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Evaluation of Neuroprotective Property of Madhunashini Vati in Diabetic Rat

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Keywords: Madhunashini Vati (MV), Streptozotocin (STZ), Neuroprotection, Thiobarbituric Acid Reactive Substances (TBARS).

ABSTRACT

The present study was designed to evaluate the Neuroprotection property of Madhunashini Vati in Diabetic Rat. Eight groups of rat and each group comprising of six animals were employed in the present study. During the experiment, different doses of Madhunashini Vati (125, 250, 500mg/kg) was administered. Insulin (4IU/kg body weight s.c) was administered daily before the global ischemia in diabetic rats. Rats were treated with Streptozotocin (STZ) 65mg/kg i.p for the induction of Diabetes for 14 days. Rats were anesthetized by using chloral hydrate (400 mg/kg, i.p) and Global cerebral ischemia was induced by occluding the carotid arteries, reperfusion was allowed for 24 h. At the end of the study, total Serum Glucose level, Serum Triglyceride level, Thiobarbituric Acid Reactive Substances (TBARS) and Cerebral Infarct size was estimated for the Neuroprotection property. Administration of Madhunashini Vati, showed a reduction in the serum glucose level, serum triglycerides level, cerebral infarct size and increases in body weight. In the present study, we observed that the madhunashini Vati significantly improved cerebral ischemia. Results indicate that Madhunashini Vati (MV) is a good neuroprotective and could be used as a treatment in brain stroke. And it is likely to be responsible for the free radical scavenging activity and rejuvenate the beta cells observed.

INTRODUCTION

Stroke

Stroke is a long term complication of diabetes [1]. A patient with Diabetes has a higher risk of stroke as compared to non-Diabetes. Diabetes and stroke both affects the blood vessels and both are associated with other risk factors such as hypertension, obesity, arterial fibrillation and dyslipidemia [2]. Different types of molecular signaling mechanism activated by hyperglycemia are Increased polyol pathway flux, Protein kinase C activation, Increased hexosamine pathway flux and Increased Advanced glycation end products (AGEs) formation [3] have demonstrated that elevation of extracellular glucose level leads to increased activity of Protein kinase C (PKC) and the mechanism of the activation is due to an enhanced de-novo synthesis of diacylglycerol (DAG). Protein kinase C has been associated with vascular alteration such as an increase in permeability, contractility, cell growth and apoptosis, angiogenesis, adhesion of leukocytes and activation of cytokine caused by different PKC isoforms [4]. Hyperglycemia increases polyol pathway flux, forming sorbitol and NADH. NADH and glucose stimulate the formation of Diacyclglycerol which in turn activates protein kinase C. Advanced glycation end products (AGEs) that are produced by the glycation of proteins which also stimulate PKC or cause reactive oxygen species (ROS) activation directly. PKC is also activated by fatty acids and angiotensin II [5].

Drug Profile: (Madhunashini Vati) *Madhunashini Vati* is a herbal drug formulation that is used for diabetes mellitus. It is a combination of medicinal plants such as namely *Tinospora cordifolia, Momordica muricata, Trigonella foenum graecum, Curcuma longa, Caesalpinia bonducella,* along with Shilajit. It has a highly antidiabetic activity with an equal proportion of shilajit. It has free radical scavenging activity of rejuvenation of cells that initiates to multiply the beta cells in the pancreas with improving their potential to secrete insulin. *momordica charantia* and *Tinospora cordifolia* presence reported to decrease hyperglycemia and long term treatment of shilajit enhance the pancreatotrophic action (increases the number of beta cells) that may result in the secretion of a large quantity of insulin in response to hyperglycemia. Madhunashini formulation has proved to be effective in nephrotoxicity [6].

Madhunashini as an antidiabetic herbal formulation has evaluated for acute and chronic toxicity studies in mice and rats respectively. In mice, the dose of madhunashini formulation (MD-19) was found to be nontoxic up to 4000mg/kg. For oral route, in mice, LD50 was

found to be 7500mg/kg and 4500mg/kg i.p. In rats, the oral dose was found to be 1000mg/kg for 30 days did not show any toxicity effect. It showed also a dose-dependent cardiac stimulant activity with a significant positive chronotropic and inotropic activity [(7)]. Madhunashini Vati is an ayurvedic formulation which activates the pancreas and helps to secrete a potential and balanced quantity of Insulin, through which extra glucose gets converted into glycogen[(8)]. Insulin renders diabetic rats resistant through arrested nitric oxide reaction with superoxide and forms peroxynitrite in acute ischemic stroke. Insulin improved superoxide production and microvascular functions. Insulin improved endothelial dysfunction in the brain. It improves the bioavailability of NO in the brain which causes the vasodilation [(9)]. Insulin treatment in diabetic animals, decrease in lipid peroxides and neurolipofuscin deposition and restored the calcium levels and the membrane fluidity also [(10)].

MATERIALS & METHODS:-

Procurement of Madhunashini Vati (Formulation)

The marketed preparation of Madhunashini Vati was procured from Local market Patanjali (Dehradun).

Procurement of Animals

Albino Wistar rat of either sex weighing 80-100g was procured in the animal house facility of Shri Guru Ram Rai Institute of Technology and Sciences, Patel Nagar, Dehradun. Animals were acclimatized in the animal house facility of the department and housed in polypropylene cages with husk bedding (renewed every 48 h) under 12:12 light-dark cycle at 25º C ± 5º C. and will be fed with standard Commercial pellet and water *ad libitum.*

The Experimental protocol was approved by *Institution Animal Ethics Committee* (Registration No. M.PH/IAEC/01/2015/EEC-02) and care of animals was as per guidelines of Committee for Control and Supervision of Experiments on Animals (Regd. No. 264/CPCSEA).

