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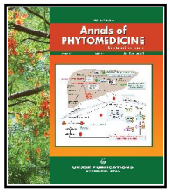

Original Article : Open Access

Assessment of comparative antidiabetic activity of herbomineral formulations (*Tryushanadya Lauha* and *Tryushanadya Mandur*) in wistar rats

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Abstract

Tryushanadya Lauha is a classical herbomineral Ayurvedic formulation, indicated for treating diabetes mellitus. The experiment was designed to investigate the antidiabetic effect of *Tryushanadya Lauha* (TL) and *Tryushanadya Mandura* (TM). The antidiabetic effect of TL and TM was studied in thirty wistar rats by oral glucose tolerance test (OGTT). The study was done in groups which were vehicle control, disease control, test drugs and standard treatment. The test drugs, TL and TM are given at 500 mg/kg along with adjuvants. The standard drug used was metformin at the dose of 500 mg/kg. The medicines were administered orally. The blood glucose level was assessed at 0, 30 and 90 min. The results were compared before and after in each group by applying paired t-test and multiple comparisons, the Turkey test, which showed no significant difference between among all groups. However, a paired t-test showed a significant result in the standard group and TL (p>0.01), but a non-significant result was observed in the TM group. In conclusion, TL as mentioned in classical text holds a potential treatment for diabetes.

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia (fasting plasma glucose ≥ 126 mg/dl and/or 200 mg/dl, 2 h after 75 g oral glucose), balance and sometimes ketonaemia (Tripathi, 2013). Diabetes is a syndrome that encompasses all clinical diseases marked by an increase in urine production, whether or not this rise is accompanied by an increase in micturition frequency.

WHO has defined diabetes as a chronic metabolic condition characterised by high levels of blood glucose (or blood sugar), which causes catastrophic damage to the heart, blood vessels, eyes, kidneys, and nerves over time. Type 2 diabetes is the most common, usually affecting adults, and occurs when the body develops insulin resistance or fails to produce enough insulin. Type 2 diabetes have increased substantially in countries of all income levels over the last three decades. Diabetes affects around 422 million people globally, with the majority residing in low- and middle-income countries, and diabetes is directly responsible for 1.5 million fatalities per year. Diabetes has been progressively increasing in both the number of cases and the prevalence during the last few decades. Blood glucose control through a combination of diet, physical activity, and, if necessary, medication; blood pressure and lipid control to reduce cardiovascular risk and other complications; and regular screening for damage to the eyes, kidneys, and feet to facilitate early treatment are among the interventions. Many recent medications used to treat diabetes had adverse effects (Kaul *et al*., 2013; Mounika and Hymavathi, 2021).

In diabetes, the two primary symptoms, polyurea and turbidity of the urine are observed (Sharma and Prajapathi, 2014). Diabetes mellitus is a chronic metabolic condition with significant social, health and economic effects. It is estimated that 285 million people worldwide (about 6.4% of the adult population worldwide) were affected by this disease in 2010 (Lovic *et al*., 2020). Type 2 diabetes mellitus requires dietary changes, including a diet high in carbohydrate complexes, protein, fibre, and low in fat, which does not induce a rapid increase in blood glucose levels (Mounika and Hymavathi, 2021). Due to the various factors involved in diabetes mellitus, there is a need for an integrated approach to its management. Under the traditional system of Indian medicine, many herbal and herbomineral formulations are mentioned and researched for diabetes management (Wanjari *et al*., 2016 ; Subramoniam *et al*., 2014). In Ayurveda for the treatment of diabetes, *Loha Bhasma* (incinerated ash of iron) is used and it is present in many different formulations. As it has the ability to combat the cause of diabetes, it is utilised to treat diabetes as mentioned in numerous Ayurveda texts. Herbomineral formulations are quick in action in less dose. Herbomineral complexes are shown to be more stable and interactive than ordinary herbs since they have a quicker therapeutic effect and a longer shelf-life (Chaudhary and Singh, 2010). Herbomineral formulation involves pharmaceutical technology that transforms metals and minerals into incinerated ash by means of detoxification methods, turning them into powders, and incineration, which nullifies the metallic properties and makes it suitable for internal administration (Gupta *et al*., 2012). This pharmaceutical technology results in the formations of many herbomineral formulations.

Tryushanadya Lauha (TL) is one of the herbomineral formulations described under the Traditional system of Indian medicine. TL is

