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Effect of Madhuriktha on Dexamathasone and Fructose Induced Insulin Resistance in Rats

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ABSTRACT

Madhuriktha a polyherbal formulation has been examined to investigate the effect of insulin resistance induced by dexamethasone and fructose. Dexamethasone was administered at a dose of 10 mg/kg S.C for 10 days and fructose was feed as 10% w/v aqueous solution in feeding bottles for 20 days. At end of the experimental period, body weight changes and serum biochemical parameters like glucose, insulin, triglycerides, cholesterol HDL and LDL levels were estimated. Madhuriktha at 200mg/kg and 400 mg/kg showed significant effect by normalizing the rise in serum glucose, insulin, triglyceride, cholesterol, HDL and LDL levels and improved the body weights.

Key words: Cholesterol, dexamethasone, fructose, glucose, insulin resistance, madhuriktha.

INTRODUCTION

Diabetes is a chronic disease, which occurs when the pancreas do not produce enough insulin, or when the body cannot effectively use the insulin it produces. This leads to an increased concentration of glucose in the blood (hyperglycemia). Diabetes is classified clinically as Type-1 characterized by insulin deficiency and Type-2 characterized by insulin inefficiency [1]. According to WHO diabetes is the world's largest endocrine disorder with abnormal carbohydrate, fat and protein metabolism [2]. It is estimated that by the year 2020 there will be approximately 250 million people affected with type-2-diabetes mellitus [3]. Insulin resistance plays an important role in the development of diabetes mellitus-2. Hormones such as catecholamine, glycogen, cortisol and thyroxin either act directly or influence other hormones their by causing disregulation of carbohydrate metabolism, elevation in glucose levels leading to insulin resistance. Insulin resistance is a common pathological state in which target cells fail to respond to ordinary levels of circulating insulin resulting in disregulation in lipid homeostasis and glucose regulation [4-5].

Exposure to glucocorticoids in large amounts inhibits insulin secretion from pancreatic-β-cells, decreases glucose utilization; stimulates glycogen secretion, lipolysis, proteolysis and hepatic glucose production. All glucocorticoids including dexamethasone cause insulin resistance by decreasing glycogen synthesis. Free fatty acids may also be elevated because of impaired insulin-dependent down regulation of lipolysis, hence leading to increased triglyceride levels in muscle and tissues. Triglycerides intern are reported to be potent inhibitors of insulin signaling, causing acquired insulin resistance [6-7]. High fructose consumption increases likelihood of weight gain and reduces circulating leptin concentration leading to insulin resistance [8] associated with hyperinsuliemia, hypertriglyceridemia and hyperglycemia [9]. Fructose alters the several enzymes and hepatic carbohydrate metabolism causing hepatic insulin resistance.

Numerous herbal like *Momordica charantia, Tectonia grandis, Tinospora cardifolia, Occimum sanctum, oryza sativa, Panax ginseng, Allium sativum, Cinnamomum zeylanicum* and formulations like Triphala, SH01D, D-400 have been used by people of various cultures to treat diabetes [10]. Madhuriktha is a commercially available marketed polyherbal formulation procured from Samraksha Ayurvedic Pharmacy (Mfg dt: June 2009, Batcch no: 1001, Exp dt: May 2011) formulated with plants mentioned in ayurveda. It contains aqueous extracts equivalent to Gumar (*Gymnema sylvestre* leaves) 200 mg, Saptarangi (*Salacia chinensis* roots) 200 mg, Jamun (*Syzgium cumini* roots) 100 mg, Karela (*Momordica charantia* seeds) 50 mg, Fenugreek (*Trigonella foenum graecum* seeds) 100 mg, Manjistha (*Rubia cordifolia* roots) 100 mg, Gudichi (*Tinospora cardifolia* stem) 30 mg, Gokhuru (*Tribulus terrestris* fruit) 30 mg, chitrak (*Plumbago zeylanica* roots) 20 mg, Haridica (*Curcuma longa* rhizome) 25mg, Ashwagandha (*Withania somnifera* roots) 30 mg, Amla (*Emblica officinalis* fruit) 30 mg, Myrobalan (*Terminalia chebula* fruit) 25 mg, chandan (*Santalum album* wood) 40 mg, Punarnava (*Boerhaavia diffusa* seeds) 20 mg. Though Madhuriktha formulation has been claimed to possess Antidiabetic activity due to the presence of multiple ingredients, experimental study in laboratory animals with insulin resistance has not been done. Hence the present was conducted to find out the effect of Madhuriktha formulation on dexamethasone and fructose induced insulin resistance.