Induction of Type-I Diabetes Mellitus with STZ (Streptozotocin)

A single dose of streptozotocin (65mg/kg) prepared in citrate buffer (pH 4.5, 0.1 M) was intraperitoneally administered into an overnight fasted rat to induce diabetes in all group except control group. All the rats were allowed free access to water, pallet diet and

maintained at room temperature in polypropylene cages. STZ treated rats were also fed glucose solution 10% for 12 hr to avoid hypoglycemia. Rats having serum glucose more than 200mg/dl after 2 weeks of induction were considered as diabetic and selected for further study.

Induction of global cerebral ischemia:

Rats were anesthetized by using chloral hydrate (400 mg/kg, i.p). A midline ventral incision was made in the neck to expose the right and left common carotid arteries, which were isolated from surrounding tissue and vagus nerve. A cotton thread was passed below both the carotid arteries. Global cerebral ischemia was induced by occluding the carotid arteries. After 17 min of global cerebral ischemia, reperfusion was allowed for 24 h. After 17 min, an incision was sutured back in layers. The sutured area was cleaned with 70% ethanol and was sprayed with antiseptic dusting powder. The animals were shifted individually to their home cage and were allowed to recover overnight. The animals were kept on a heating pad during surgery to maintain the body temperature, to avoid the effect of temperature variations on the final results [(11)].

EXPERIMENTAL PROTOCOL:

Control and Diabetic rat were randomly selected and divided into eight groups and each group comprises 6 animals.

Group I (Normal Control group): Normal Animals.

Group II (Sham Control): the Only surgical procedure was performed (Carotid artery was exposed for 17 min).

Group III (Diabetic Control group): Rats were treated with streptozotocin (65mg/kg. i.p) for the induction of type-1 diabetes.

Group IV (Diabetes + stroke-induced group): 17 min global ischemia was induced in diabetic rats followed by 24hr reperfusion.

Group V (Standard drug-treated group in diabetes + stroke-induced): Insulin (4IU/kg body weight s.c) was administered daily for seven days before the global ischemia in diabetic rats.

Group VI (Test group): Madhunashini Vati (125mg/kg body weight p.o) was administered daily for seven days before the global ischemia in diabetic rats.

Group VII (Test group): Madhunashini Vati (250mg/kg body weight p.o) was administered daily for seven days before the global ischemia in diabetic rat.

Group VIII (Test group): Madhunashini Vati (500mg/kg body weight p.o) was administered daily for seven days before the global ischemia in diabetic rats.

Insulin (standard) and Madhunashini Vati (test drug) were administered for the next seven days starting from 2nd week of STZ injection, before the global ischemia in diabetic rats.

Collection of blood sample:

Blood from the experimental rat was withdrawn by retro-orbital plexus techniques under mild anesthesia using heparinized capillary glass tubes. Serum was separated by centrifugation [12].

Preparation of Post-Mitochondrial Supernatant:

Animals were sacrificed by cervical dislocation and then the brains were removed and cooled 0.9% saline and brain was kept on ice and subsequently blotted on filter paper, then weighed and homogenized in cold phosphate buffer (0.1M, pH 7.4) by using homogenizer. The homogenate was centrifuged at 10,000 rpm for 15 min at 4℃ and post-mitochondrial supernatant was kept on ice until assayed and used for biochemical estimation weighed and homogenized in phosphate buffer (pH 7.4). The homogenate was then centrifuged at 10,000 rpm for 15 min at 4^0C and post-mitochondrial supernatant was kept on ice until assayed and used for biochemical estimation. [13].

ANALYSIS OF BIOCHEMICAL PARAMETERS

Estimation of Glucose level: Serum glucose level was estimated by glucose oxidase/peroxidase method by using commercially available enzymatic glucose oxidase-peroxidase method. The blank, standard and test sample was prepared according to the standard procedure as mentioned in the standard serum glucose estimation kit. The absorbance was measured against blank 540 nm using spectrophotometer [14].

Estimation of tissue TBARS level: it was estimated by using the method describe by Slater and Sawyer. 2.0ml of the tissue homogenate called (supernatant) was added to 2.0ml of

freshly prepared 10% w/v trichloroacetic acid (TCA) and the mixture was allowed to stand in an ice bath for 15 minutes. After 15 minutes, the precipitate was separated through centrifugation and 2.0ml of the clear supernatant solution was mixed with 2.0ml, freshly prepared of thiobarbituric acid (TBA). Then the resulting solution was heated in a boiling water bath for mainly 10 minutes. Then it was immediately, cooled in an ice bath for 5 minutes. The developed color was measured at 532nm [11, 15].

Assessment of cerebral infarct size: At the end of 24 h of reperfusion after global cerebral ischemia, the animals were sacrificed by cervical dislocation. Brain samples were immediately sliced and slices were incubated with 1% triphenyltetrazoliumchloride (TTC) at 37 °C in 0.2M Tris buffer (pH7.4) for 20 min. TTC is converted to red form zone pigment by NAD and lactate dehydrogenase and thus stained the viable cells deep red. The Infarcted cells lost the enzyme as well as a cofactor and thus remain unstained dull yellow. The average area of each brain slice was calculated by counting the number of squares on either side. Similarly, the number of squares falling over the non-stained dull yellow area was also counted. Infarcted area was expressed as a percentage of the total brain volume. Whole-brain slices were weighed. Infarcted dull yellow part was dissected out and weighed. Infarct size was expressed as a percentage of the total wet weight of the brain [11].