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indicated in diabetes mellitus. TL comprises of incinerated ash of iron as a main ingredient along with *P. longum, P. nigrum* , *Z. officinale* , *P. chaba* , *P. Corylifolia* , *P. zeylanica* , rock salt, sodium carbonate, ammonium chloride, sodium sulphate (Govinda, 2017; Swer *et al*., 2021). Research had proven that all herbal ingredients of TL are having anti-hyperglycaemia and anti-hyperlipidaemic activity. Only *Loha Bhasma* (incinerated ash of iron) is insufficient to treat all causes and consequences of diabetes. A multi-ingredient combination may be more superior to only *Loha Bhasma* (incinerated ash of iron). The characteristics of incinerated ash of cast iron and ash of slag of iron (Mandura) are similar in properties (Vagbhatta *et al.,* 2011). As the previous study of comparison of them show that incinerated ash of slag of iron (*Mandura*) have better effect (Sarkar *et al*., 2007). As the origin of slag of iron is from cast iron (*Loha*), so it has the property of iron. However, for preparation of incinerated ash of cast iron is very time-consuming and laborious as compared to the preparation of ash of slag of iron. Hence, by using cast iron in place of iron can be used and both the preparations are studied. The OGTT is employed in basic research, mostly to study animal glucose homeostasis. The glucose-insulin index derived from the OGTT in animals was used to identify insulin resistance and insulin sensitivity. Although, animal research can help us to understand the underlying causes of human disease. There are significant disparities between species when it comes to metabolic control. As a result, the OGTT is limited in basic research. Over the last century, the OGTT has been used to diagnose impaired glucose tolerance (IGT) by measuring plasma glucose concentrations following either an overnight fast or glucose loading (Kuo *et al*., 2021). Animal studies provide us guidelines and assist us in answering questions about the extent of use of herbomineral preparation in terms of safety and efficacy. The findings of animal experiments have greatly aided modern medicine in understanding the drug's benefits and drawbacks. This can also be applied to our ancient formulation to determine the magnitude of dose response in the human body (Prakash *et al.,* 2019). Wistar rats are one of the most commonly utilised rat strains in research. They are well-known for their calm demeanour, homogeneous size, and general ease of handling. Wistar rats are an important tool in biomedical research because they are used to evaluate human and animal health effects (King *et al*., 2012). Hence, to assess the efficacy of TL and it is modified form of TM were studied for antidiabetic activity in wistar rats and the results were compared.

2. Materials and Methods

2.1 Collection and preparation of TL and TM

The dried herbs, salts and minerals were procured from a pharmaceutical unit of Mahatma Gandhi Ayurved College Hospital and Research Centre (MGACHRC), Wardha. The fresh herbs required for the study were collected from medicinal plant garden attached to MGACHRC. Medicine preparations (TL and TM) and analysis of them were carried out at the pharmaceutical unit of MGACHRC.

2.2 Method of preparation

Table 1 shows the ingredients, parts and quantity used for preparation. All the ingredients are made into fine powders separately and sieved through cotton clothes. Fine powders of all ingredients were taken in a vessel in the mentioned quantity and mixed thoroughly and continuously till they attained a homogeneous mixture. The mixture was sieved through 100 Mesh. Organoleptic characters were observed, formulations were analysed and stored in air-tight containers labelled as TL and TM for antidaibetic study.

Table 1: Show the ingredients, part and quantity use for the preparation of TL

S.No.	Ingredients		Part used	Quantity
	Tryushanadya Lauha	***Tryushanadya Mandura***	Fruit	15 g
1.	*Pippali* (*P. longum*)	*Pippali* (*P. longum*)	Fruit	15 g
2.	*Maricha* (*P. nigrum*)	*Maricha* (*P. nigrum*)	Fruit	15 g
3.	*Sunthi* (*Z. officinale*)	*Sunthi* (*Z. officinale*)	Stem	15 g
4.	*Cavya* (*P. chaba*.)	*Cavya* (*P. chaba*)	Roots	15 g
5.	*Chitraka* (*P. zeylanica*)	*Chitraka* (*P. zeylanica*)	Seeds	15 g
6.	*Bakuchi* (*P. corylifolia*)	*Bakuchi* (*P. corylifolia*)	Raw	15 g
7.	*Vida lavana* (Ammonium chloride)	*Vida lavana* (Ammonium chloride)	Raw	15 g
8.	*Saindhava lavana* (Sodium chloride)	*Saindhava lavana* (Sodium chloride)	Raw	15 g
9.	*Aubhidha lavana* (Sodium carbonate)	*Aubhidha lavana* (Sodium carbonate)	Raw	15 g
10.	*Sauvarchala lavana* (Sodium sulphate)	*Sauvarchala lavana* (Sodium sulphate)	Raw	165 g
11.	*Loha Bhasma* (Incinerated ash of iron)	*Mandura Bhasma* (Incinerated ash of Slag of iron)	Ash	315 g

2.3 Experiment design

Healthy adult wistar rats (190-300 g) of either sex between 2 to 3 months of age were used for the investigations. The animals were acclimatised for a week. They were housed in groups in polypropylene cages, maintained under standard conditions (12 h light and dark cycle, temperature 25 ± 1° C; humidity 40-60%) and fed with a standard rat pellet diet and purified water *ad libitum*. The experimental study was carried out at the animal house, DMCP, DMIHER, Wardha. The study was approved by an Institutional Animals Ethics Committee (IAEC) DMIMS/CPCSEA-IAEC DA/20-

21/16 of DMIHER, Sawangi, Wardha and performed according to CPCSEA (Committee for Control and Supervision of Experiments on Animals) Guidelines (Pereira, *et al*., 2004). An experimental study was done in five groups containing 6 wistar rats (3 males and 3 females), a total of 30 wistar rats. The groups, drug dose and adjuvant were presented in Table 2.