MATERIALS AND METHODS

Animals

Male wistar albino rats weighing 160-200 gm were procured from NIN. The animals were maintained at standard environmental conditions of temperature, relative humidity, dark and light cycles. They were fed with standard diet and water *ad libitum* during experimental period. The experimental protocol was approved by Institutional Animal ethics Committee (IAEA) of G. Pulla Reddy College of Pharmacy (Reg No.CPCSEA320-01/10)

Dose selection

Madhuriktha at doses of 200 and 400 mg/kg b.w were used for study based on human being dose.

Experimental design

Dexamethasone induced insulin resistance in rats (Shalam et al., 2006).

All rats were weighed before treatment and then were divided into four groups of six animals each. Animals were kept for overnight fasting one day prior to experiment.

Group I: Normal control received water and fed with normal diet.
Group II: Dexamethasone control received dexamethasone sodium phosphate 10 mg/kg, s.c for 10 days.
Group III: Received madhuriktha at a dose of 200 mg/kg, p.o along with dexamethasone sodium phosphate 10 mg/kg, s.c for 10 days.
Group IV: Received madhuriktha at a dose of 400 mg/kg, p.o along with dexamethasone sodium phosphate 10 mg/kg, s.c for 10 days.

On the11th day of the experiment, the animals were weighed and later anaesthetized with ether, blood was collected from retro orbital puncture and serum was separated for the estimation of glucose, insulin, triglycerides, cholesterol HDL and LDL. All the biochemical parameters were performed using span diagnostic reagent kits. Serum insulin was estimated by Immunochem radioimmunoassay method using standard kit obtained from BRIT, BARC, Mumbai, India.

Fructose induced insulin resistance in rats (Shalam et al., 2006).

All rats were weighed before treatment and then were divided into four groups of six animals each.

Group I: Normal control received water and fed with normal diet.
Group II: Fructose control fed with 10% w/v fructose solution ad libitum in feeding bottles for 20 days.
Group III: Animals were fed with 10% w/v fructose solution ad libitum in feeding bottles and treated with madhuriktha at a dose of 200 mg/kg, p.o for 20 days.
Group IV: Animals were fed with 10% w/v fructose solution ad libitum in feeding bottles and treated with madhuriktha at a dose of 400 mg/kg, p.o for 20 days.

All the animals were fasted for half an hour prior to the madhuriktha administration. On 21st day all the animals were weighed an later anaesthetized with ether, blood was collected from retro orbital puncture and serum was separated for the estimation of glucose, triglycerides, cholesterol, HDL and LDL using span diagnostic reagent kits. Serum insulin was estimated by Immunochem radioimmunoassay method using standard kit obtained from BRIT, BARC, Mumbai, India. The animals were sacrificed by cervical dislocation, liver was washed immediately with 3ml of 30% KOH solution for determining glycogen content using anthrone reagent.

Statistical analysis

The results were expressed as Mean ± SEM and analysis was carried out by one-way ANOVA. Post-hock analysis was done by Dunett's multiple comparison tests to estimate the significance of difference between various individual groups. ***P*<0.05 was considered significant.

RESULTS

Effect of madhuriktha on dexamethasone induced insulin resistance model.

Animals treated with Dexamethasone significantly increased serum glucose, insulin, triglycerides and cholesterol when compared to normal control group. No changes were observed in HDL and LDL levels. All rats treated with dexamethasone and madhuriktha at dose levels of 200 and 400 mg/kg b.w p.o showed significant decrease (P<0.05) in the levels of serum glucose, insulin, triglycerides and cholesterol and the activity was found to be dose dependent. Decrease in body weight was observed in dexamethasone group when compared to normal control group. Madhuriktha at a dose of 200 and 400 mg/kg b.w inhibited dexamethasone induced decrease in body weight when compared to disease control. Results are shown in table1 and figure 1-3.