Estimation of Serum Triglyceride level: Triglyceride level was estimated by using commercially available triglycerides enzymatic assay kit. The absorbance of the colored complex is measured at 505 nm [16].

STATISTICAL ANALYSIS:

All the entire data were expressed as mean standard error mean (SEM). All the data were analyzed through one way ANOVA, followed by Tukey's test for significance except body weight and glucose level. Data of Bodyweight and glucose level was analyzed through two way ANOVA, followed by Bonferroni’s multiple comparison test using graph pad prism version 5.3 software.

RESULTS:

Effect of Madhunashini Vati on body weight (g):

Administration of streptozotocin in the rat produced a significant decrease in body weight in rats as compared to normal control [Table & Fig no. 5.1]. Treatment with madhunashini Vati in diabetic rats (125, 500 mg/kg p.o) showed significant (P≤0.01) increase in body weight as compared to the diabetic control group and 250mg/kg p.o showed significant (P≤0.05) increase in body weight as compared to diabetic + stroke group [Table & Fig no. 5.2].

Table No. 5.1: Effect of Streptozotocin on body weight in rat Bodyweight (g) mean ± SEM

Group	Day 0	Day 7	Day 14
Normal control	120±0.991	129±0.972	135±1.202
Sham control	100±0.970	103±0.960	104±0.988
Diabetic control	116±1.321	109±0.991[a]	102±1.232[a]

* Day 0 represents, no STZ was administered. The same day, STZ was administered and body weight was measured on 0, 7th and 14th day. Values are expressed as mean ± SEM, n=6, **a** represents P≤0.01: As compared to the normal control group.

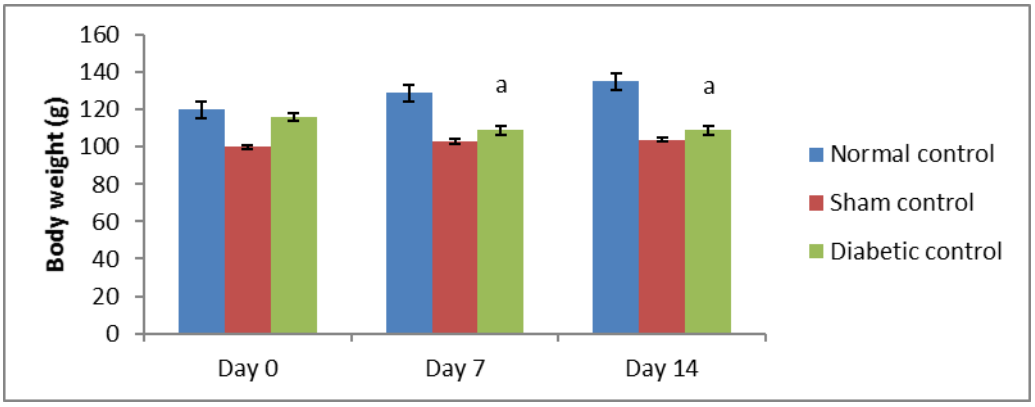

Fig. No. 5.1: Effect of streptozotocin on Bodyweight of the rat

Control group represents, there was no STZ administered. Sham control represents as there was surgical procedure was performed. Administration of STZ produced a significant decrease in body weight of rats as compared to normal control. Values are expressed as mean ± SEM, n=6, **a** represents P≤0.01: As compared to the normal control group.

Table No. 5.2: Effect of Madhunashini Vati on body weight in Diabetic rat Bodyweight (g) mean ± SEM

Group	Day 0	Day 7	Day 14
Diabetic Control	116±1.321	109±0.991	102±0.942
Diabetic + Stroke	119±1.623	111±1.132[a]	105±0.982[a]
Standard (Insulin) in diabetic rat	130±0.922	136±1.423 [b]	140±1.665[b]
MD(125mg/kg)in diabetic rat	122±1.510	124 ±1.399[b]	128±1.021[b]
MD(250mg/kg)in diabetic rat	125±1.151	129 ±1.344 [c]	134±1.211 [c]
MD(500mg/kg)in diabetic rat	123±0.933	127±0.972 [b]	130±0.912 [b]

*Day 0 represents, no STZ was administered. The same day, STZ was administered and after administered of STZ, on day 0, 7th and 14th-day body weight were measured. Values are expressed as mean ± SEM, n=6, **a** represents P≤0.001: As compared to Diabetic control group. **b** represents P≤0.01 As compared to Diabetic + stroke group. **c** represents P≤0.05: As compared to diabetic + stroke control.

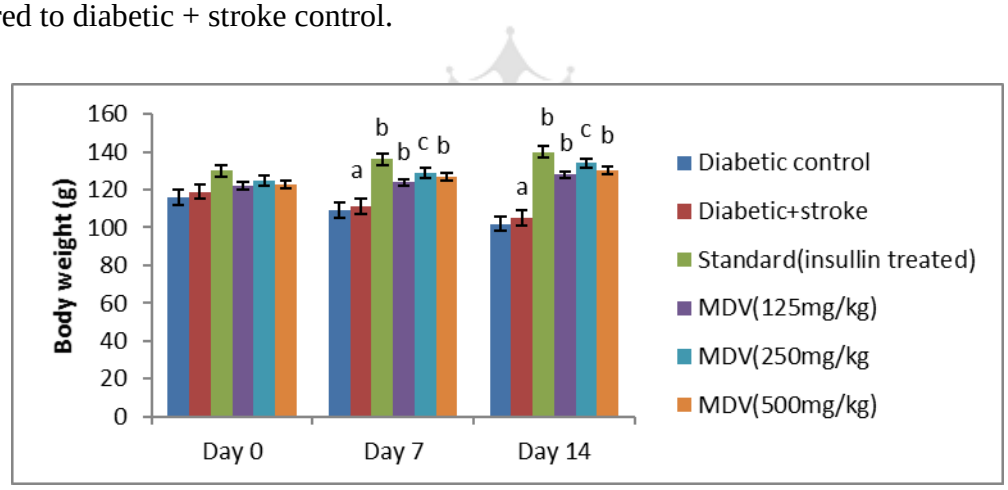

Fig. No. 5.2: Effect of Madhunashini Vati on Bodyweight of Diabetic rat.