Table 2: Showing the grouping and treatment given

Groups	Name of group	Drugs	Induction drug (glucose) with dose	No. of animal	Test drug dose	Vehicle duration	Study
Group I	Disease control	-	4 g/kg with 3 ml Distilled water	6	-	-	1 day
Group II	Standard control	Metformin	4 g/kg with 3 ml Distilled water	6	5.1 mg/g	3 ml Distilled water	1 day
Group III	Vehicle control	Ghee and Honey	4 g/kg with 3 ml Distilled water	6	-	1 ml (Honey) 3 ml (Ghee)	1 day
Group IV	Test drug 1	*Trushanadya Lauha* (TL)	4 g/kg 3 ml Distilled water	6	5.1 mg/g	1 ml (Honey) 3 ml (Ghee)	1 day
Group V	Test drug 2	*Trushanadya Mandura* (TM)	4 g/kg 3 ml Distilled water	6	5.1 mg/g	1 ml (Honey) 3 ml (Ghee)	1 day

2.4 Methods

The study was referred from the glucose tolerance test in mice: Sex, drugs and protocol by Kennard *et al*. (2022). The OGTT was used to test new antidiabetic treatments on stimulated blood glucose concentrations and is one of the most commonly undertaken experiments in metabolic research. It is also used both in clinical practice and research to assess glucose tolerance.

2.5 Procedure

OGTT was performed on wistar rats of either sex and the body weight of the rats was measured which was between 190-300 g. Then, the rats were marked to make the rats easily distinguishable and transferred into the cage. The wistar rats were separated into five groups. Each group contains 6 wistar rats of either sex. The wistar rats were fasted overnight before testing, *i.e*., 16 h by removing their food and only water was kept in the cage.

2.6 Animal dose conversion from human dose (Paget and Barnes)

To calculate animal dose, human dose mean is multiplied by Conversion factor (6.2). To find out the dose of each rat in grams, dose in rat multiplied by weight/1000. In 1000 g of rat's dose is 5.1 mg/kg. If, the weight of the rat is 210 g, then multiply by 5.1 and divide by 1000 is 1.07 mg, *i.e*., a dose of that particular weight of rats.

2.7 Preparation of medicine

I. Disease control group - Glucose 4 g was dissolved in 10 ml distilled water.

II. Standard control group - (Metformin 500 mg) 5.1 mg/kg dissolved in distilled water.

III. Vehicle control group- 3 ml ghee and 1 ml honey.

IV. Test drug 1-TL (*Tryushanadya Lauha* 500 mg) 5.1 mg/kg mixed with unequal quantity of ghee and honey (3 ml + 1 ml).

V. Test drug 2-TM (*Tryushanadya Mandura* 500 mg) 5.1 mg/kg mixed with unequal quantity of ghee and honey (3 ml + 1 ml).

The next day, blood glucose was measured with the help of a glucometer. With the aseptic precaution, the vein at the tail was pricked, blood glucose was measured and noted considering 0 min. Each of the rats was fed with experimental drugs except group I. They were administered according to the body weight of animals by oral routes with the help of an intragastric tube. Later after 30 min, wistar rats were fed with glucose orally at the dose of 4 g/kg. Then, the blood glucose was estimated at 30, and 90 min, respectively, and noted.

2.8 Statistical analysis

Statistical analysis was done by using descriptive and inferential statistics using Student's paired t-test, one-way ANOVA and Multiple Comparison: The Tukey Test and software used in the analysis was SPSS 27.0 version and $p<0.05$ is considered as the level of significance.

3. Results

3.1 Effect of TL and TM on wistar rats by OGTT

For the oral glucose tolerance test, the blood samples were analysed for glucose content at 0, 30 and 90 min, respectively. The glucose level, after oral administration of glucose in control and treated rats is given in Table 3. The blood sugar levels of TL and TM-treated groups were compared along with the standard and vehicle groups. Standard drug, *i.e*., metformin has been used for many years to treat diabetes and enhance insulin sensitivity (Cusi and De Fornzo, 1998). Ghee and honey in unequal quantities were used as vehicle control.

