Effect of Combination Dose of Basil, Turmeric and Aloevera on Biochemical Parameters in Streptozotocin Induced Diabetic Rats

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Abstract: Diabetes mellitus (DM) is a chronic metabolic disorder affecting the health of a large population worldwide. In the present study the effect of combination of basil, turmeric and aloe vera in different doses on the biochemical parameters were investigated in streptozotocin induced diabetic rats. The effect of these doses on fasting blood glucose (FBG), lipid profile, urea, creatinine and BUN content were investigated in streptozotocin induced rat and found significant effect(p<0.05). Maximum reduction of FBG level was found with high dose. It has been observed from the experimental results that reduction in TG, LDL% are highly significant (0.001<p<0.000). Total cholesterol level was also found to decrease significantly (p<0.05) whereas HDL-C level increases. A significant decrease in creatinine, urea and BUN were also observed. The results indicate the favourable effect of the combined herbal preparation in the treatment of diabetes mellitus.

Keywords: Diabetes Mellitus, Streptozotocin, C. longa, O. sanctum

1. Introduction

Diabetes Mellitus (DM) is a common chronic medical condition characterized by hyperglycemia, glycosuria, hyperlipidemia, polyuria, polyphagia, polydipsia, negative nitrogen balance and sometimes ketonemia. Insulin and glucagon are two hormones produced by the pancreas which controls the blood glucose level. Elevated level of sugar in blood increases insulin secretion and blood sugar level is normalized. Diabetes is a condition with impairment of insulin secretion or defective insulin response. DM has been found to be associated with many complications including production of ROS. There are many expensive drugs available in the market, as well as insulin that are used for treatment of diabetes but some of them can cause side effects like disturbances in the functioning of vital organs. 'So, World Health Organization advocated exploring herbal remedy and naturally occurring medicinal plant for the treatment of diabetes in a better way with minimum side effects' [1]. On the other hand, medicinal plants have been used from ancient time in the herbal medicine and some of them have therapeutic potential and experimentally documented. There are some preliminary studies to suggest that oral administration of Aloe vera might be effective in reducing blood glucose levels in diabetic patients and a study was designed to investigate the protective effect of Aloe vera in lowering blood glucose levels in streptozotocin induced albino rat [2-4]. Aloe vera extract is able to decrease blood glucose levels significantly and to protect animals against diabetic complications [5]. Aloe vera gel has a longterm effect on lowering blood glucose level and is useful for the treatment of type 2 diabetes mellitus [6]. Water extract A.vera results in a definite hypoglycemic, of hyperinsulinemic effect [7]. Turmeric is also beneficial for diabetic complications. It has been found that orally ingested curcumin improves glycemic control in mouse models of type 2 diabetes [8].

Objective of the present sudy is to assess the efficacy of combination herbal preparation in different doses in the treatment of diabetes mellitus.

2. Methodology

Laboratory tests performed on rats involve the safety of chemicals. As rats are mammals, their system reacts to these chemicals in a similar fashion as humans. In order to determine the dosage of a herb for human consumption, it may be tested on rats or other mammal. Healthy adult Wistar albino rats of 90-120 gram weight were used for the study. They were fed with a standard diet and water. Twenty five rats were made diabetic by intraperitoneal injection of freshly prepared streptozotocin (STZ), 40 mg/kg body weight. Then after 7 days 2nd dose was given. The diabetic rats were divided into following four groups, each having five rats. Five diabetic rats, which expired during the experiment, were excluded from the study. The dosing schedule used was once per day. The aqueous solution was fed through oral gavage for 21 days. Time of dosage was at10:00 a.m. to 10:30 a.m. Oral gavage was performed using a ball ended feeding needle. The rat was restrained in a straight line to facilitate introduction of the gavage needle. The needle was introduced in the space between the left incisors and molars, and gently it was directed caudally toward the right ramous of the mandible. Once the desired position was attained, the dose was injected and the syringe The animal was monitored after the was withdrawn. procedure to ensure that there are no adverse effects. The blood glucose level and body weight was measured once in every week. Blood was collected at the beginning, after 7 days, 14 days, retro-orbitally from the inner canthus of the eye using capillaries (Mucaps) in EDTA vial and at the end of the experiment after 21 days they were sacrificed by cervical dislocation under light ether anesthesia and blood was collected. Group no of rats and - Dose details are given in the Table 1 [9].

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3. Results

Estimation of biochemical parameters

Glucose was estimated at the beginning, after 7 days, 14 days and end of the experiment by using Glucometer (Contour ^{TS} Blood Glucose meter) and Contour ^{TS} Blood Glucose strip. Total cholesterol (TC), high-density lipoprotein cholesterol, (HDL-C) and triglycerides (TG) were estimated by enzymatic methods employing kits from Span diagnostics. LDL cholesterol was estimated by using Friedewald formula (1972) as follows: LDL in mg % = total cholesterol – (HDL-C –1/5 triglycerides).Urea and creatinine were estimated by enzymatic methods employing kits from Span diagnostics. BUN was calculated by the standard formula as follows: BUN concentration mg/dl= Urea concentration mg/dl X 0.467 [10].

