Efficacy of a Polyherbal Formulation Madhumehari in Attenuating Diabetic Nephropathy.

Reema Mitra*, Papiya Mitra Mazumder1, Upendra K. Jain2

1Birla Institute of Technology and Sciences, Mesra, Ranchi.
2Chandigarh College of Pharmacy, Chandigarh Group of Colleges, Landran, Mohali.

ABSTRACT
Oxidative stress in kidneys increases during diabetic nephropathy. Polyherbal formulation Madhumehari was assessed for its ability to decrease oxidative stress in kidney homogenates of diabetic rats. Animals were rendered diabetic by a single i.p. injection of 60 mg/kg b.w. streptozotocin. After 72 hours of STZ injection blood glucose was estimated. The animals having fasting blood sugar above 200mg/dl were included in the study. Treatment was started 7 days after the induction of diabetes. The animals were divided into the following groups with 6 rats in each group:

- Group –I: Normal control rats
- Group –II: Diabetic control
- Group-III: Diabetic animals received Madhumehari at a dose of 500 mg/kg b.w.

Assessment of renal oxidative stress: The kidneys were isolated from each animal. The renal cortical homogenates were centrifuged at 5000 rpm for 10 min at 4°C. The resulting supernatant was used for determination of:

- Malondialdehyde (MDA)
- Catalase
- Superoxide Dismutase (SOD)
- Glutathione –S- Transferase

Treatment with Madhumehari showed significantly decreased MDA level and GST level showing a reversal of peroxidation. SOD and Catalase level was significantly increased as compared to the diabetic control group. The results of the study indicate that Madhumehari was beneficial in decreasing the oxidative stress in the treatment group as compared to the
diabetic control group. Thus Madhumehari may be useful in preventing renal complications in diabetic patients.

**KEYWORDS**: Diabetic Nephropathy, Madhumehari, Oxidative stress, In–vivo antioxidant enzymes.

### 1. INTRODUCTION

Diabetic nephropathy is a syndrome characterized by a secondary renal disease in patients with diabetes mellitus. It is the leading cause of kidney disease in patients starting renal replacement therapy and affects approximately 40% of type 1 and type 2 diabetic patients. Diabetic nephropathy is indicated by the presence of microalbuminuria (UAE, 30 mg/day or 20µg/min.) in the absence of other renal disease.[1] Oxidative stress has been reported to play a key role in pathogenesis of diabetic nephropathy. Hyperglycaemia increases the production of reactive oxygen species (ROS) and also attenuates antioxidative mechanisms through glycation of the scavenging enzymes.[2] The ayurvedic preparations usually contain a variety of herbal and non herbal ingredients that incorporate synergistic, potentiative, agonistic, antagonistic pharmacological agents and hence act on a variety of targets by various modes and mechanisms. In diabetic nephropathy it becomes important to have therapeutics addressing oxidative stress in concurrence with reducing the blood glucose level i.e. maintaining a near normal glycaemic state. The ayurvedic system of medicine chooses medicinal plants that possess apart from Kapha (eg: antidysglycaemic and antidyslipidemic properties) and Pitta (enzyme inhibitors/ modulators) modifying activities strong Vata Nashak (antioxidant) properties also, in order to address holistically, the cause and complications of diabetes mellitus. In the present study Madhumehari was chosen which already has proven efficacy against diabetes.[3][4] It has been suggested that the current belief ‘one disease – one drug’ may no longer hold true in the future and that polyherbal formulations designed in a rational manner could also be investigated as an alternative in multi- target therapeutics and prophylaxis.[5] The present formulation chosen Madhumehari contains extracts from a number of different plants which have antidiabetic, anti-inflamatory, antioxidant, free radical scavenging, diuretic and immunomodulatory properties. So, a combination of these plants may prove to be more efficacious in the treatment of diabetes and thus in prevention of the long term complications like diabetic nephropathy.
2. METHODOLOGY

2.1. Ayurvedic formulations
MADHUMEHARI (Shree Baidyanath Ayurved Bhawan Pvt. Ltd.) was purchased from a registered ayurvedic medicine store.

2.2. Animals
Male inbred albino rats weighing between 150-180 g were procured from Animal House of Birla Institute of Technology, Mesra, Ranchi and were housed in polypropylene cages with one animal in each cage. They were kept under controlled environmental condition of 25 ± 2 °C and 45-55% relative humidity with natural light / dark cycle and allowed free access to food (standard pellet diet, Hindustan Lever Ltd., India) and water and acclimatized for at least a week before the commencement of the experiment (Reg no.621 / 02 / ac / CPCSEA). All experiments were performed subject to prior approval of the Institutional Animal Ethics committee.