Control group represents, there was no STZ administered. Sham control represents as there was surgical procedure was performed. Administration of STZ produced a significant decrease in body weight of rats as compared to normal control. Administration of madhunashini Vati in diabetic rat (125mg/kg, 250mg/kg, 500mg/kg p.o) produced significant (P< 0.001) increases in body weight as compared to diabetic control group. Values are expressed as mean ± SEM, n=6, **a** represents P≤0.001: As compared to Diabetic control group. **b** represents P≤0.01: As compared to Diabetic control. **c** represents P≤0.05: As compared to diabetic + stroke group.

Table No. 5.3: Effect of Streptozotocin on Serum Glucose level in Diabetic rat Fasting Serum Glucose level (mg/dl) mean ± SEM

Group	Day 0 (After 14 days STZ administration)	Day 7 (After 21 days STZ administration)	Day 14 (After 28 days STZ administration)
Normal control	93.55±0.95	94.23±0.99	92.22±0.94
Sham control	96.65±0.98	95.98±1.01	95.65±0.90
Diabetic control	90.42±2.11	170.15±1.72[a]	207.32±0.91[a]

*Day 0 represents, no STZ was administered. The same day, STZ was administered and after administered of STZ, on day 0, 7th and 14th-day serum glucose level were measured. Values are expressed as mean± SEM, (n=6). 'a' represents P≤ 0.001: As compared to the normal control group.

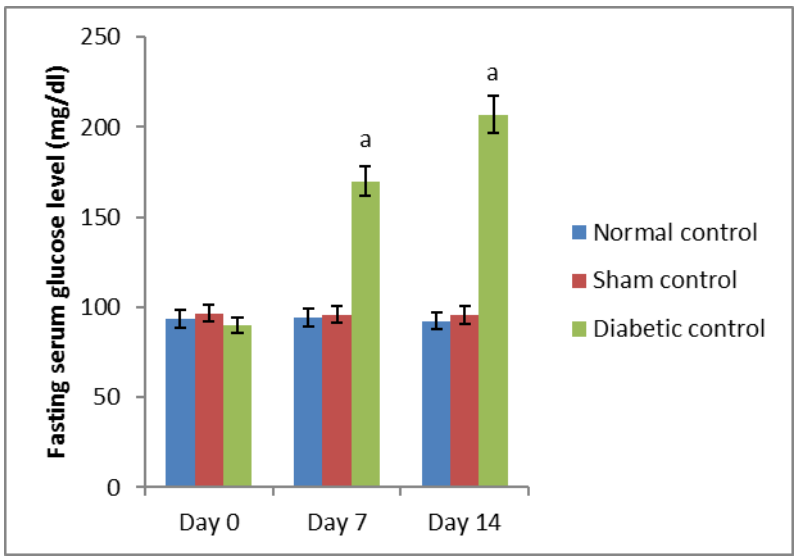

Fig. No. 5.3: Effect of streptozotocin on serum Glucose level in Diabetic rat.

Control group represents, there was no STZ administered. Administration of STZ produced significant (P≤0.001) increases in fasting serum glucose level in rats as compared to normal control. Values are expressed as mean± SEM, (n=6). 'a' represents P≤0.001: As compared to the normal control group.

Table No. 5.4: Effect of Madhunashini Vati on Serum Glucose level in Diabetic rat Fasting Serum Glucose level (mg/dl) mean ± SEM

Group	Day 0 (After 14 days STZ administration)	Day 7 (After 21 days STZ administration)	Day 14 (After 28 days STZ administration)
Diabetic control	207.42±2.11	220.15±1.72[a]	249.32±0.91[a]
Diabetic + stroke	200.07±1.87	217.53±1.98	243.34±1.28
Standard(Insulin) in diabetic rat	202.35±2.21	197.21±1.53 [b]	185.35±1.65[b]
MDV(125mg/kg) in diabetic rat	205.25±2.25	194.46±2.87 [b]	182.24±1.92 [b]
MDV(250mg/kg) in diabetic rat	201.33±2.28	192.23±1.99 [b]	186.42±1.20 [b]
MDV(500mg/kg) in diabetic rat	202.46±2.21	199.32±1.56 [b]	187.45±1.12 [b]

*Day 0 represents, administration of Insulin and MDV (madhunashini Vati) in diabetic rat and after 14th-day serum glucose level was measured. Values are expressed as mean ± SEM, (n=6). **'a'** represents P≤ 0.001: As compared to the control group. **b** represents P≤ 0.001: As compared to the normal control group.

