

**CHANCES OF REDUCTION IN CARDIOVASCULAR RISK BY  
DRAKSHARISHTA IN INDUCED DIABETIC CONDITION**

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**Summary**

The objective of the present study was to evaluate the effect of Draksharishta-T and Draksharishta-M prepared by traditional and modern methods respectively and Dabur Draksharishta on fasting blood glucose and serum lipid profile in alloxan induced diabetic rats. Oral administration of Draksharishta-T, Draksharishta-M and Dabur Draksharishta ( 2 ml/kg p.o.) for 21 days caused a significant decrease in fasting blood glucose (FBG) and showed significant rise in blood glutathione level (GSH) in diabetic rats. Glibenclamide was used as a standard antidiabetic drug (10 mg/kg, p.o). These preparations also caused significant reduction in serum cholesterol, LDL and triglycerides and showed significant rise in serum HDL level in diabetic albino rats. Thus all these preparations were able to maintain the tested parameters near to the normal level significantly.

**Key words:** Cardiovascular risk, Blood glucose, anti-diabetic, Glutathione, Lipid profile, Draksharishta, alloxan

## Introduction

Diabetics have accelerated levels of oxidative stress and this contributes massively to most cardiovascular, neurological, retinal and renal diabetic complications<sup>1</sup>. Diabetes mellitus is a heterogeneous metabolic disorder as old as mankind and its incidence is considered to be high all over the world<sup>2</sup>. It is characterized by hyperglycemia. Hyperglycemia significantly diminishes glutathione levels lowering defences against oxidative stress<sup>3</sup>.

A multitude of herbs, spices and other plant material has been described for the treatment of diabetes throughout the world<sup>4</sup>. Furthermore, after the recommendations made by WHO (World Health Organization) on diabetes mellitus, investigations on hypoglycemic agents from medicinal plants have become more important. The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents the risk factor for coronary heart disease. Moreover, diabetic patients experience a two to three fold increase in cardiovascular morbidity and mortality in comparison to non diabetics<sup>5</sup>.

Draksharishta is a polyherbal hydroalcoholic preparation and is used as blood purifier, in the treatment of anaemia and advised as a choice of remedy in respiratory problems. The chief ingredient of Draksharishta is draksha, dried fruits of *Vitis vinifera*<sup>6</sup>. The composition and properties of fruits of *Vitis vinifera*, have been extensively investigated and it was reported that they contain large amount of phenolic compounds as catechins, epicatechin, quercetin, and gallic acid, dimeric, trimeric and tetrameric procyanidins<sup>7</sup>. These compounds have many favourable effects on human health such as lowering of human low density lipoproteins, reduction of heart disease and cancer<sup>8-11</sup>.

Therefore we undertook the present investigation to evaluate the antidiabetic effect of Draksharishta-T and Draksharishta-M prepared by traditional and modern methods respectively and also Dabur Draksharishta in alloxan induced hyperglycemic rats. Effect of these preparations was also evaluated on the serum lipid profile of alloxan induced diabetic rats.

## Materials and Methods

### Preparation of Draksharishta

#### Draksharishta-T

This was prepared by the method as given in Ayurvedic Formulary of India<sup>6</sup>. The ingredients of Draksharishta were procured from Local market, Jamnagar. Identification of all the individual plant material was done as per Ayurvedic Pharmacopoeia of India. Authentification of all these ingredients was done in the Botany Department of Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow. Prepared herbarium has been deposited in the CIMAP for future reference. According to this method, dried fruits of *Vitis vinifera* after proper crushing were placed in polished vessel of brass along with prescribed quantity of water (13L), and allowed to steep overnight. After overnight steeping, this material was warmed at medium flame until the water for decoction reduced to one fourth of the prescribed quantity (3.25L), then the heating was stopped and it was filtered through unstarched muslin cloth in cleaned and fumigated vessel and after that jaggery was added and mixed properly.

Then Dhataki flowers (*Woodfordia floribunda*) and prescribed quantity of coarsely powdered prakshepa dravyas as *Cinnamomum zeyleynicum*( stem bark), *Elettaria cardamomum*( seeds), *Cinnamomum tamala* ( leaves) , *Mesua ferrea* ( stamens), *Callicarpa macrophylla* ( flowers), *Piper nigrum* ( fruits), *Piper longum* (fruits), *Embelia ribes* (fruits) were added and this sweet filtered fluid was placed for fermentation in incubator for fifteen days at  $33^{\circ}\text{C}\pm 1^{\circ}\text{C}$ . After fifteen days completion of fermentation was confirmed by standard tests<sup>12</sup>.