Statistical Analysis

The results are analysed using mean<u>+SD</u> for all experiments and represented graphically. The significance of difference between data pairs was evaluated by analysis of variance (ANOVA).

Treated groups			Dose of	
	turmeric	aloe vera	tulsi	
T_0 (Diabetic control)	-	-	-	
T_1 (low dose mg /kg body weight)	50	100	50	
T_2 (medium dose mg /kg body	100	200	100	
weight)				
T_3 (high dose mg /kg body	150	300	200	
weight)				

Table 1: Group no and - Dose detail

The effects of oral administration of aqueous extract of combination doses of herbal preparation in streptozotocin induced diabetic rat are shown in **Table 2**. Whereas in diabetic untreated rat (T₀) Blood glucose continued to rise from 220+24mg/dl to 243+14.1mg/dl after 14days, and 244.67+44mg/dl after 21days, in diabetic rats having treatment with combination herbal preparation at low dose (T₁), blood glucose drecreases significantly (p=0.000) from 257.3+26.3mg/dl, to 222.67+47.43 mg/dl after 7days, 183+12.56 mg/dl and 153+6.37mg/dl after 14 days and 21 days respectively. When treated with medium dose (T₂) of combination herb, blood glucose level increases from

183+12.56 mg/dl and 153+6.37mg/dl after 14 days and 21 days respectively. When treated with medium dose (T₂) of combination herb, blood glucose level increases from 214.75+49.5mg/dl to 235.3+39.1mg/dl at the beginning of the experiment, and then decreases significantly (p=0.001) to 145.7+10.68 mg/dl and 122.3+11.02 mg/dl after 14 and 21 days respectively. With high dose treatment (T₃) the blood glucose fell significantly (p=0.000) from 244.37+28.3 mg/dl at day 01 to 182+7.56 mg/dl, 151.5+10.08 mg/dl and 138.5+6.57 mg/dl for day 07,day 14 and day 21 respectively in a consistant manner (**Fig. 1**).

 Table 2: Blood glucose level after treatment with combination herbal preparation in streptozotocin induced diabetic rat

Blood glucose mg/dl , mean \pm SD						
Group	0 days	07 days	14 days	21 days	F	p value
Diabetic untreated (T_0)	220 <u>+</u> 24	243 <u>+</u> 14	243 <u>+</u> 14.1	244.67 <u>+</u> 44	4.44	0.170
Low $dose(T_l)$	257.3 <u>+</u> 26.3	222.67 <u>+</u> 47.43	183 <u>+</u> 12.56	153 <u>+</u> 6.37	59.459***	0.000
Medium $dose(T_2)$	214.75 <u>+</u> 49.5	235.3 <u>+</u> 39.1	145.7 <u>+</u> 10.68	122.3 <u>+</u> 11.02	30.669***	0.001
High $dose(T_3)$	244.37 <u>+</u> 28.3	182+7.56	151.5 <u>+</u> 10.08	138.5+6.57	54.886***	0.000

***=1% level of significance, **= 5% level of significance and *=10% level of significance

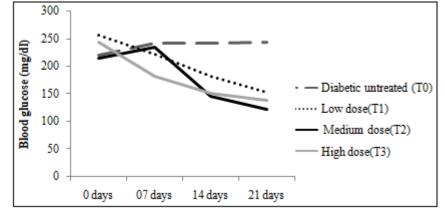


Figure 1: Effect of aqueous extract of combination herbal preparation on blood glucose level in diabetic control, and streptozotocin - induced treated diabetic rats.

Table 3 shows the effect of aqueous extract of combination hearbal preparation on lipid profile level in different doses, measured at the end of experiment in diabetic control and treated rats. Study result revealed that HDL-C level was 19.07 ± 1.29 mg/dl for untreated rat whereas after treatment

with herbal extract HDL-C level increases significantly for all three doses. A significant reduction (p=0.000) in the triglyceride and LDL % level with low, medium and high dose and in total cholesterol level with medium and high dose were observed (**Fig 2**)

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Group	T ₀	T_1	T_2	T ₃
HDL-C(mg/dl)	19.07 <u>+</u> 1.29	43.66 <u>+</u> 5.33	33.6 <u>+</u> 4.8	34.6 <u>+</u> 4.6
F		58.516***	24.659**	30.522***
<i>P</i> value		0.002	0.008	0.005
TG(mg/dl)	251.8 <u>+</u> 16.31	92.04 <u>+</u> 6.01	82.83 <u>+</u> 4.01	61.08 <u>+</u> 7.5
F		253.156***	303.338***	338.222***
<i>P</i> value		0.000	0.000	0.000
Total Cholesterol (mg/dl)	78.3 <u>+</u> 8.95	90.14 <u>+</u> 12.8	61.7 <u>+</u> 2.72	46.63 <u>+</u> 6.01
F		1.713	9.434**	25.841**
<i>P</i> value		0. 261	.037	.007
LDL%(mg/dl)	109.59 <u>+</u> 9.82	64.89 <u>+</u> 16.99	44.66 <u>+</u> 4.96	24.25 <u>+</u> 8.32
F		15.559**	104.433***	131.754***
<i>P</i> value		.017	.001	.000