Table I. Effect of madhuriktha on serum glucose, insulin, triglycerides, cholesterol and body weight change on dexamethasone induced insulin resistance in rats[a]

S.No	Groups	Serum glucose (mg/dl)	Serum insulin (mg/dl)	Serum triglycerides (mg/dl)	Serum cholesterol (mg/dl)	Body weight change (gm)
1	Normal control	72 ±0.49	0.713±0.0145	62.3±0.55	114.5±0.62	16.6±3.3
2	Dexamethasone control	129.8±1.74	18.96±0.088	122±0.57	140.5±0.61	-30.66±3.073
3	Dexamethasone + madhuriktha 200mg/kg	80.5±0.42**	0.908±0.0047**	85±0.81**	79±0.73*	25.1±0.09**
4	Dexamethasone + madhuriktha 400mg/kg	76.6±1.22**	0.884±0.0109**	75±0.57**	76±1.09*	26.83±0.85**

*[a]Values are expressed as Mean ±SEM, n=6, *P < 0.05, **P < 0.01 when compared to dexamethasone control using one-way ANOVA Dunett's test*

Figure I. Effect of madhuriktha on serum glucose in dexamethasone induced insulin resistance in rats

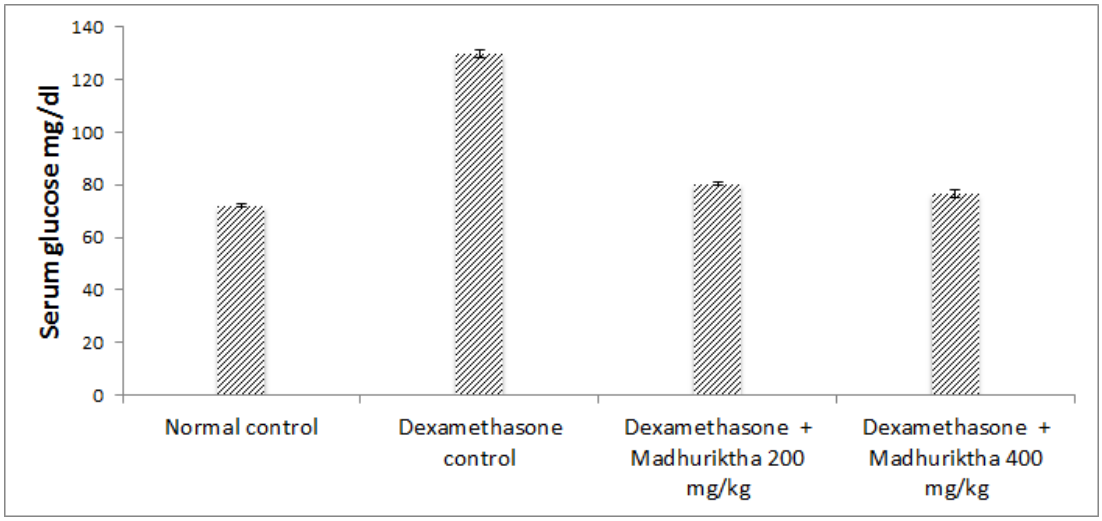


Table II. Effect of madhuriktha on serum glucose, insulin, triglycerides, cholesterol, HDL and LDL and glycogen and body weight in fructose induced insulin resistance[a]

S.No	Groups	Serum glucose (mg/dl)	Serum insulin (mg/dl)	Serum triglycerides (mg/dl)	Serum cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Glycogen levels (µg/mg of tissue) liver	Body weight change (gm)
1	Normal control	53.5 ±6.71	0.839±0.005	71.5±0.86	78±0.73	40.44±0.298	22.1±0.1514	31.6±1.31	13.6±2.83
2	Fructose control	103.5±1.54	20.28±0.243	100.3±0.94	81±1.91	33.5±0.138	63.73±0.24	21.08±0.55	28.66±2.1
3	Fructose feeding +madhuriktha 200mg/kg	97.6±0.42**	1.078±0.0135**	82.5±0.81**	78±0.73*	34.35±0.082*	61.62±0.158**	25.1±0.9*	18.2±3.23**
4	Fructose feeding +madhuriktha 400mg/kg	75.5±1.22**	0.951±0.0129**	73.6±0.57**	77.5±1.09*	38.41±0.1553**	29±0.1883**	26.83±0.85*	15.3±2.2**

*[a]Values are expressed as Mean ±SEM, n=6, *P < 0.05, **P < 0.01 when compared to fructose control using one-way ANOVA Dunett's test*

Figure II. Effect of madhuriktha on serum insulin in dexamethasone induced insulin resistance in rats.