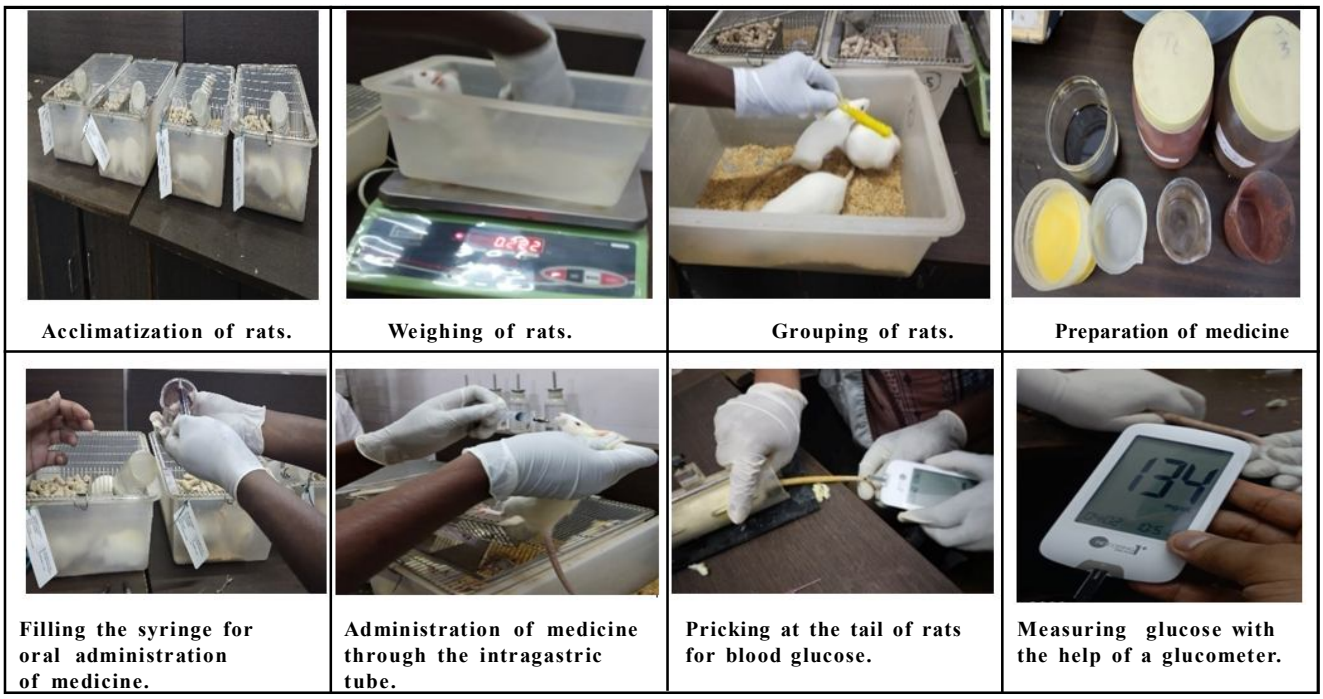

Figure 1: Showing images of experimental study.

Table 3: Effect of glucose in wistar rats by OGTT

S.No.	Groups	Glucose levels (mg/dl)		
		0 min	30 min	90 min
I.	Disease control group	119.00 ± 8.60	163.83 ± 42.86	138.00 ± 19.25
II.	Standard control group	124.00 ± 8.39	149.00 ± 7.56	142.00 ± 18.63
III.	Vehicle control group	123.16 ± 23.14	144.83 ± 17.45	126.33 ± 19.31
IV.	Test drug -TL	120.00 ± 13.92	150.66 ± 19.05	131.16 ± 18.75
V.	Test drug -TM	123.66 ± 16.57	157.66 ± 21.92	143.33 ± 26.56

Note: All values represent means ± S.D. of the mean (n=6).

The effect of TL and TM on glucose levels was determined by comparison of normal control, standard, test drugs and vehicle. In data analysis, when comparing OGTT by applying Student's paired T-test showed that group I (Disease control) was non-significant at 30 and 90 min as shown in Table 4 and the same has been shown in Figure 1which shows that the glucose level was increased in 30 min and decreased in 90 min. Group II (metformin) showed a significant difference at 30 and 90 min when the Student's paired t-test was applied, as shown in Table 5 and Figure 3. Group III (Vehicle control) showed a significance at 30 and non-significant at 90 min as shown in Table 6 and Figure 4. Group IV (TL) showed significant decrease in glucose levels at 30 and 90 min, as shown in Table 7 and Figure 5. The significant result of TL suggests that it has hypoglycaemic effect. Group V (TM) showed significant results at 30 and not statistically significant at 90 min as shown in Table 8 and Figure 6. These results suggest that hypoglycaemic effect of TL is better as compared with TM. One way ANOVA showed that the comparison of all groups, a non-significant difference was found as shown in Table 10. Multiple comparison test that is the Tukey tests showed that there is a non-significant difference between all the test groups, as shown in Table 11. In figure 6 when OGTT in group III and group IV were compared at 30 min and 90 min, showed observable reductions in glucose were approximately equal to the 0 min. However, the test drug, TL and standard drug, metformin depressed the peak of blood glucose level at 90 min after glucose loading. The Students paired t-test showed the significant result of standard metformin and test drug TL (p>0.01). Both metformin and TL are found equally significant.

Figure 2: Comparison of mean oral glucose tolerance test score in Group I at 30 min and 90 min with 0 min.

Note: The X-axis represents the mean glucose level and the Y-axis represents the time.

Table 4: Comparison of oral glucose tolerance test score in Group I at 30 min and 90 min with 0 min by applying Student's paired t-test

Time	Mean	N	Std. deviation	Std. error of mean	Mean difference	t-value
0 min	119.00	6	8.60	3.51	-	-
30 min	163.83	6	42.86	17.49	44.83 ± 44.82	2.45, p=0.058, NS

Table 5: Comparison of oral glucose tolerance test score in Group II at 30 min and 90 min with 0 min by applying Student's paired t-test

Time	Mean	N	Std. deviation	Std. error of mean	Mean difference	t-value
0 min	124	6	8.39	3.42	-	-
30 min	149	6	7.56	3.08	25±4.51	13.55, p = 0.0001, S

