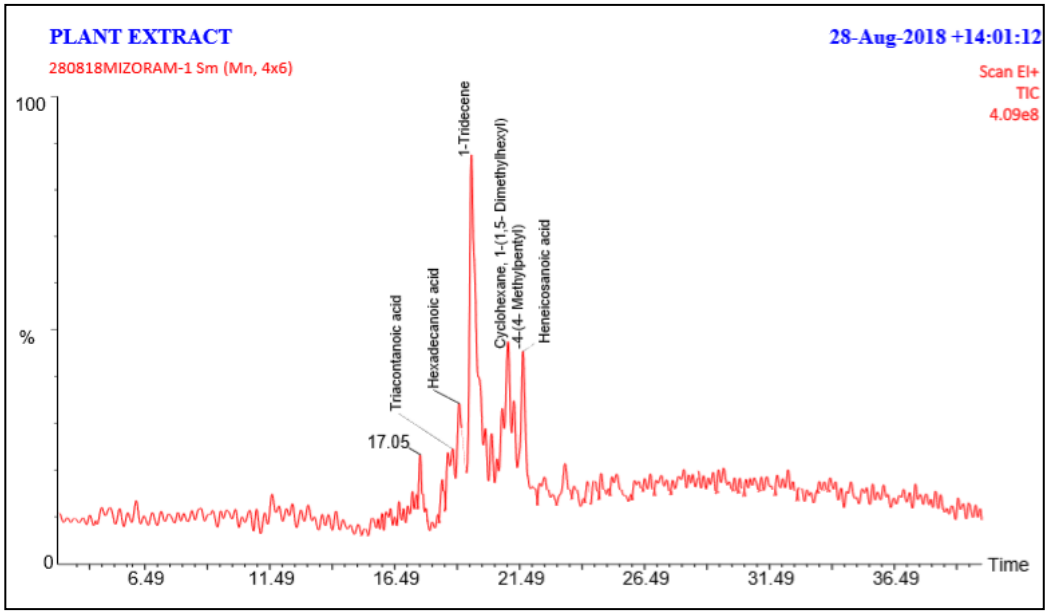


Fig 9: Detection of anti-diabetic compounds by GC-MS

Discussion

Diabetes Mellitus is a metabolic disease characterized by loss of glucose homeostasis with the disorder of carbohydrates, fat, protein metabolism resulting from defects in insulin (Barcelo and Rajpathak, 2001) [6]. In our research, the induction of diabetes in rats was done by single intraperitoneal injection of STZ (40mg/kg b.w) to determine the anti-diabetic activity of *Scurrula parasitica*.

The ethanolic extract of *Scurrula Parasitica* confirm the presence of alkaloids, tannins, saponins, flavonoids, reducing sugar, phytosterols, terpenoid, and phenol but glycosides were absent. Medicinal plants contains secondary metabolites, which play a pivotal role against diseases and pathogens, phytochemical screening reveals the active constituents present in plants that are known to be responsible for various activities such as antimicrobial, anticancer, antioxidant, anti-diabetic and antifungal (Hossain and Nagooru, 2011) [13].

To determine the anti-diabetic potential of the plant, ethanol extract of *Scurrula parasitica*, on normal and diabetic rats was performed by creating experimental design with multiple doses.

From the results of the acute toxicity experiment, doses of 100 and 200mg/kg of ethanol extract of *Scurrula parasitica* were selected for the experiment. The ethanol extracts given at doses of 100 and 200 mg/kg displayed significant role in lowering of the blood glucose level and bringing the body weight back to normal against STZ induced diabetic rats and the effects were compared with metformin. The most significant reduction in the glucose levels was observed in ethanol extract *Scurrula parasitica* at the dose of 200 mg/kg (Table 2). The loss in body weight was observed due to the induction of streptozotocin, but the administration of the extract to diabetic rats was controlled by treatment with the extract of *Scurrula parasitica*, which resulted in increase in body weight.

Lipids play an essential role in the pathogenesis of diabetes, of which Hypertriglyceridemia and hypercholesterolemia occurs most frequently (Al-Shamaony *et al*., 1994) [3]. The uncharacteristically high concentrations of serum lipids in diabetic rats are generally due to an increase in the mobilization of free fatty acids from the peripheral fat depots as insulin inhibits the hormone-sensitive lipase (Pushparaj *et al*., 2000) [22]. Excess fatty acids in the serum of diabetic rats are converted into phospholipids and cholesterol in the liver along with excess triglycerides formed at the same occasion in the liver is discharged into the blood as lipoproteins (Bopanna *et al*., 1997) [7]. Total cholesterol, triglycerides and LDL cholesterol of the streptozotocin-induced diabetes rats treated with ESP (100 or 200 mg/kg) showed considerable (p<0.05) reduction compared to untreated diabetic rats and diabetic control rats (Table 4). Coronary threats also arise due to an increase in HDL cholesterol (Rajalingram *et al*., 1993) [23]. The high flavonoid contents of *Scurrula parasitica* might be the reason for the reduction activity in the levels of serum triglyceride and VLDL, flavonoids are recognized for their antioxidant activity (Afanas'ev *et al*., 1995) [1]. In the current investigation, the plant extract enhanced the levels of HDL cholesterol. (Table 3)

As there in an increase in glucose levels, the level of SGOT and SGPT also rises. Regeneration process takes place after the treatment with *Scurrula parasitica* extract an SGPT, SGOT levels were brought back to normal. *Xanthosomas agittifolium* also comprise of similar results, when experimented with diabetic rats [25]. Decrease in ALP level shows its steadiness function against the damage caused by STZ. (Table 4)

Conclusion

Scurrula parasitica is a well known parasitic plant which is used for treatment of diabetes by the people of Mizoram and the current investigation, shows significant results in lowering of blood glucose, triglyceride, cholesterol, LDL, ALP, SGOT and SGPT and increases the body weight and level of HDL after the 21 day experiment. 5 anti-diabetic compounds were

detected by the GCMS analysis and these maybe the reason for the plant to have anti-diabetic activity. Further study to isolate the compounds is important for formulating of synthetic drugs which can be used for treatment of diabetes.

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