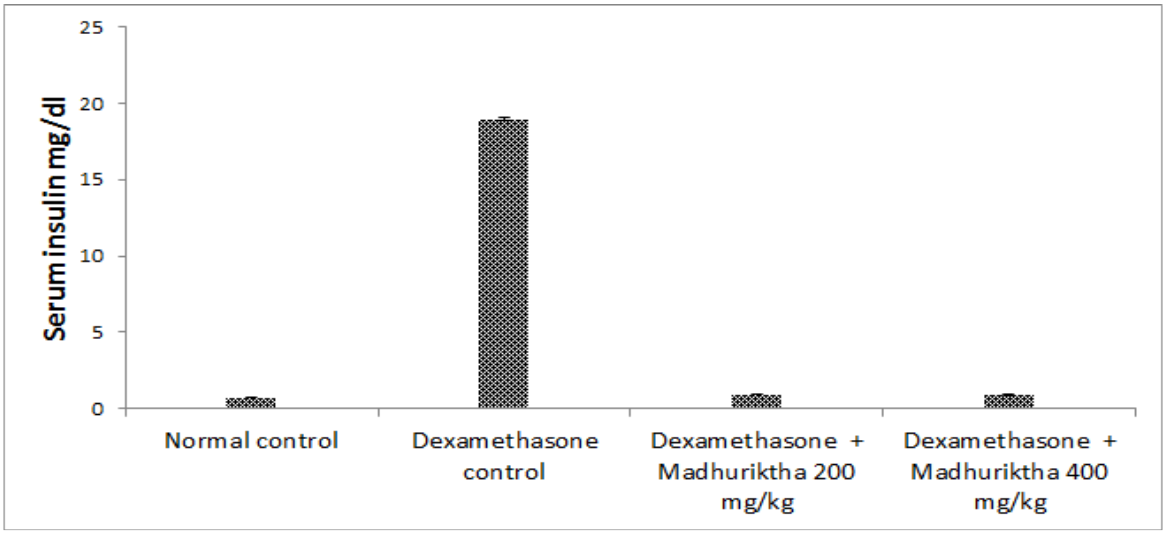


Figure III. Effect of madhuriktha on serum triglycerides and cholesterol in dexamethasone induced insulin resistance in rats

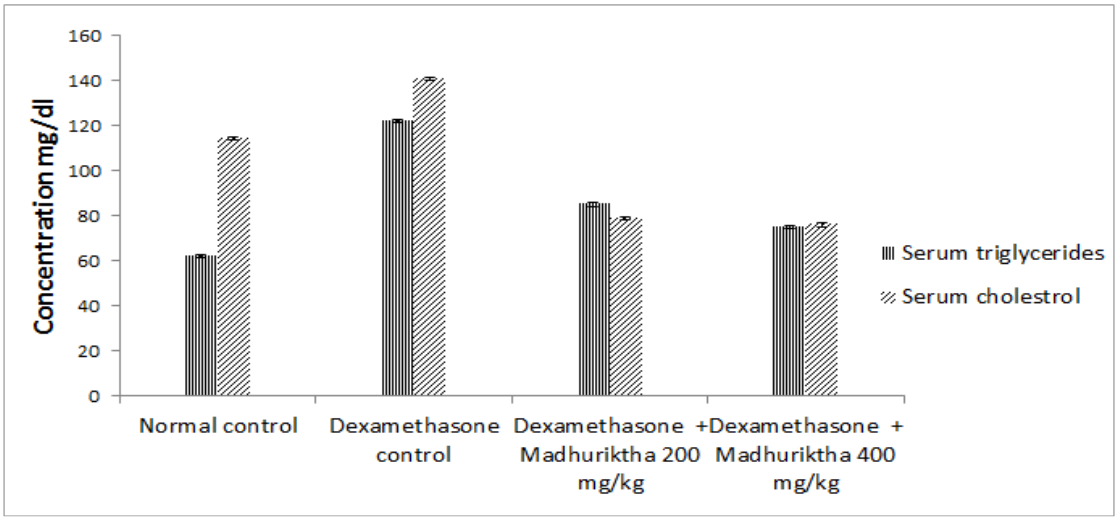


Figure IV. Effect of madhuriktha on serum glucose in fructose induced insulin resistance in rats