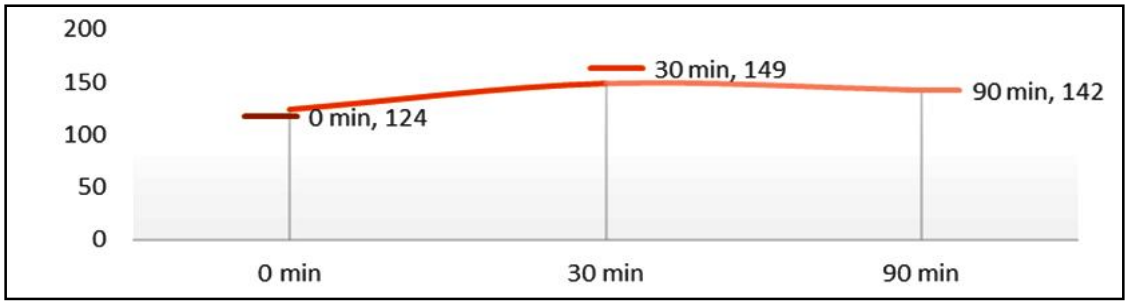

Figure 3: Comparison of mean oral glucose tolerance test score in Group II at 30 min and 90 min with 0 min.

Table 6: Comparison of oral glucose tolerance test score in Group III at 30 min and 90 min with 0 min by applying Student's paired t-test

Time	Mean	N	Std. deviation	Std. error of mean	Mean difference	*t*-value
0 min	123.16	6	23.14	9.44	-	-
30 min	144.83	6	17.45	7.12	21.66 ± 13.64	4.00, p = 0.010, S
90 min	126.33	6	19.31	7.88	3.16 ± 20.93	0.37, p = 0.72, NS

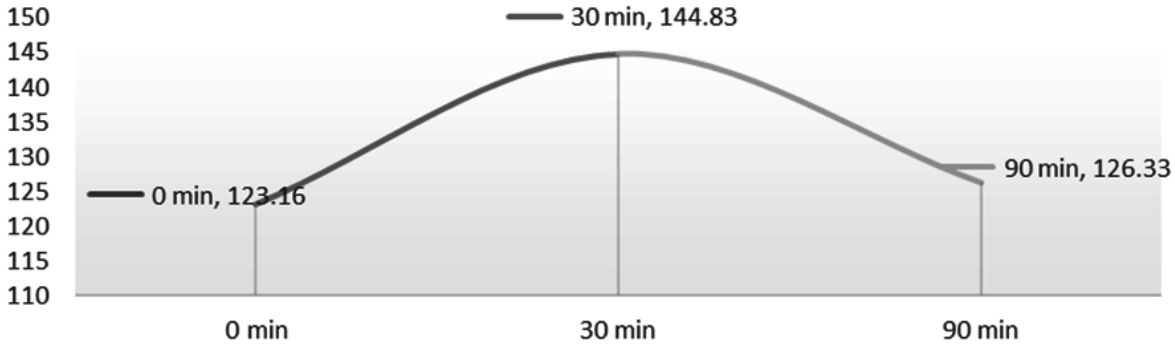

Figure 4: Comparison of mean oral glucose tolerance test score in Group III at 30 min and 90 min with 0 min.

Table 7: Comparison of oral glucose tolerance test score in Group IV at 30 min and 90 min with 0 min by applying Student's paired t-test

Time	Mean	N	Std. deviation	Std. error of mean	Mean difference	t-value
0 min	120	6	13.92	5.68	-	-
30 min	150.66	6	19.05	7.77	30.66 ± 8.64	8.69, p = 0.0001, S
90 min	131.16	6	18.78	7.65	11.16 ± 6.52	4.19, p = 0.009, S

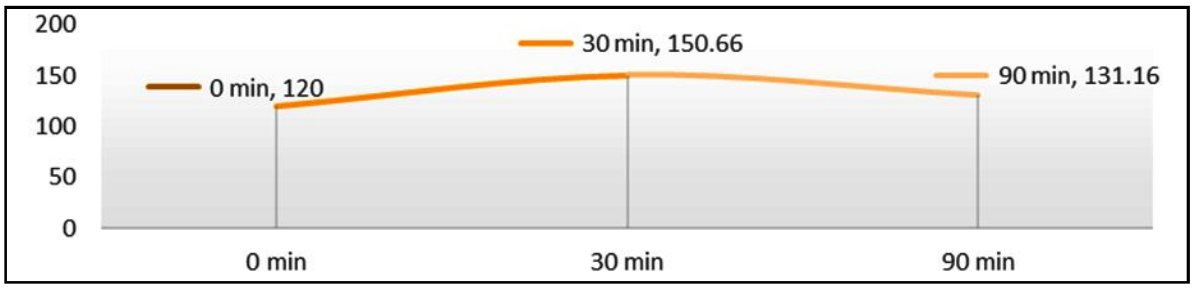

Figure 5: Comparison of mean oral glucose tolerance test score in Group IV at 30 min and 90 min with 0 min.