The fermented preparation was filtered with unstarched muslin cloth and kept in cleaned covered vessel for further next seven days. Then, it was poured in clean amber coloured glass bottles previously rinsed with ethyl alcohol, packed and labelled properly.

### **Draksharishta-M**

Method of preparation was same as followed with Draksharishta -T, only dhataki flowers were replaced with Yeast for inducing fermentation<sup>13</sup>.

### **Animals**

Adult Wistar albino rats, weighing between 200-220g of either sex were acclimatized to normal environmental conditions in the animal house for one week. The animals were housed in standard polypropylene cages and maintained under controlled room temperature ( $22^{\circ}\text{C}\pm 2^{\circ}\text{C}$ ) and humidity ( $55\pm 5\%$ ) with 12:12 hour light and dark cycle. All the animals were given a standard chow diet (Hindustan Lever Limited), and water *ad libitum*. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Government of India were followed and prior permission was granted from the Institutional Animal Ethics Committee (CPCSEA No. 07/09).

### **Induction of Diabetes**

The animals were fasted for 18 h and made diabetic by injecting Alloxan monohydrate (150mg/kg, i.p.) dissolved in sterile normal saline. In order to stave off the hypoglycaemia during the first day, 5% w/v glucose solution was given orally to diabetic rats after four to six hours of alloxan administration. The diabetic state was confirmed when the blood sugar level was greater than 180 mg/dl<sup>14</sup>.

### **Treatment Protocol**

All the animals were randomly divided into the six groups with six animals in each group. All the three types of Draksharishta as Draksharishta-T, Draksharishta-M and Dabur Draksharishta were given at a dose of 2 ml/kg body weight, orally daily for a period of 21 days to different groups of diabetic animals.

- Group I : Normal animals received normal saline as vehicle (2 ml/kg, p.o.)
- Group II : Diabetic animals received normal saline as vehicle (2 ml/kg, p.o.)
- Group III : Diabetic animals received glibenclamide (10 mg/kg, p.o.)<sup>15</sup>
- Group IV : Diabetic animals received Draksharishta-T (2 ml/kg, p.o.)
- Group V : Diabetic animals received Draksharishta-M (2 ml/kg, p.o.)
- Group VI : Diabetic animals received Dabur Draksharishta (2 ml/kg, p.o.)

Albino rats were made diabetic by a single intra-peritoneal injection of Alloxan monohydrate (Loba Chemie, Mumbai). Alloxan was first weighed individually for each animal according to the weight and then solubilized with 0.2ml saline just prior to injection.

Two days after Alloxan monohydrate injection rats with blood glucose level of greater than 180mg/dl were selected for the present study. Treatment with Draksharishta-T, Draksharishta-M and Dabur Draksharishta was started 48h after Alloxan injection. Blood samples were drawn at weekly interval until the end of study i.e three weeks. Fasting blood glucose (FBG), blood glutathione (GSH) estimation and body weight measurement were done on day 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> of the study. On 21<sup>st</sup> day blood was collected by retro orbital plexus under mild ether anaesthesia and fasting blood glucose was estimated<sup>16</sup>. Serum was separated and analyzed for serum cholesterol<sup>17</sup>, serum triglycerides<sup>18</sup>, serum HDL<sup>19</sup>, serum LDL<sup>19</sup>, serum creatinine<sup>20</sup>, serum urea<sup>21</sup>, serum alkaline phosphatase<sup>22</sup>, blood glutathione<sup>23</sup> by using Span and Erba diagnostic kits.

### **Drugs and Chemicals**

Alloxan monohydrate was purchased from Loba Chemie, Mumbai. Standard antidiabetic drug glibenclamide was obtained from Ranbaxy Research Laboratories, Gurgaon, India.

### **Statistical Analysis**

The results were expressed as mean±SEM. Statistical analysis of data among the various groups was performed by using one way analysis of variance (ANOVA) followed by the Tukey test of significance.

## **Results**

### **Effect of Different Types of Draksharishta on FBG and GSH of Diabetic rats**

All types of Draksharishta as Draksharishta-T, Draksharishta-M and Dabur Draksharishta produced significant ( $P<0.001$ ) reduction in FBG level in alloxan induced diabetic rats [Table 1]. Glibenclamide produced significant reduction in FBG level (60%) which was highest as compared to that produced by all types of Draksharishta [Table 1]. In our study, in alloxan treated diabetic rats the blood Glutathione level (GSH) decreased significantly ( $P<0.001$ ). Draksharishta-T, Draksharishta-M, Dabur Draksharishta (2 ml/kg p.o.) and glibenclamide (10 mg/kg p.o.) showed significant increase in GSH level on both 14<sup>th</sup> and 21<sup>st</sup> day of treatment (Table 1).