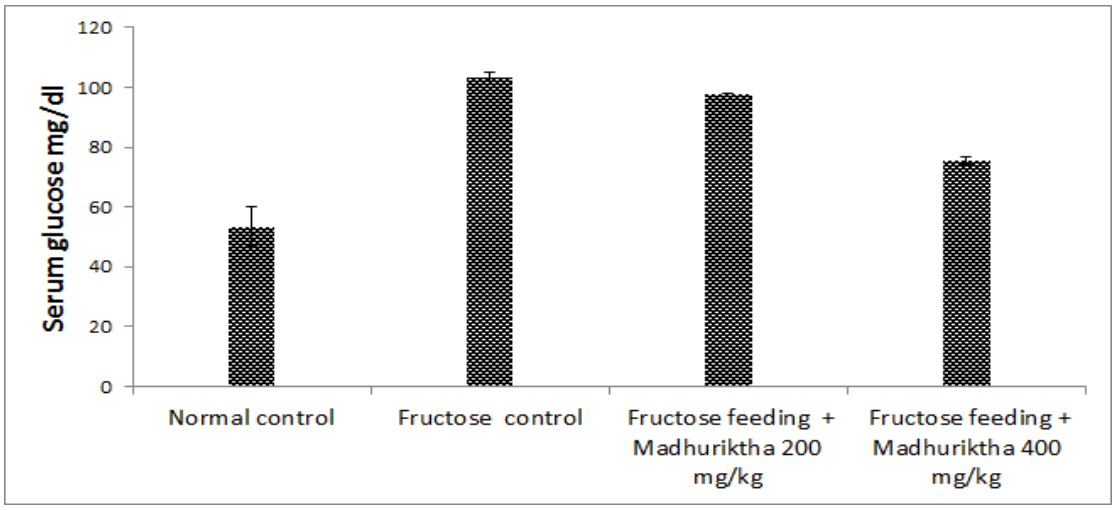


Figure V. Effect of madhuriktha on serum insulin in fructose induced insulin resistance in rats

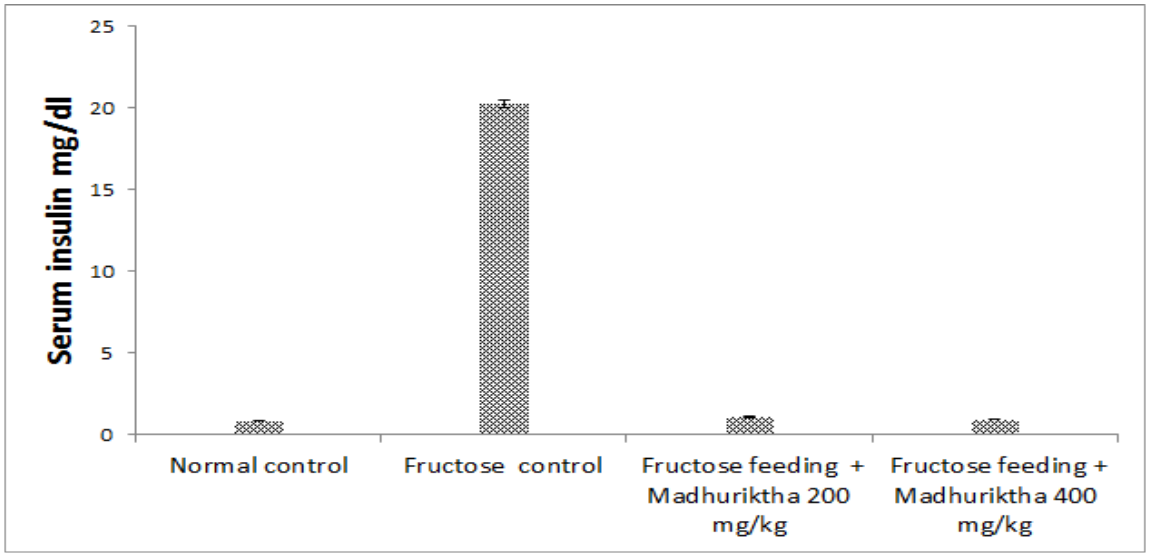
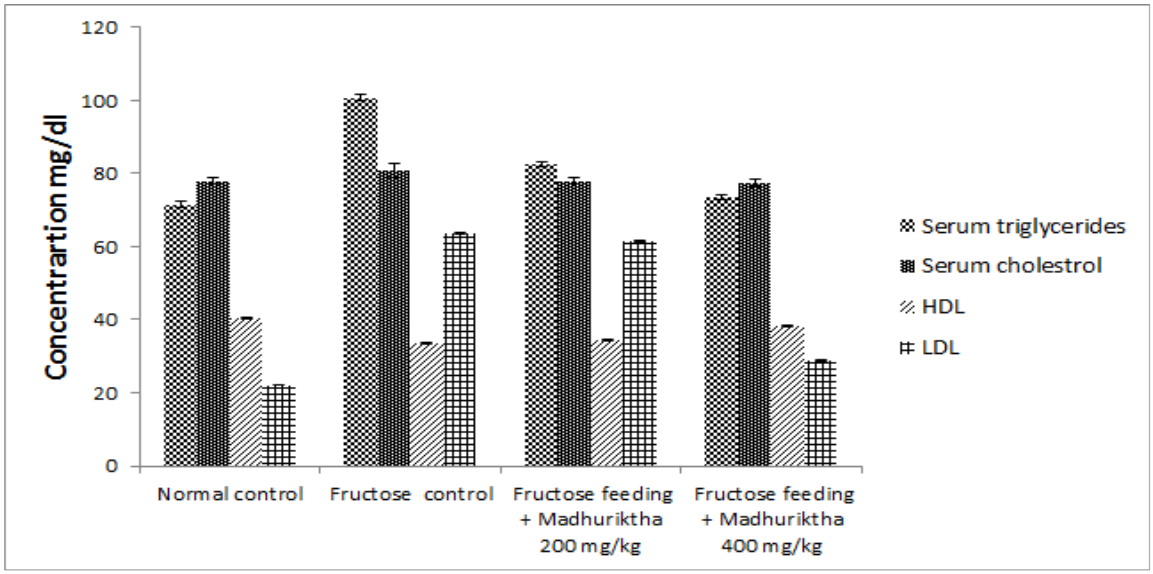