Table 8: Comparison of oral glucose tolerance test score in Group V at 30 min and 90 min with 0 min by applying Student's paired t-test

Time	Mean	N	Std. deviation	Std. error of mean	Mean difference	*t*-value
0 min	123.66	6	16.57	6.76	-	-
30 min	157.66	6	21.92	8.95	34 ± 17.08	4.87, p = 0.005, S
90 min	143.33	6	26.56	10.81	19.66 ± 21.33	2.25, p = 0.074, NS

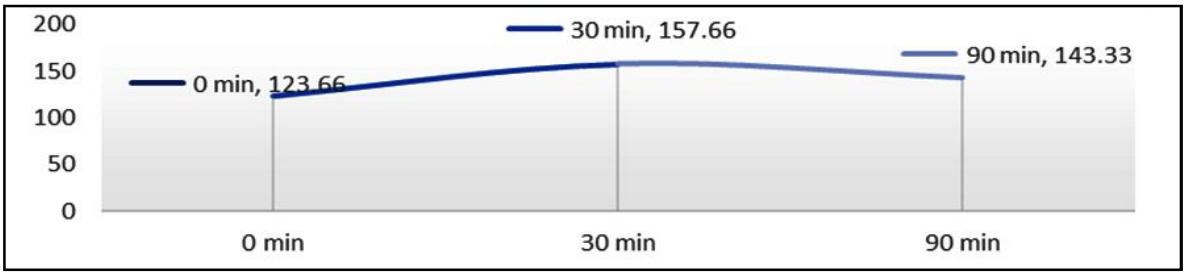

Figure 6: Comparison of mean oral glucose tolerance test score in group V at 30 min and 90 min with 0 min.

Table 9: Comparison of oral glucose tolerance test score in five Groups at 30 min and 90 min with 0 min by descriptive statistics

		N	Mean	Std. deviation	Std. error	95% Confidence interval for mean		Min	Max
						Lower bound	Upper bound		
0 min	Group I	6	119.00	8.60	3.51	109.97	128.02	106.00	129.00
	Group II	6	124.00	8.39	3.42	115.19	132.80	113.00	137.00
	Group III	6	123.16	23.14	9.44	98.87	147.45	100.00	153.00
	Group IV	6	120.00	13.92	5.68	105.38	134.61	98.00	136.00
	Group V	6	123.66	16.57	6.76	106.27	141.05	98.00	140.00
30 min	Group I	6	163.83	42.86	17.49	118.8	208.81	130.00	245.00
	Group II	6	149.00	7.56	3.08	141.03	156.93	139.00	161.00
	Group III	6	144.83	17.45	7.12	126.51	163.14	120.00	165.00
	Group IV	6	150.66	19.05	7.77	130.67	170.66	118.00	172.00
	Group V	6	157.66	21.92	8.95	134.65	180.67	130.00	197.00
90 min	Group I	6	138.00	19.25	7.86	117.79	158.20	110.00	166.00
	Group II	6	142.00	18.63	7.60	122.44	161.55	120.00	175.00
	Group III	6	126.33	19.31	7.88	106.06	146.60	106.00	154.00
	Group IV	6	131.16	18.75	7.65	111.48	150.84	104.00	151.00
	Group V	6	143.33	26.56	10.80	115.45	171.21	100.00	183.00

Table 10: Statistical analysis by oneway ANOVA

Source of variation		Sum of squares	Df	Mean square	F	*p*-value
0 min	Between groups	126.800	4	31.700	0.138	0.967, **NS**
	Within groups	5744.167	25	229.767		
	Total	5870.967	29			
30 min	Between groups	1362.467	4	340.617	0.560	0.694, **NS**
	Within groups	15214.333	25	608.573		
	Total	16576.800	29			
90 min	Between groups	1262.667	4	315.667	0.735	0.577, **NS**
	Within groups	10743.500	25	429.740		
	Total	12006.167	29			

Table 11: Statistical analysis by Multiple comparison test: Turkey test

Dependent variable	Group		Mean difference	Std. error	*p*-value	95% Confidence interval	
						Lower bound	Upper bound
0 min	Group I	Group II	-5.00	8.75	0.978, **NS**	-30.70	20.70
		Group III	-4.16	8.75	0.989, **NS**	-29.86	21.53
		Group IV	-1.00	8.75	1.000, **NS**	-26.70	24.70
		Group V	-4.667	8.75	0.983, **NS**	-30.36	21.03
	Group II	Group III	0.833	8.75	1.000, **NS**	-24.86	26.53
		Group IV	4.000	8.75	0.990, **NS**	-21.70	29.70
		Group V	0.333	8.75	1.000, **NS**	-25.36	26.03
	Group III	Group IV	3.16	8.75	0.996, **NS**	-22.53	28.86
		Group V	-0.50	8.75	1.000, **NS**	-26.20	25.20
	Group IV	Group V	-3.66	8.75	0.993, **NS**	-29.36	22.03
30 min	Group I	Group II	14.83	14.24	0.834, **NS**	-26.99	56.66
		Group III	19.00	14.24	0.673, **NS**	-22.82	60.82
		Group IV	13.16	14.24	0.885, **NS**	-28.66	54.99
		Group V	6.16	14.24	0.992, **NS**	-35.66	47.99
	Group II	Group III	4.16	14.24	0.998, **NS**	-37.66	45.99
		Group IV	-1.66	14.24	1.000, **NS**	-43.49	40.16
		Group V	-8.66	14.24	0.972, **NS**	-50.49	33.16
	Group III	Group IV	-5.83	14.241	0.994, **NS**	-47.66	35.99
		Group V	-12.83	14.24	0.894, **NS**	-54.66	28.99
	Group IV	Group V	-7.00	14.241	0.987, **NS**	-48.82	34.82
90 min	Group I	Group II	-4.00	11.96	0.997, **NS**	-39.15	31.15
		Group III	11.66	11.96	0.864, **NS**	-23.48	46.81
		Group IV	6.83	11.96	0.978, **NS**	-28.31	41.98
		Group V	-5.33	11.96	0.991, **NS**	-40.48	29.81
	Group II	Group III	15.66	11.96	0.688, **NS**	-19.48	50.81
		Group IV	10.83	11.96	0.892, **NS**	-24.31	45.98
		Group V	-1.33	11.96	1.000, **NS**	-36.48	33.81
	Group III	Group IV	-4.83	11.96	0.994, **NS**	-39.98	30.31
		Group V	-17.00	11.96	0.621, **NS**	-52.15	18.15
	Group IV	Group V	-12.16	11.96	0.845, **NS**	-47.31	22.98