### **Effect of Different Types of Draksharishta on Body weight of Diabetic rats**

All the Draksharishta preparations as Draksharishta-T, Draksharishta-M, Dabur Draksharishta and glibenclamide exhibited significant antihyperglycemic activity in alloxan induced diabetic rats without causing significant change in body weight (Table 2).

### **Effect of Different Types of Draksharishta on Serum Lipid Profile of Diabetic rats**

Draksharishta-T and Draksharishta-M at the dose of 2ml/kg body weight orally significantly reduced total serum cholesterol (40% and 39%), serum LDL (48% and 48%) and triglycerides (42% and 41%) when compared to diabetic control. The increase in HDL was (36% and 34%) with Draksharishta-T and Draksharishta-M

respectively. Draksharishta-T and Draksharishta-M also significantly reduced serum creatinine (64% and 63%), serum urea (44% and 42%) and serum alkaline phosphatase levels (53% and 52%) [Table 3]. Dabur Draksharishta produced similar effects on the serum lipid profile of diabetic rats as that of Draksharishta- T and Draksharishta-M whereas glibenclamide caused highest significant ( $P < 0.001$ ) reduction in the levels of serum cholesterol, triglyceride and LDL as well as significant improvement in the level of HDL- cholesterol in diabetic rats (Table 3).

**Table 1: Effect of Draksharishta-T and Draksharishta-M on Fasting blood glucose and Blood glutathione level of diabetic rats**

Different groups and treatment(p.o.)	Fasting blood glucose and Blood glutathione level ( mg/dl) ( mean±SEM)							
	Day 1		Day 7		Day 14		Day 21	
	FBG	GSH	FBG	GSH	FBG	GSH	FBG	GSH
Normal + Normal saline(2ml/kg)	66.8±2.1	27.71±0.7	72.01±2.2	24.25±0.67	73.5±2.8	25.0±0.49	75.6±2.3	24.5±0.39
Diabetic control + normal saline (2ml/kg)	205.15±3.15	14.17±0.71	267.72±2.95	14.01±2.1	285.79±4.85	13.7±2.7	291.70±6.5	12.79±2.03
Diabetic + glibenclamide (10mg/kg)	202.15±3.0	17.8±1.0	180.63±6.1	20.48±1.8	136.16±2.3	22.73±1.2	116.72±4.2*	25.63±2.6*
Diabetic + Draksharishta-T(2ml/kg)	202.25±1.85	15.42±2.14	198.0±0.75	15.95±2.27	182.15±2.96	16.36±2.85	173.0±2.41*	16.84±1.82*
Diabetic + Draksharishta-M (2ml/kg)	202.56±2.41	16.32±1.87	197.14±1.48	16.54±1.89	180.62±3.24	16.76±1.94	171.0±1.96*	16.97±2.48*
Diabetic + Dabur Draksharishta (2ml/kg)	202.34±1.92	15.62±1.82	197.52±2.16	15.98±2.41	181.27±1.48	16.45±3.14	172±3.21*	16.91±2.48*

\* $P < 0.001$  as compared to diabetic control group, n = 6 in each group

**Table 2: Effect of Draksharishta-T and Draksharishta- M on body weight of alloxan induced diabetic rats**

Different groups and treatment (p.o.)	Average Body Weight( g)( mean±SEM)			
	Day 1	Day 7	Day 14	Day 21
Normal + Normal saline(2ml/kg)	200.2±3.4	203.4±2.6	204.12±4.2	206.19±4.5
Diabetic control + normal saline (2ml/kg)	200.5±4.6	169.43±3.4	160.47±4.2	142.7±2.4
Diabetic + glibenclamide (10mg/kg)	208.4±2.4	194.27±1.6	190.8±2.7	187.37±3.4*
Diabetic + Draksharishta-T(2ml/kg)	209.2±2.6	194.15±3.8	182.14±2.4	180.24±2.4*
Diabetic + Draksharishta-M (2ml/kg)	209.5±4.2	195.27±2.8	183.79±3.5	181.17±3.2*
Diabetic + Dabur Draksharishta (2ml/kg)	209.0±3.14	194.24±2.14	182.27±1.86	180.12±3.17*

\* $P < 0.001$  as compared to diabetic control group, n = 6 in each group

**Table 3: Effect of Draksharishta-T and Draksharishta- M on serum lipid profile of diabetic rats after three weeks of treatment**