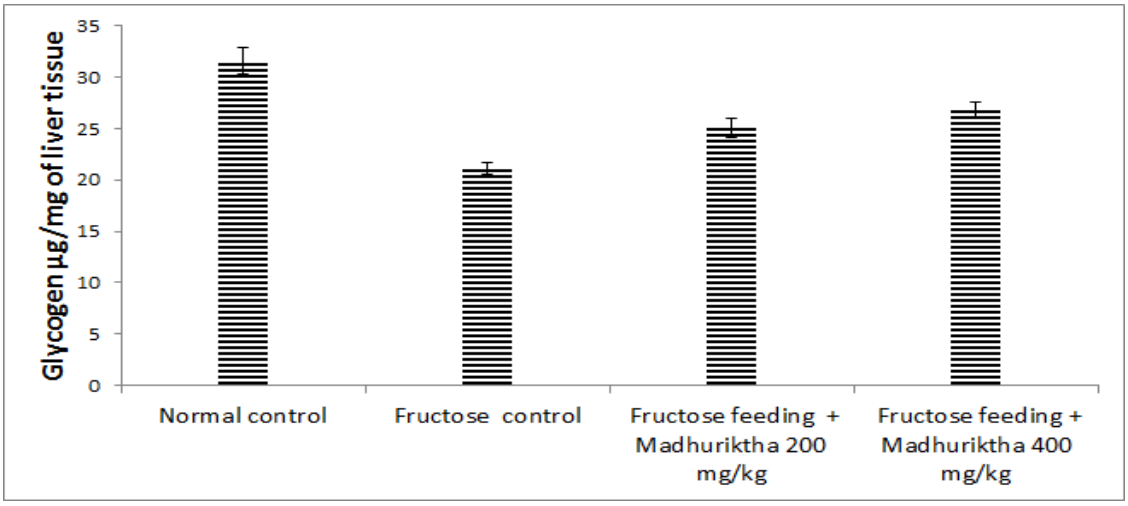
Figure VI. Effect of madhuriktha on serum triglycerides, cholesterol, HDL and LDL in fructose induced insulin resistance in rats



Effect of madhuriktha on fructose induced insulin resistance model.