4. Discussion

The present study was designed and conducted to evaluate the effect of TL and TM on the blood glucose of wistar rats for antidiabetic action (Mishra, 2007). The origin of Mandura Bhasma lies in Loha (iron), and prior studies have indicated its effectiveness in certain therapeutic applications equal or better than Loha Bhasma (Madhava, 1999). Considering this present study, seeks to prepare TL and modified form as TM. Diabetes mellitus, on the other hand, is defined as a clinical illness characterised by hyperglycaemia with or without glycosuria caused by faulty insulin either in quantity or effectively, and characterised by polyuria, polyphagia, and polydipsia (Murthy and Singh, 1989). Diabetes mellitus is still a major health issue throughout the world, including in the tropics (Yadav and Srivastava, 2014). There has been an ongoing search for new antidiabetic drugs manufactured from plants (Rani *et al*., 2023). According to the World Health Organisation (WHO), up to 80% of developing-countries use plants and their 110 products as Traditional medicine for primary healthcare (Tiwari *et al*., 2015; Thakur *et al*., 2020). TL is indicated in diabetes in Traditional system of Indian medicine but was not evident. With the background, an attempt has been made to prepare TL and TM and find out their antidiabetic activity.

The results obtained from OGTT, offer a compelling insight into the relative effectiveness of TL in comparison to TM when it comes to reducing blood glucose levels. This finding carries significant implications for the management of diabetes. During the OGTT, a widely accepted method for evaluating how the body processes glucose, the data indicate that TL exerts a considerably more pronounced effect in regulating blood glucose levels. This statistical significance underscores the practical importance of TL as a potentially potent intervention for diabetes. The notable contrast in effectiveness between TL and TM raises pertinent questions about the specific components or mechanisms within TL that contribute to its superior antidiabetic action. Unravelling these mechanisms could hold the key to unlocking new avenues for diabetes treatment and management, potentially leading to more effective and tailored therapeutic approaches. These findings underscore the necessity for further research, both to elucidate the precise factors responsible for the remarkable antidiabetic effects of TL and to explore ways to enhance the therapeutic potential of TM. Such knowledge can pave the way for the development of more potent and precisely targeted treatments, ultimately benefitting individuals living with diabetes.

TL contains herbal ingredients that have antidiabetic activity. *P. longum* has potent anti-hyperglycaemic and antilipid peroxidative activity in alloxan-induced diabetic rats (Nabi *et al*., 2013), *P. nigrum* one of the most studied spices, has a variety of therapeutic characteristics (Singh *et al*., 2012). It has antidiabetic properties also been confirmed *in vivo* as well as hypolipidemic activity as evidenced by decrease in the level of cholesterol, triglycerides, and low-density lipoprotein and increase in high-density lipoprotein (Takoree *et al*., 2019), *Z. officinale* study on diabetic rats model had shown that an aqueous extract of ginger has potential hypoglycaemic properties (Almin *et al*., 2006). *P. corylifolia* study on the sub and mildly diabetic rats indicates that treatment with the PC seed extract decreases glucose levels. The long-term study on severely diabetic rats also shows a decrease in glucose and correction of the associated disturbed lipid profile (Dhar *et al*., 2013), *P. zeylanica* study showed that plumbagin possessed antidiabetic activity in STZ-induced diabetic rats (Sunil *et al*., 2012). *P. chaba* study confirms the hypoglycaemic effect with other beneficial effects in diabetic mice. These results suggest that the acetone and ethanol extract of *P. chaba* may improve the metabolic disruption produced by diabetes (Haque, *et al*., 2018), incinerated ash of iron case study, there is a drastic reduction in the blood sugar level value, indicating the antihyperglycaemic action (Bineesh *et al*., 2021). So, the presence of active moieties in the individual plants might be contributing an important role in the attenuation of hyperglycaemia. Also, phytochemicals like steroids, alkaloids, phenols and flavonoids are present in TL. These compounds were reported to have antihyperglycaemic activity and antidiabetic activities. Hence, the cumulative synergism may be obtained. The mechanism may be by increasing insulin secretion, antioxidant action, or altering carbohydrate metabolism or peripheral glucose utilization. Thus, the antihyperglycaemic effect of the TL may be attributed to the cumulative effect of these constituent plants.