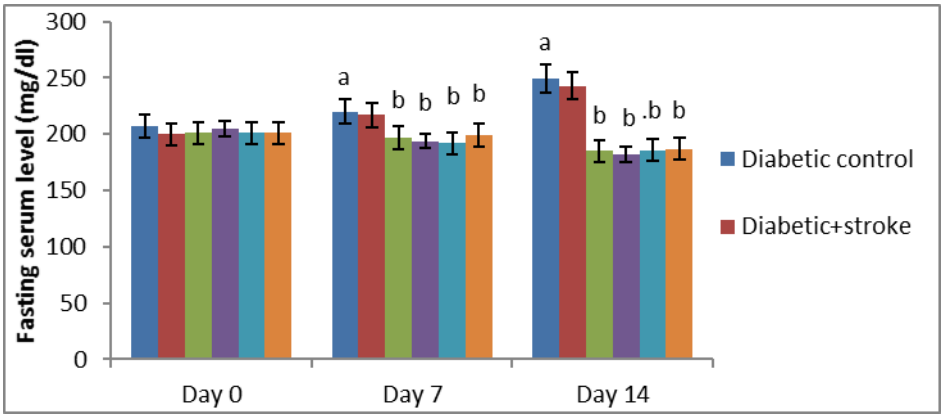

Fig. No. 5.4: Effect of Madhunashini Vati (MDV) on Serum Glucose level in Diabetic rat

Administration of MDV in diabetic rat (125mg/kg, 250mg/kg, 500 mg/kg p.o) treatment did not produced a significant effect on serum glucose level in diabetic rats on day 0. Then, the administration of MDV treatment in diabetic rats on day 7th and 14th showed a significant (P<0.001) lowering the serum glucose level as compared with diabetic control. Values are

expressed as mean ± SEM, (n=6).). **a** represents P≤ 0.001: As compared to the control group**. b** represents P≤0.001: As compared to Diabetic control.

Table No. 5.5: Effect of Madhunashini Vati on triglycerides level in Diabetic rat

Group	Triglycerides (mg/dl)
Normal control	114.01±2.01
Sham control	112.13±2.12
Diabetic control	185.25±2.52[a]
Diabetic + Stroke	195.57±2.62[a]
Standard (Insulin treated)	165.32±2.52[b]
MD (125mg/kg)	152.37±2.35[b]
MD(250mg/kg)	148.44±2.66[b]
MD(500mg/kg)	141.22±2.88[b]

*Control group represents, the brain was removed without performed surgical procedure. Sham group represent, the surgical procedure was performed in each rat, both carotid arteries were isolated and exposed for 17min followed 24 hr reperfusion and the brain was removed to estimate the triglycerides level. Administration of MDV madhunashini Vati (125mg/kg, 250mg/kg, 500mg/kg p.o) in diabetic rat and after 7 days triglycerides level was measured. Values are expressed as mean ± SEM (n=6). **a** represents P≤0.001: As compared to the normal control group. **b** represents P≤0.001: As compared to the diabetic control group.

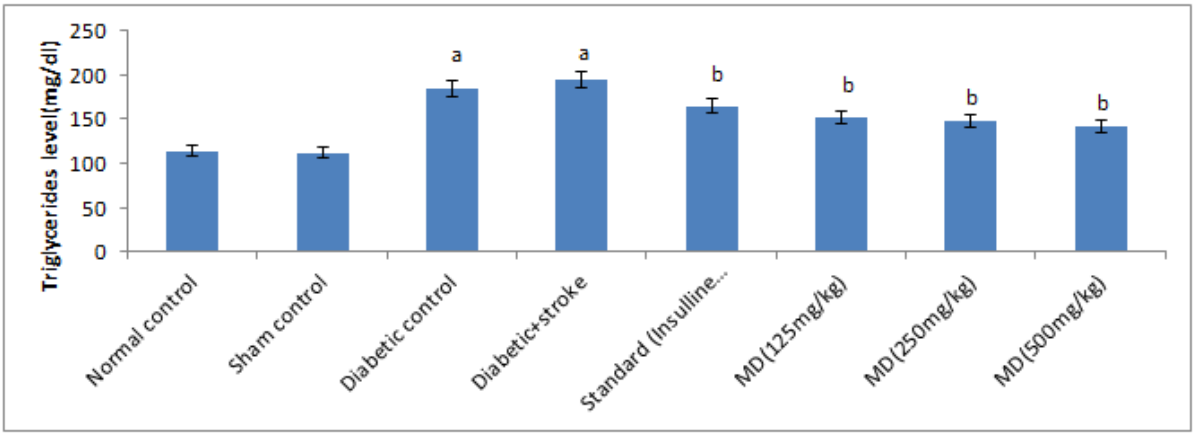

Fig. No. 5.5: Effect of Mdhunashini Vati on triglycerides level in Diabetic rat.

Administration of STZ in the rat produced a significant increase in serum triglycerides level in rats as compared to normal control. Administration of MDV (125mg/kg, 250mg/kg,

500mg/kg p.o) in diabetics rats showed a significant (P≤0.001) lowering of serum triglycerides level after 7 days as compared with diabetic control. Values are expressed as mean ± SEM (n=6). **a** represents P≤0.001: As compared to the normal control group. **b** represents P≤0.001: As compared to the diabetic control group.

Table No. 5.6: Effect of Madhunashini Vati on global cerebral I/R injury-induced cerebral infarct size in Diabetic rat by weight method.