2.3. Treatment protocol
Overnight fasted animals were rendered diabetic by a single i.p. injection of 60 mg/kg body weight Streptozotocin freshly prepared in 0.1 M citrate buffer (pH 4.5). The STZ injected animals were then given 5% w/v glucose solution for 5-6 hours following the injection to prevent initial drug induced hypoglycemic mortality. After 72 hours of STZ injection blood was drawn from the tail vein of rats and fasting blood glucose was estimated by a calibrated glucometer [SD Check Gold, Standard Diagnostics]. The animals having fasting blood sugar above 200mg/dl were included in the study. Treatment was started 7 days after the induction of diabetes. The biochemical and pharmacological experiments were carried out from the 0th day (7th day after diabetes induction) till the 40th day of the experiment.

Drugs were given every day for a period of 40 days by the oral route by oral gavage using a 5ml syringe. The animals were divided into the following groups with 6 rats in each group:

Group –I: Normal control rats which were kept untreated.
Group –II: Diabetic animals received citrate buffer for the entire period of treatment and served as the diabetic control group.
Group-III: Diabetic animals received Madhumehari at a dose of 500 mg/kg b.w. by the oral route.
2.4. Assessment of renal oxidative stress

The kidneys were isolated from each animal and their fresh weight was recorded. Then the kidneys were kept at ~80 °C and subsequently homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The renal cortical homogenates were centrifuged at 5000 rpm for 10 min at 4°C. The resulting supernatant was used for determination of:

- Malondialdehyde (MDA)
- Catalase
- Superoxide Dismutase (SOD)
- Glutathione –S- Transferase

2.4.1. Assay of Malondialdehyde (MDA)

Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS), using malondialdehyde (MDA) as standard. Thus, 1 ml of homogenized kidney tissue in 2 ml of normal saline was mixed with 24% TCA and centrifuged at 2,000 rpm for 20 mins. To 2 ml of protein–free supernatant, 1 ml of fresh TBA (0.67%) reagent was added, mixed thoroughly and heated at 95° C for 1 hour, in water–bath. The suspension was then cooled to room temperature, centrifuged at 2,000 rpm for 10 mins, and the pink coloured supernatant was taken for spectroscopic measurement at 532 nm for the assay of MDA. Lipid peroxide is expressed in terms of nM of MDA /mg of kidney tissue.

2.4.2. Catalase Assay

Catalase activity in the gastric tissue was determined according to the method followed by Lück. The gastric tissue was scrapped off and homogenized in ice-cold normal saline medium. The solution was then centrifuged for 10 mins at 3,000rpm and the supernatant was collected for estimation. The supernatant (100µl) was added to a solution containing 3 ml of H₂O₂ -phosphate buffer mixture (50 mM phosphate buffer, pH 7.0 and 30 % H₂O₂). The change in optical density at 240 nm per unit time, was taken as a measure of catalase activity. The concentration of the buffer –H₂O₂ was standardized to get the optical density at 240 nm to 0.500± 0.010 (d=1cm).

2.4.3. Superoxide Dismutase Assay

An indirect method of inhibiting auto-oxidation of epinephrine to its adrenochrome was used to assay Superoxide dismutase activity in the kidney homogenate. Auto-oxidation of epinephrine was initiated by adding 1ml of Fenton reagent. To a mixture of epinephrine (3 x10⁻⁴ M), Na₂CO₃ (10⁻³M), EDTA (10⁻⁴M), and 1.0ml of deionised water Fenton reagent was
added. The auto-oxidation was read in a spectrophotometer at 480 nm every 30 sec for 5 min. A graph of absorbance against time was plotted for each, and the initial rate of auto-oxidation calculated. One unit of Superoxide dismutase activity was defined as the concentration of the enzyme (mg protein/ml) in the homogenate that caused 50 % reduction in the auto-oxidation of epinephrine. Superoxide dismutase activity was subsequently calculated for each sample.

2.4.4. GST Assay

GST activity was determined by measuring the increase in absorbance at 340 nm using 1-chloro-2, 4-dinitrobenzene (CDNB) as the substrate, according to the method of [11. Briefly, 1 mM CDNB was added to buffer containing 1 mM GSH and an aliquot of sample to be tested. Upon addition of CDNB, the change in absorbance at 340 nm was measured as a function of time. The extinction coefficient for this reaction is 9.6 mM$^{-1}$cm$^{-1}$.