 Table 3: Effect of aqueous extract of combination herbal preparation on lipid profile level in diabetic control, and streptozotocin - induced treated diabetic rats

***=1% level of significance, **= 5% level of significance and *=10% level of significance

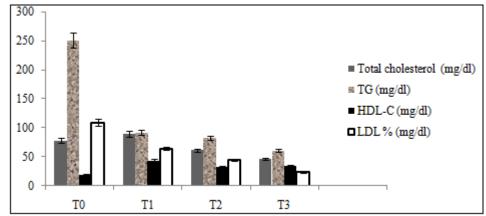


Figure 2: Effect of aqueous extract of combination herb on lipid profile in Control, and streptozotocin induced treated diabetic rats. (HDL= High density lipoprotein, LDL =Low density lipoprotein)

The effect of aqueous extract of combination hearbal preparation in different doses, on the value of urea, creatinine and BUN measured at the end of experiment in control, diabetic untreated and treated rats are shown in **Table 4**. A significant reduction in creatinine, urea and BUN was observed in the study. At the end of the experiment creatinine level was 1.28 ± 0.13 mg/dl for diabetic untreated rat whereas after treatment with combination hearbal

preparation it was 0.719 ± 0.009 mg/dl (55.465***), 0.577 ± 0.01 mg/dl (86.004***) and 0.36 ± 0.07 mg/dl (114.097***) for low, medium and high dose respectively. Urea level was found to be 66.4 ± 4.1 mg/dl for diabetic untreated rat and decreases upto 56.33 ± 3.70 mg/dl, (9.924**) and 42.7 ± 3.63 mg/dl (56.020***) for T₂ and T₃ respectively (**Fig 3 & Fig 4**).

 Table 4: Effect of aqueous extract of combination herbal preparation on urea, creatinine and BUN in diabetic control, and streptozotocin - induced treated diabetic rats

Sucpu	streptozotoeni induced treated diabetie rats				
Group	T_{θ}	T_{I}	T_2	T_3	
Creatinine(mg/dl)	1.28 <u>+</u> 0.13	0.719 <u>+</u> 0.009	0.577 <u>+</u> 0.01	0.36 <u>+</u> 0.07	
F		55.465***	86.004***	114.097***	
P value		0.002	0.001	0.000	
Urea(mg/dl)	66.4 <u>+</u> 4.1	75.6 <u>+</u> 3.88	56.33 <u>+</u> 3.70	42.7 <u>+</u> 3.63	
F		8.052	9.924**	56.020***	
P value		0. 047	0.035	0.002	
BUN(mg/dl)	31 <u>+</u> 1.91	35.33 <u>+</u> 1.8	26.3 <u>+</u> 1.73	19.94 <u>+</u> 1.69	
F		8.052**	9.924**	56.020***	
P value		0.047	0.035	0.002	

***=1% level of significance, **= 5% level of significance and *=10% level of significance

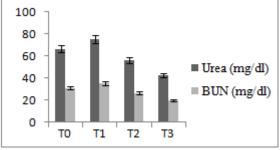


Figure 3: Effect of aqueous extract of combination herb on urea and BUN in control, and streptozotocin - induced treated diabetic rats

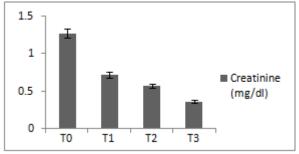


Figure 4: Effect of aqueous extract of combination herb on creatinine in control, and streptozotocin – induced treated diabetic rat

4. Conclusion

In the present investigation the effect of combination herbal preparation on diabetes was studied in adult Wister albino rats. Experimental result showed the anti-diabetic and antihyperlipidemic potential of combination herbal preparation on streptozotocin induced diabetic rats. Treatment of the diabetic animals with aloe vera, turmeric or C. longa and basil or O. sanctum for 21days showed reversal of blood glucose level. A significant reduction in lipid profile levels was observed in the study. Study result revealed that the HDL-C level increases significantly after treatment with the combination herbal preparation which is an important part of the study because diabetes is often associated with cardiovascular diseases and elevation in HDL-C level indicates lowering the chance of cardiovascular diseases. Reduction in TG, total cholesterol and LDL% also accounts for its anti-hyperlipidemic activity. Parameters like urea and creatinine and BUN that are closely associated with diabetic complication were also found to decrease significantly with combination herbal supplementation.

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