Group	Cerebral infarct size (by weight %)
Normal control	2.45±0.92
Sham control	2.52±0.95
Diabetic control	32.21±2.54[a]
Diabetic + Stroke	44.45±2.82[a]
Standard (Insulin treated)	34.91±1.22[c]
MD (125mg/kg)	40.75±1.12[b]
MD(250mg/kg)	36.42±1.09[b]
MD(500mg/kg)	35.45±2.12[b]

*Control group represents, the brain was removed without performed surgical procedure. Sham group represent, the surgical procedure was performed in each rat, both carotid arteries were isolated and exposed for 17min followed 24 hr reperfusion and brain was removed to estimate the cerebral infarct size by weight method. Administration of MDV madhunashini Vati (125mg/kg, 250mg/kg, 500mg/kg p.o) in diabetic rat and after 7 days cerebral infarct size was measured by weight method. Values are expressed as mean ± SEM (n=6). **a** represents P≤0.01: As compared to the normal control group. **b** represents P≤0.001: As compared to diabetic + stroke control group. **c represents** P≤0.05: As compared to diabetes + stroke group.

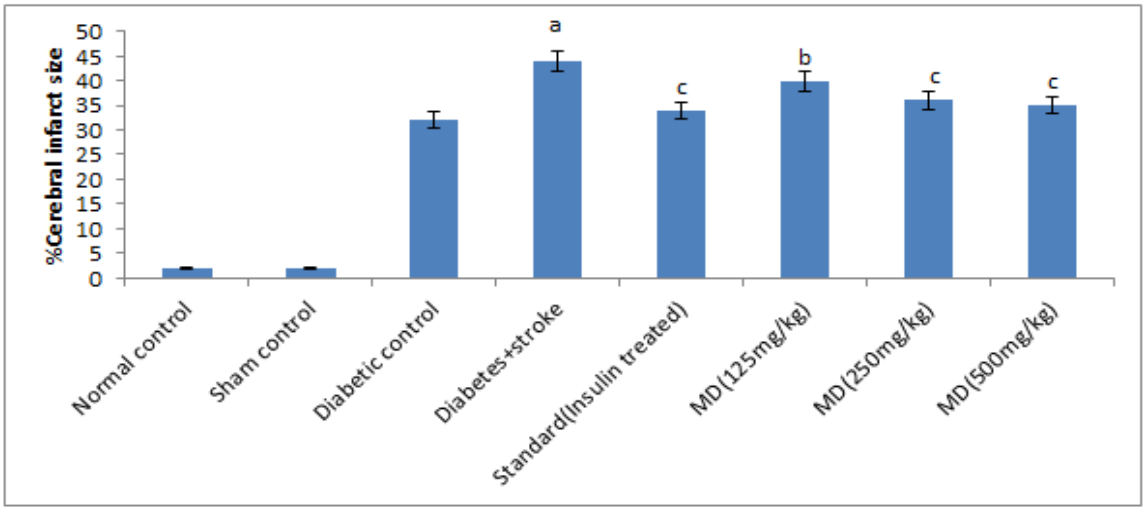

Fig. No. 5.6: Effect of Madhunashini Vati on global cerebral I/R injury-induced cerebral infarct size in Diabetic rat by weight method.

Global cerebral ischemia of 17 min followed by 24 hr reperfusion produced a significant increase in cerebral size in Diabetic + stroke group as compared to the normal control group and diabetic group. There are no significant differences in the control and sham group by both weight and volume methods. After 7 days prior to treatment of madhunashini Vati (125mg/kg, 250mg/kg, 500mg/kg p.o) in diabetic rats significantly ($P<0.001$, $P<0.01$) decreases the cerebral infarct size as compared to Diabetes + stroke group. Values are expressed as mean ± SEM (n=6). **a** represents $P\leq0.01$: As compared to the normal control group. **b** represents $P\leq0.001$: As compared to diabetic + stroke group. **c represents** $P\leq0.05$: As compared to diabetes + stroke group.

Table No. 5.7: Effect of Madhunashini Vati on global cerebral I/R injury-induced cerebral infarct size in Diabetic rat by volume method.

Group	**Cerebral infarct size (by volume %)**
Normal control	2.25±0.94
Sham control	2.45±0.97
Diabetic control	35.48±1.95
Diabetic + Stroke	46.55 ± 2.05^{a}
Standard (Insulin treated)	36.11 ± 0.98^{c}
MD (125mg/kg)	42.05 ± 1.05^{b}
MD(250mg/kg)	38.05 ± 2.21^{b}
MD(500mg/kg)	35.34 ± 2.16^{b}

*Control group, the brain was removed without performed surgical procedure. Sham group represent, the surgical procedure was performed in each rat, both carotid arteries were isolated and exposed for 17min followed 24 hr reperfusion and brain was removed to estimate the cerebral infarct size by volume method. Administration of MDV madhunashini Vati (125mg/kg, 250mg/kg, 500mg/kg p.o) in diabetic rat and after 7 days cerebral infarct size was measured by volume method. Values are expressed as mean ± SEM (n=6). **a** represents P≤0.001: As compared to the normal control group. **b** represents P≤0.001: As compared to diabetic + stroke group. **c** represents P≤0.05: As compared to diabetes + stroke group.

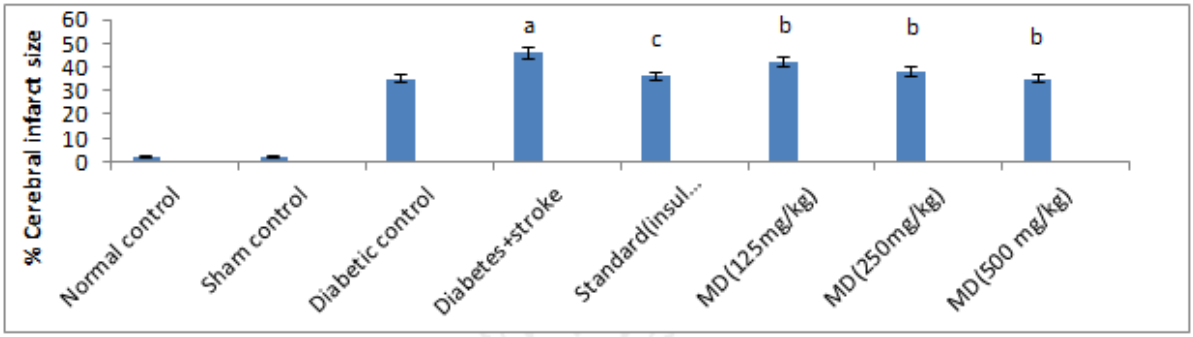

Fig. No. 5.7: Effect of Madhunashini Vati on global cerebral I/R injury-induced cerebral infarct size in Diabetic rat by volume method.