3. Statistical analysis

All results were expressed as mean ± standard error of mean. All statistical calculations were done by One Way Analysis of Variance followed by Dunnett’s test using Statistica 8 (Statsoft inc. USA).

4. RESULTS

Malondialdehyde

The MDA (nM / mg of renal tissue) was found to be significantly elevated (p < 0.01) in the diabetic control rats as compared to the normal controls (Fig.1). On treatment with the two formulations Diabecon and Madhumehari it was found that the MDA level was significantly decreased (p < 0.01) showing a reversal of peroxidation.

![MDA level in kidney homogenate](https://example.com/image)

Fig.1: Level of MDA (nM / mg of renal tissue) in kidney homogenate after 40 days treatment with the selected doses Madhumehari in STZ diabetic rats.
Superoxide dismutase (SOD)
The Superoxide dismutase level (u / ml) was found to be significantly decreased (p < 0.01) in the diabetic control rats as compared to the normal controls (Fig.2). On treatment with the two formulations Diabecon and Madhumehar it was found that the SOD level was significantly increased (p < 0.01) as compared to the diabetic control group.

![Superoxide dismutase level in kidney homogenate](image1)

**Fig.2:** Level of Superoxide dismutase in kidney homogenate after 40 days treatment with the selected doses of the two ayurvedic formulation in STZ diabetic rats.

Catalase
The Catalase activity was found to be significantly decreased (p < 0.01) in the diabetic control rats as compared to the normal controls (Fig.3). On treatment with the two formulations Diabecon and Madhumehar it was found that the Catalase level was significantly increased (p < 0.01) as compared to the diabetic control group.

![Catalase level in kidney homogenate](image2)

**Fig.3:** Level of Catalase in kidney homogenate after 40 days treatment with the selected doses of the two ayurvedic formulation in STZ diabetic rats.
**Glutathione – S – Transferase**

The Glutathione – S – Transferase (GST) activity was increased in the diabetic control group as compared to the normal control and the result was statistically significant (p < 0.05) (Fig.4). On treatment with the two formulations Diabecon and Madhumehari it was found that there was a significant decrease as compared to the diabetic control group (p < 0.05 in Diabecon and p < 0.01 in Madhumehari).

![Glutathione-S-Transferase activity in renal homogenate](image)

**Figure 4: Level of Glutathione – S - Transferase in kidney homogenate after 40 days treatment with the selected doses of the two ayurvedic formulation in STZ diabetic rats.**

5. **DISCUSSION**

Chronic hyperglycemia, a well-recognized pathogenetic factor of long-term complications in diabetes mellitus, is reported to generate not only more reactive oxygen species (ROS) but also attenuate anti-oxidative mechanisms through glycation of the scavenging enzymes. Lipid peroxidation of unsaturated fatty acids, one of the major reactions in vivo, has been proven to be an index of increased oxidative stress and the subsequent cytotoxicity. The present study showed that there were significantly higher levels of lipid peroxides (estimated in terms of thiobarbituric acid reactive species taking MDA as standard) in renal homogenates suggesting increased oxidative stress in diabetic kidneys. Treatment with both Diabecon and Madhumehari decreased the levels of lipid peroxides. Catalase is an enzymatic antioxidant widely distributed in all animal tissues. Catalase decomposes hydrogen peroxide and helps protect the tissues from highly reactive hydroxyl radicals. In the study a significant lowering of catalase activity occurred in the diabetic control animals as compared to the normal animals signifying an increased oxidative stress in diabetic kidney. In the diabetic animals treated with Diabecon and Madhumehari there was a significant increase in the levels of
catalase enzyme as compared to the diabetic control group. Glutathione-S-transferases can work as endogenous antioxidant to protect cells from oxidative stress. There have been reports of increased levels of glutathione-S-transferases even during the early stages of diabetes probably in response to oxidative stress triggered by hyperglycaemia or other toxic effects of glucose.[12] In the present study treatment with Diabecon and Madhumehari produced a significant decrease in the levels of Glutathione-S-transferase indicating an improvement in oxidative stress condition.

The results of the study indicate that the two formulations were beneficial in decreasing the oxidative stress in the treatment group as compared to the diabetic control group. Thus Diabecon and Madhumehari may be useful in preventing the occurrence of renal complications in diabetic patients.

REFERENCES