The use of the OGTT in animals in fundamental research has primarily focused on glucose homeostasis. Unlike in clinical practise, the OGTT has not been used in basic research for diagnosis. The glucose-insulin index derived from the OGTT in animals was used to identify IR and insulin sensitivity. Diabetic animals were then employed to test the activity of a drug under investigation. The OGTT glucose curve form is solely used as a guideline (Kuo *et al*., 2021).

The impact of TL and TM on glucose levels was ascertained through a comparative analysis involving disease control, standard, test drugs, and a vehicle control group. Data analysis, specifically, the comparison of OGTT, by applying Student's paired t-test, revealed that Group I (Disease control) exhibited non-significant differences at both 30 and 90 min, as illustrated in table 4 and Figure 3. The glucose level increased at 30 min and decreased at 90 min in this group. In contrast, Group II (metformin) demonstrated a significant disparity at 30 and 90 min when Student's paired t-test was applied, as indicated in Table 5 and Figure 4. Group III (Vehicle control group) showed significance at 30 min and non-significant at 90 min, as shown in Table 6 and Figure 5. Group IV (TL) exhibited a significant decrease in glucose levels at both 30 and 90 min, as shown in Table 7 and Figure 6. The significant outcome for TL suggests its hypoglycaemic effect.

Group V (TM) displayed significance at 30 min but no statistical significance at 90 min, as demonstrated in Table 8. These findings suggest that the hypoglycaemic effect of TL is superior to that of TM. Further analysis using one-way ANOVA, as presented in Table 10, revealed a non-significant difference when comparing all the groups. Multiple comparison tests, specifically the Tukey test confirmed that there was no significant variation between the test groups. In Figure 8, a comparison of OGTT results in Group III and Group IV at 30 and 90 min showed an observable reduction in glucose levels, approximately equal to the baseline (0 min). Notably, both the test drugs, TL and the standard drug, metformin suppressed the peak of blood glucose levels at 90 min following glucose loading. The Student paired t-test indicated a significant result for both standard metformin and the test drug, TL ($p > 0.01$), suggesting that they are equally effective.

The results of the study showed that there is a significant difference in pre and post-values of glucose levels in the groups, metformin and TL. When compared before and after blood glucose was observed at 90 min, there is a slight decrease in blood glucose, this can be interpreted that TL was able to normalize the impaired glucose tolerance in glucose after administration of glucose; in rats. Standard drug metformin, a standard anti-hyperglycaemic agent, did show an effect on glucose levels. In Student paired t-test shows the significant result that TL is better than TM while ANOVA shows non-significant which can be interpreted that the drugs are as good as one another or less effective as one another. These showed significant result in TL and metformin, suggesting their antidiabetic action. Oral anti-hyperglycemic agent, metformin may show some side effect and cannot be taken for a longer duration as it is harmed the organ of the body and end in hypoglycaemia (Stowers *et al*., 1977). However, ayurvedic formulations can be given for a long duration, if given by following proper standard operating procedure (Srivastava *et al*., 2012).

In Ayurveda, diabetes is a vitiated humours fatty predominant disease that involves others tissue of the body. Due to heaviness, reaches the urinary bladder mixed with urine and increase urinary flow (Chauhan *et al*., 2017). The compound formulations under Ayurveda helps to increase the bioavailability of the medicine by their properties and removes the minute channel blockage at the cellular level. They improve digestion, digest the unwanted materials in the body, dry and scrape the excess fatty tissues which is the main cause in diabetes and eliminate all the waste out of the body (Govindadas *et al*., 2017). Salts have the power to infiltrate the tiniest pathways in the body and aid in mobilising humour from the upper regions of the body and bring vitiated humour in the downward direction (Prabhakar *et al*., 2022). Ghee and honey as adjuvant act as bioenhancers that help in absorption, assimilation reduces the adverse effect of drugs, facilitate transportation to the target site and increase the action of the drug (Rosy *et al*., 2010). This prevents the humours/tissues from moving against their normal state and abundance of the humours by removing the minute channel blockage of fatty tissue and promoting physical strength by restoring physical vitality and rejuvenating the body. This brings the humours to their normal state and helps in treating the diabetes (Sharma and Chandola, 2011).

5. Conclusion

The present study investigated the potential antidiabetic effect of TL and TM in wistar rats. The study showed a significant result that TL has better antidiabetic activity as compared to TM. TL as mentioned in classic holds promising antidiabetic action. TM also shows promising result in experimental study, but statistically does not found significance as compared with metformin and TL. The present study still has certain limitations including lack of estimation of glucose level at 60 min, 120 min or some more time interval, biochemical analysis and histopathology of the pancreas. The mechanisms underlying the antidiabetic activity of compound formulations, TL and TM need more experimental exploration to provide a comprehensive understanding of their mode of action. Further preclinical and clinical studies are needed for the assessment of TL and TM.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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