Global cerebral ischemia of 17 min followed by 24 hr reperfusion produced a significant increase in cerebral size in Diabetic + stroke group as compared to the normal control group and diabetic group. There are no significant differences in the control and sham group by both weight and volume methods. After 7 days prior to treatment of madhunashini Vati (125mg/kg, 250mg/kg, 500mg/kg p.o) in diabetic rats significantly (P≤0.001, P≤0.01) decreases the cerebral infarct size as compared to Diabetes + stroke group. Values are expressed as mean ± SEM (n=6). **a** represents P≤0.001: As compared to the normal control group. **b** represents P≤0.001: As compared to diabetic + stroke group. **c** represents P≤0.05: As compared to diabetes + stroke group.

Table No. 5.8: Effect of Madhunashini Vati on Increases in Thiobarbituric acid reactive substances (TBARS) level in Diabetic rat

Group	TBAR level (nmol/mg protein)
Normal control	2.82± 0.94
Sham control	2.86± 0.92
Diabetic control	4.12± 1.23[a]
Diabetic + Stroke	8.35± 1.21[b]
Standard (Insulin treated)	5.18± 0.99[c]
MD (125mg/kg)	4.07± 0.99[c]
MD(250mg/kg)	5.38±0.94[c]
MD(500mg/kg)	3.58± 1.23[c]

*Control group, the brain was removed without performed surgical procedure. Sham group represent, the surgical procedure was performed in each rat, both carotid arteries were isolated and exposed for 17min followed 24 hr reperfusion and the brain was removed to estimate the TBARS level in diabetic rats. Administration of MDV madhunashini Vati (125mg/kg, 250mg/kg, 500mg/kg p.o) in diabetic rat and after 7 days TBARs level was measured. Values are expressed as mean ± SEM, (n=6). **a** represents P≤0.001: As compared to the normal control group. **b** represents P≤0.001: As compared to the diabetic control group. **c** represents P≤0.001: As compared to diabetes + stroke group.

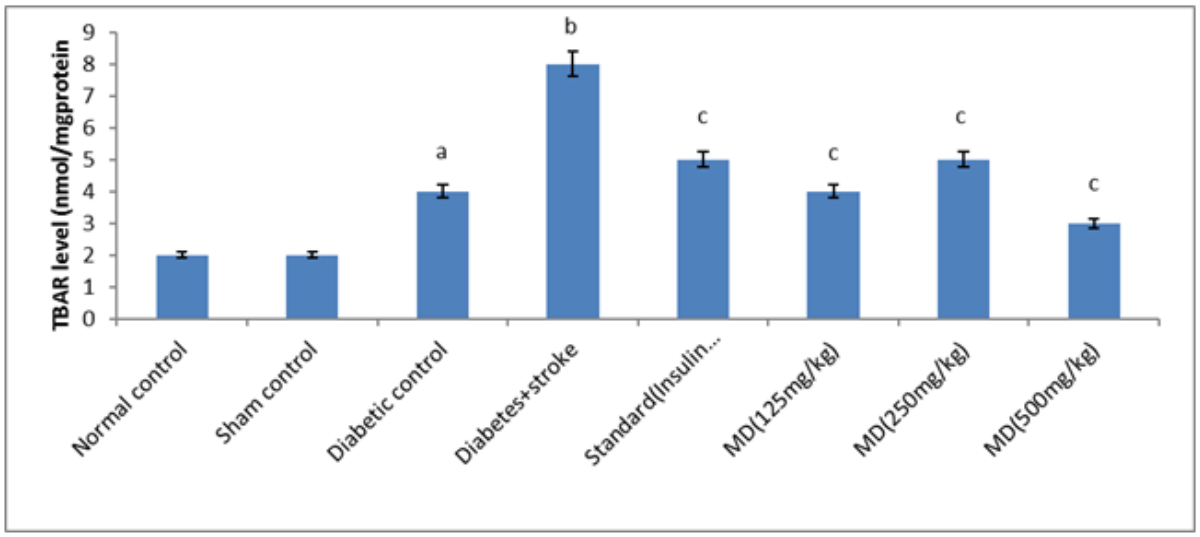

Fig. No. 5.8: Effect of Madhunashini Vati on TBAR level in Diabetic rat.

Global cerebral ischemia of 17 min followed by 24 hr reperfusion produced a significant increase in TBARS level in Diabetic and diabetic + stroke group as compared to the normal control group. There are no significant differences in the control and sham group by both weight and volume methods. After 7 days prior to treatment of madhunashini Vati (125mg/kg, 250mg/kg, 500mg/kg p.o) in diabetic rats significantly ($P \leq 0.001$, $P \leq 0.01$) decreases the TBARS level as compared to Diabetes + stroke group. Values are expressed as mean ± SEM, (n=6). **a** represents $P<0.001$: As compared to the normal control group. **b** represents $P<0.001$: As compared to the diabetic control group. **c** represents $P<0.001$: As compared to diabetes + stroke group.

