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**RESEARCH ARTICLE**

# A STUDY OF AN OXIDATIVE STRESS ON STREPTOZOTOCIN INDUCED DIABETIC RATS TREATED WITH DIACURE A POLYHERBAL FORMULATION

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**ABSTRACT**

The aim of this study was to investigate the effects of methanolic extract of Diacure a polyherbal formulation for oxidative stress in Streptozotocin induced diabetic rats. Its effect was compared with that of glibenclamide, a reference antidiabetic drug. White albino male rats (Streptozotocin induced) were administered with methanolic extract of Diacure (100mg /kg body wt) orally for 30 days. At the end of 30 days, serum insulin and level, SGOT, SGPT level in serum and liver, catalase, SOD, GPx, GSH and LPO level in serum, liver and kidney were estimated in control, alloxan induced, extract treated and glibenclamide treated rats. Oral administration of methanolic extract of Diacure for 30 days made significant changes in serum, liver and kidney of plant treated rats where compared with untreated diabetic rats. The effects produced by the extract were comparable to that of glibenclamide. In conclusion the methanolic extract of Diacure showed significant antioxidant effect in Streptozotocin induced diabetic rats.



## INTRODUCTION

Diabetes mellitus, a leading non communicable disease with multiple etiologies, affects more than 100 million people worldwide and is considered as one of the leading causes of death in the world (1). The WHO (world health organization) reported that 300 million peoples would suffer from diabetes mellitus by the year 2025 (2). India is one of the leading countries for the number of people with diabetes mellitus and it is estimated that diabetes affects approximately 57 million people by the year 2025 in India (3). Diabetes mellitus is a group of syndrome characterized by hyperglycemia and altered metabolism of carbohydrates, lipids and proteins. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries (4). There is increasing evidence that complications related to diabetes are associated with oxidative stress induced by the generation of free radicals (5). Free radicals may also form via auto-oxidation of unsaturated lipids in plasma and membrane lipids. The free radicals produced may react with polyunsaturated fatty aids in all membranes leading to lipid peroxidation (6). The level of lipid peroxidation in cells is controlled by various cellular defense mechanisms consisting of enzymatic and non-enzymatic scavenger systems (7).

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The levels of these defense mechanisms are altered in diabetes and therefore, the in effective scavenging of free radicals plays a crucial role in determining tissue injury (8). Antioxidants thus play an important role to protect the human body against damage caused by reactive oxygen species (ROS) (9). The endogenous antioxidant enzymes (eg: SOD, CAT, GSH & GPX) are responsible for the detoxification of deleterious oxygen radicals (10). In diabetes, oxidative stress has been found to be mainly due to an increased production of oxygen radicals and a sharp reduction of anti oxidant defenses (11). Hence, compounds with both hypoglycemic and antioxidative properties would be useful antidiabetic agents (12). Many plant extracts and plant products have been shown to have significant antioxidant activity (13), which may be an important property of plant medicines associated with the treatment of several ill fated diseases including diabetes. Thus herbal plants are considered useful means to prevent and/or ameliorate certain disorders, such as diabetes, atherosclerosis and other complications (14). The present investigation was to assess antioxidant efficacy of methanolic extract of diacure a polyherbal formulation in streptozotocin induced diabetic rats after 30 days of treatment and the effect produced by methanolic extract of diacure was compared with glibenclamide.

## MATERIALS AND METHODS

### Drugs and Chemicals

Streptozotocin was purchased from Ponmani Chemical Pvt., Ltd., Trichy. All other chemicals and reagents used were of analytical grade.

### Plant Material

The fresh plant materials was collected from Perambalur, Tamilnadu, India. The plant was identified, authenticated and the voucher specimen has been kept in our laboratory for future reference. The leaves were shade dried, powdered and passed through a 40 mesh sieve, and kept in a well closed container for further extraction.

### Preparation of plant extract

500g of dried, powdered plant material diacure, a polyherbal formulation a combination of 11 medicinal plants are mixed 1:1 ratio,the medicinal plants are listed in the table given below, were extracted successively with methanol using soxhelt apparatus. The residual extract was suspended in water for overnight and filtered. The filtrate was dried and was stored at $4^0$C until used. A known volume of the residual extract is suspended in distilled water and was orally administrated to the animals during the experimental period. Table showing the Diacure a polyherbal formulation combination of 11 medicinal plants