Fructose feeding to disease control rats has shown significance increase in serum glucose, insulin, triglycerides, cholesterol, HDL and LDL levels when compared to normal control rats. Animals treated with madhuriktha for 20 days at a dose of 200 and 400 mg/kg b.w showed significant ($P<0.05$) decrease in serum glucose, insulin, triglycerides, cholesterol levels and the activity was found to be dose dependent. In disease control there was a steep increase in bodyweight, however bodyweight were reverted to near normal when treated with madhuriktha at 200 and 400 mg/kg b.w. The decrease in glycogen levels was also significantly ($P<0.05$) prevented in a dose dependent manner as compared to disease control. Results are shown in table2 and figure 4-7.

Figure VI. Effect of madhuriktha on glycogen level in fructose induced insulin resistance in rats.



DISCUSSION

Insulin resistance precedes the development of type-2- diabetes, obesity, atherosclerosis and other associated cardiovascular diseases. Thus interventions to decrease insulin resistance may postpone the development of diabetes and its complications. Treatment with herbs has been a better choice because they are effective with fewer side effects and are affordable as compared to presently used synthetic oral antidiabetic drugs. Among the various constituents of Madhuriktha, reports suggest that the aqueous leaf extract of *Gymnema sylvestre* showed significant reduction of glucose, cholesterol, triglycerides and LDL levels in diabetic rats [11]. The ethanolic extract of *Curcuma longa* lowered blood sugar levels [12], *Tinospora cordifolia* had significant antihyperglycemic effect [13]. *Momordica charantia* at a dose of 400 mg/day had prevented hyperglycemia, hyperinsulinemia in rats fed with fructose rich diet [14], *Emblica officinalis* had antiatherosclerotic and lipid lowering properties in rats fed with atherogenic diet.

Dexamethasone is a potent and highly selective glucocorticoid used in the treatment of inflammation. High exposure to glucocorticoids impairs insulin sensitivity, contributing to the generation of metabolic syndrome including insulin resistance and hypertension [15]. The mechanism by which dexamethasone induces peripheral insulin resistance is by inhibiting GLUT-4 translocation, increasing lipase activity in adipose tissue their by causing impairment of endothelium-dependent vasodilation [16]. Dexamethasone increases the triglycerides levels causing an imbalance in lipid metabolism leading to hyperlipidemia [17] and increases glucose levels leading to hyperglycemia [18] Pharmacological doses of glucocorticoids induces ob gene expression in rat adipose tissue within 24 hrs and is followed by a complex metabolic changes resulting in decrease in food consumption causing reduction in body weight accompanied by diabetes and development of Insulin resistance with enhanced glucose and triglycerides levels [7 &19]. In the present study administration of dexamethasone for 10days resulted in increased glucose, insulin, triglycerides, cholesterol levels and decrease in body weight. Madhuriktha at a dose of 200 and 400 mg/kg significantly prevented the rise in glucose, triglycerides and cholesterol levels. Further Madhuriktha also prevented the progressive decrease in the body weight caused by dexamethasone.

High fructose consumption leads to obesity and metabolic abnormalities as observed in insulin resistance syndrome. Fructose as such doesn't stimulate insulin secretion from pancreatic-β-cells, leptin a adipose derived hormone production is regulated by insulin in response to meals, consumption of foods and beverages containing fructose reduces circulating leptin concentration leading to insulin resistance [8-9]. The rats fed with high fructose diet provide an animal model of insulin resistance associated with weight gain, hyperinsulinemia, hyperlipidemia, hyperglycemia [9]. The use of 10% w/v fructose in drinking water for a period of 20 days significantly raised glucose, insulin, triglycerides, cholesterol and bodyweights with a decline in liver glycogen levels. Administration of madhuriktha at 200 and 400 mg/kg b.w prevented the development of hyperglycemia, hyperinsulinemia and hypertriglyceridemia. Madhuriktha might have improved insulin sensitivity in peripheral tissues, as this was evident from the results showing decreased glucose and insulin production and increased glycogen stores.

Thus the above results indicate that madhuriktha a polyherbal formulation has preventive effect on both dexamethasone and fructose induced insulin resistance.

CONCLUSION

Madhuriktha a polyherbal formulation at 200mg/kg and 400mg/kg prevented the development of hyperglycemia, hyperinsulinemia, hypercholesteremia and hypertriglyceridemia in dexamethasone induced and fructose induced insulin resistance models. Body weighs were decreased in dexamethasone model and increased in fructose model. So this polyherbal formulation could be a better choice and might be useful in prevention of insulin resistance in non-diabetic states such as obesity and impaired glucose tolerance.

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