DISCUSSION

In the present study, to evaluate the neuroprotective effect of Madhunashini Vati in streptozotocin-induced and global cerebral ischemia/reperfusion injury-induced in the rat. It is well known as that diabetes has a higher risk of stroke. Diabetes and stroke both affects the blood vessels and both are associated with other risk factors such as hypertension, obesity, arterial fibrillation and dyslipidemia [(2)]. Overproduction of ROS by mitochondria causes the impairment of ETC (electron transport chain), which leads to decreased ATP production and increased the formation of free radicals, altered calcium homeostasis and mitochondrial dysfunction [(17)]. Many synthetic drugs can protect against oxidative damage, but they have side effects also. Numerous clinical and preclinical studies using especially tissue plasminogen activator thrombolytic agents examined pharmacological recanalization and restoration of cerebral circulation or reperfusion within minutes of the onset of treatment. It is also recently was shown to be effective and approved for the urgent treatment of ischemic stroke in humans. But the less effective, widely applicable and safe pharmacological treatments of ischemic patients that may grow interested in the traditional medicines. It has been suggested that in the brain, herbal medicines or their products may improve the microcirculation, protect ischemic reperfusion injury also and possess neuroprotective properties that lead to inhibit apoptosis. So there is an alternative solution for this problem is to consume natural antioxidants as much as possible from food supplements and traditional medicines [(18)].

In this study madhunashini, vati was used as a test drug in rats. As per the previous reports that madhunashini vati combination herbal formulation namely *Tinospora cordifolia, Momordica muricata, Trigonella foenum graecum, Curcuma longa, Caesalpinia bonducella,*

along with Shilajit and has an anti-diabetic activity due to the presence of shilajit. It has free radical scavenging activity of rejuvenation of cells that initiates to multiply the beta cells in the pancreas with improving their potential to secrete insulin. *momordica charantia* and *Tinospora cordifolia* presence reported to decrease hyperglycemia and long term treatment of shilajit enhance the pancreatotrophic action (increases the number of beta cells) that may result in the secretion of a large quantity of insulin in response to hyperglycemia [(6)]. And the previous study also investigated that, *Trigonella foenum graecum* seeds one of the ingredients of madhunashini vati has neuroprotective property. It has shown the presence of steroid saponins in TSP *Trigonella foenum graecum* seeds, which rejuvenate the pancreas beta cells and stimulate the insulin secretion [(10)]. Diabetes mellitus (Type 1) was chemically induced in rat by administrating single dose of streptozotocin (STZ 65mg/kg i.p) which produces cytotoxicity to beta cells of islets of Langerhans by increasing the activity of xanthine oxidase and poly ADP- ribose polymerase (PARP), that causes apoptotic and necrotic cell death in beta cells of pancreas [(19, 20)]. In this study, Anti-diabetic effects of Madhunashini Vati was evaluated because diabetes is the leading cause of brain damage due to several mechanisms.

Madhunashini Vati showed a reduction in the serum glucose level, serum triglycerides level, MDA (malondialdehyde), cerebral infarct size and increases in body weight as compared to diabetic control and diabetes + stroke group. Low dose shows less effective as compared to medium dose and high dose. But a medium dose of madhunashini vati (250mg/kg p.o) in diabetic rat showed significant increases in body weight as compared with low and high dose. Madhunashini Vati (125mg/kg, 250mg/kg, 500mg/kg p.o) did not show any significant effect on body weight on day 0 but it showed a significant increase in body weight on 7^{th} and 14^{th} day. Madhunashini vati (125mg/kg, 250mg/kg, 500mg/kg p.o) in diabetic rat showed a significant decreases in serum glucose level as compared with diabetic group. On the 14^{th} day, madhunashini vati (125mg/kg p.o) showed a significant decrease in serum glucose level as compared with medium and a high dose of madhunshini vati in diabetic rat. In the present study, we observed that the madhunashini Vati significantly improved cerebral ischemia. The diabetic control group showed a significant difference as compared to normal control and sham control group when the increased level of markers of oxidative stress (brain damage) was observed and this finding of the present study suggest the diabetes mellitus play a key role in the pathogenesis of oxidative stress and brain stroke. Results indicate that Madhunashini Vati is a good neuroprotective and could be used as a treatment in brain stroke.

And it is likely to be responsible for the free radical scavenging activity and rejuvenate the beta cells observed.

CONCLUSION

From the above discussion and results, it can be concluded that:

- Madhunashini Vati shows a Neuroprotective as well as the anti-diabetic effect in streptozotocin-induced diabetic rat.

- Treatment with Madhunashini Vati (125mg/kg, p.o) shows to lower serum glucose level due to maybe increased glucose uptake by the tissues and its utilization and it also increases the body weight, also it has good scavenger of free radicals.

- There was a significant decrease in cerebral infarct size and triglycerides level at the dose of 500mg/kg p.o of madhunashini vati, which are the main markers of brain damage.

- The present study results constitute the main evidence for the therapeutic potential of Madhunashini Vati in Global Cerebral Ischemia/ Reperfusion Injury. The study supports an important concept that the onset of neurodegenerative due to diabetes may be delayed or mitigated with the use of anti-diabetic drugs with free radicals scavenger that protects against the brain damage, oxidative stress, and degeneration of neurons.

- Madunashini vati was found to be effective as neuroprotective because it might have scavenging activity of free radicals and rejuvenate the beta cells that initiates to multiply the beta cells in the pancreas with improving their potential to secrete insulin which acts as neuroprotection.

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CONFLICT OF INTEREST: Nil

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