| S. No | Name of the plant | Common name | Family | Part of the plant used |
|---|---|---|---|---|
| 1 | *Syzigium cumini* | Jamun | Myrtaceae | Seed |
| 2 | *Azardiricta indica* | Neem | Meliaceae | Leaves |
| 3 | *Ocimum tenuiflorum* | Tulsi | Lamiaceae | Leaves |
| 4 | *Abitulon indicum* | Tuthi | Malvaceae | Leaves |
| 5 | *Cassia auriculata* | Cassia | Ceasalpinaceae | Flowers |
| 6 | *Ficus bengalensis* | Aalam | Moraceae | Seed |
| 7 | *Tinospora cardifolia* | Seendal | Convolvulaceae | Root |
| 8 | *Phyllanthus emblica* | Nelli | Euphorbiaceae | Seed |
| 9 | *Trigonella foena graceum* | Fenugreek | Leguminasae | Seed |
| 10 | *Curcuma longa* | Turmeric | Zingiberaceae | Rhizome |
| 11 | *Phyllanthus niruri* | Keelanelli | Euphorbiaceae | leaves |

### Animals

Male albino rats of the wistar strain weighing about 175-210g were used for this study. The rats were 10-12 weeks of age at the time of this study. They were acclimatized to the animal house conditions at least for one week before carrying out any experimental work. The rats were fed ad libitum with normal pellet (Hindustan Lever Ltd., Bangalore, India) and Water. The experiments were designed and conducted in accordance with the ethical norms approved by ministry of social justice and empowerment, government of India and International Animal ethics committee Guidelines for the investigation of experimental pain conscious animals.

### Induction of diabetes mellitus

Diabetes was induced by a single IP injection of 55 mg/kg body weight of streptozotocin. After 72 hours of streptozotocin injection, the diabetic rats (glucose level >250mg / dL ) were separated and used for the study (16).

### Experimental design

The method described by Pari and Satheesh (17) was adopted. In the experiment a total of 30 rats (18 diabetic surviving rats and 12 normal rats) were used. The rats were divided into 5 groups (6 rats / group) after the induction of streptozotocin - diabetes.

Group I : Normal untreated rats
Group II : Normal rats were given MEt 100mg/kg body weight in aqueous solution daily for 30 days.
Group III : Diabetic control
Group IV : Diabetic rats were given MEt 100mg/kg body weight in aqueous solution daily for 30 days.
Group V : Diabetic rats were given glibenclamide 600µg/kg body weight (18) in aqueous solution daily for 30 days.

On completion of 30 days of experimental period, the 18 hour fasted rats were anaesthetized and sacrificed by cervical dislocation. Blood was collected with anticoagulant (heparin) was used for serum separation and plasma was collected for insulin estimation.

### Biochemical estimation

Blood glucose was determined by the method of Sasaki etal., (19) using O-toluidine reagent. Insulin content was estimated by using RIA kit (for rats) supplied by linco research Inc. (stat diagnostics, Mumbai).AST and ALT content were estimated by the methods of Reitman and Frankel (20). Catalase was assayed according to method of Takahara et al., (21). SOD was assayed by the method of Misra and Fridovich (22). Glutathione peroxidase was assayed by the method of Rotruck etal., (23) with modifications. Total reduced glutathione was determined by the method of Ellman (24). The level of lipid peroxidation was assays by the method of Ohkawa et al., (25).

### Statistical Analysis

The values are expressed as mean $\pm$ SD for Six rats in each group. All other data were analysed with SPSS/15.0 student's software. Hypothesis testing method included one way analysis of variance (ANOVA) followed by post hoc testing performed with least significant difference (LSD) test. The 'P' value of less than 0.05 was considered to indicate statistical significance.

## RESULTS

Table 1 shows the level of blood glucose and insulin in control and experimental animals in each group .The level of blood glucose was significantly increased and the insulin level was decreased in diabetic control rats as compared to normal control rats. However the level of blood glucose and insulin retuned to near normal concentrations diabetic rats treated with MET and glibenclamide. MET showed comparable effect to that of glibenclamide. Table 2 shows that the values of AST and ALT in serum and liver. Diabetic control rats shows that the increased level of AST and ALT in serum and liver, whereas the treatment with MEt and glibenclamide in alloxan induced diabetic rats shows that the AST and ALT levels were return back to its normal range. Table 3 shows that the values of Catalase and SOD content in serum, liver and kidney of control and experimental rats in each group. A significant