Different groups and treatment (p.o.)	Serum cholesterol (mg/dl)	Serum LDL (mg/dl)	Serum HDL (mg/dl)	Serum triglycerides (mg/dl)	Serum creatinine (mg/dl)	Serum urea (mg/dl)	ALP(IU/L)
Normal + Normal saline(2ml/kg)	155.0±6.2	91.5±4.1	47.46±1.3	84.65±5.2	0.49±0.79	25.7±1.2	118.72±2.2
Diabetic control + normal saline(2ml/kg)	275.35±17.5	195.47±10.5	27.17±0.31	200.0±11.7	1.72±0.32	64.85±1.7	318.42±5.9
Diabetic + glibenclamide (10mg/kg)	146.13±6.0*	84.17±3.2*	39.17±1.71*	109.1±4.8*	0.52±0.21*	29.06±2.05*	122.63±3.92*
Diabetic + Draksharishta-T(2ml/kg)	166.24±4.5*	101.21±3.4*	36.98±1.24*	115.47±1.72*	0.62±0.36*	36.44±2.91*	150.0±5.72*
Diabetic + Draksharishta-M (2ml/kg)	168.41±3.6*	100.79±2.94*	36.52±0.72*	117.12±2.94*	0.64±0.27*	37.25±3.24*	152.14±4.72*
Diabetic + Dabur Draksharishta(2ml/kg)	167.35±2.4*	100.94±3.2*	36.84±0.86*	116.12±2.14*	0.63±0.24*	36.86±1.48*	150.8±3.42*

All values are expressed as mean± SEM, n = 6 in each group; \* $P < 0.001$  as compared to diabetic control

### Discussion

Administration of alloxan caused rapid destruction of pancreatic  $\beta$  cells in rats, which led to impaired glucose stimulated insulin release and insulin resistance, both of which are marked feature of type II diabetes<sup>24</sup>. This is because administration of alloxan (150mg/kg i.p.) led to more than 1.5 fold elevation of fasting blood glucose level, which was maintained over a period of three weeks. Three week of daily treatment of Draksharishta-T, Draksharishta-M and Dabur Drakshrishta (2ml/kg p.o.) caused significant fall in blood glucose level.

GSH, being the most important bio-molecule against chemically induced toxicity can participate in the elimination of reactive intermediates by reduction of hydro-peroxidase in the presence of glutathione per-oxidase. The most important mechanism implicated in the diabetogenic action of alloxan is by increased generation of oxygen free radicals which cause a decrease in plasma GSH concentration. Hence, drugs that could prevent the generation of these oxygen free radicals or increase the free radical scavenging enzymes may be effective in alloxan induced diabetes<sup>25</sup>.

In the present study, the observed significant increase in blood glucose level and a decrease in blood glutathione levels in diabetic rats could be due to destruction of  $\beta$  – cells by alloxan reinforcing the view that alloxan induced diabetes probably through the generation of oxygen free radicals<sup>26</sup>.

The standard anti-diabetic drug glibenclamide and the test preparations as Drakshrishta-T, Draksharishta-M and Dabur Draksharishta showed that they could prevent the development of Diabetes mellitus in albino rats, due to their antioxidant property since they showed a significant decrease in fasting blood glucose level (FBG) along with significant increase in blood glutathione level (GSH) after treatment.

Vehicle control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during 21 days [Table 2]. Alloxan caused body weight reduction, which was improved by standard drug (glibenclamide) and test preparations (Draksharishta-T, Draksharishta-M and Dabur Draksharishta) nearly equal to the normal.

In general, an increase in blood glucose level is usually accompanied by an increase in plasma cholesterol, triglyceride, LDL levels and a decrease in HDL levels as observed in diabetic patients<sup>27</sup>. The marked hyperlipidemia that characterizes the diabetic state may be the consequence of the uninhibited actions of lipolytic hormones on fat depots<sup>28</sup>. Draksharishta-T, Draksharishta-M, Dabur Draksharishta and standard anti-diabetic glibenclamide produced significant reduction in fasting blood glucose level. These Draksharishta preparations and glibenclamide also produced significant reduction in serum cholesterol, LDL and triglyceride along with significant rise in HDL level in alloxan induced diabetic rats. The improvement in the lipid profile in diabetic animals after treatment with Draksharishta-T, Draksharishta-M, Dabur Draksharishta and standard anti-diabetic glibenclamide could be beneficial in preventing diabetic complications, as well as improving lipid metabolism in diabetic patients<sup>29</sup>.

A decrease in the FBG level, improvement in the lipid profile along with significant rise in the blood glutathione level (GSH) by Draksharishta-T, Draksharishta-M and Dabur Draksharishta in alloxan induced diabetic rats suggests that these preparations could be useful as an anti-diabetic agent with cardio-protective activity. The obtained result suggests that presence of alcohol could be beneficial in the faster absorption of poly-phenolic compounds found present in Draksharishta which are responsible for showing scavenging of alloxan induced reactive oxygen species.

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