**Table 1. Blood glucose and plasma insulin level in control and experimental rats in each group**

| Groups | Glucose (mg/dl) | Insulin (IU/L) |
|---|---|---|
| Control | 97.22 ± 1.9 | 94.6 ± 0.87 |
| Normal + MEt | 99.05 ± 1.6[a] | 92.7 ± 0.61[a] |
| Diabetic control | 257.88 ± 2.8[b]* | 59.5 ± 0.70[b]* |
| Diabetic + MEt | 106.05 ± 2.0[c]* | 91.2 ± 1.06[c]* |
| Diabetic + glibenclamide | 108.26 ± 3.7[d]* | 91.81 ± 3.40[d]* |

**Table 2. Changes in the level of SGOT and SGPT content in serum and liver of control and experimental rats in each group**

| Group | SGOT(IU/L) | | SGPT(IU/L) | |
|---|---|---|---|---|
| | Serum | Liver | Serum | Liver |
| Normal control | 17.31± 0.72 | 26.64± 1.67 | 20.18± 1.17 | 18.56± 0.88 |
| Normal+MEt (100mg/kg body wt) | 17.83± 0.61[a] | 26.13± 1.01[a] | 21.66± 1.13[a] | 17.63± 1.13[a] |
| Diabetic control | 1.56±2.27[b]* | 54.50± 0.98[b]* | 54.28± 2.84[b]* | 56.9± 1.49[b]* |
| Diabetic+MEt (100mg/kg body wt) | 3.07±1.82[c]* | 28.30± 1.53[c]* | 23.48± 1.06[c]* | 22.31± 0.63[c]* |
| Diabetic+glibenclamide (600µg/kg body wt) | 4.71±0.54[d]* | 29.16± 1.20[d]* | 24.85± 1.35[d]* | 22.16± 1.41[d]* |

**Table 3. Changes in the level of Catalase and SOD content in serum, liver and kidney of control and experimental rats in each group**

| Group | Catalase(IU/L) | | | SOD(IU/L) | | |
|---|---|---|---|---|---|---|
| | Serum | Liver | Kidney | Serum | Liver | Kidney |
| Normal control | 22.23 ± 0.64 | 21.31± 1.14 | 18.10± 1.30 | 197.01± 4.19 | 198.01± 3.05 | 106.18± 1.26 |
| Normal+MEt (100mg/kg body wt) | 22.71 ± 1.31[a] | 21.68± 0.98[a] | 16.78± 0.74[a] | 198.15± 2.92[a] | 199.58± 3.14[a] | 108.23± 1.47[a] |
| Diabetic control | 11.05 ± 0.50[b]* | 8.91±0.65[b]* | 7.45± 0.92[b]* | 132.56± 5.14[b]* | 100.06± 2.79[b]* | 75.91±1.48[b]* |
| Diabetic+MEt (100mg/kg body wt) | 20.21 ± 0.65[c]* | 21.73±1.13[c]* | 15.60± 1.16[c]* | 190.61± 1.79[c]* | 189.18± 2.26[c]* | 103.55± 2.12[c]* |
| Diabetic+glibenclamide (600µg/kg body wt) | 20.95 ± 0.58[d]* | 22.80±1.08[d]* | 16.56± 0.87[d]* | 191.66± 3.44[d]* | 195.48± 1.34[d]* | 106.91± 1.97[d]* |

**Table 4. Changes in the level of glutathione peroxidase, reduced glutathione and lipid peroxidation content in control and experimental rats in each group**

| Group | GPx (IU/L) | | | GSH (IU/L) | | | LPO µmoles of MDA liberated/mg of protein | | |
|---|---|---|---|---|---|---|---|---|---|
| | Serum | Liver | Kidney | Serum | Liver | Kidney | Serum | Liver | idney |
| Normal control | 156.18± 2.79 | 157.58± 2.18 | 75.88± 1.10 | 106.30± 2.18 | 104.45± 3.13 | 39.96± 1.49 | 23.7± 4.2 | 24.6± 3.1 | 22.1± 2.7 |
| Normal+MEt (100mg/kg body wt) | 157.00± 2.43 a | 159.95± 2.15[a] | 77.38± 2.30[a] | 112.25± 2.15[a] | 113.68± 2.12[a] | 41.93± 4.97[a] | 20.1± 3.2[a] | 21.3± 2.4[a] | 20.7± 3.8[a] |
| Diabetic control | 201.46± 2.54[b]* | 96.81± 1.84[b]* | 49.61± 3.25[b]* | 93.81± 3.86[b]* | 201.48± 3.22[b]* | 68.25± 1.34[b]* | 39.2± 5.9[b]* | 42.5± 4.2[b]* | 43.1± 2.6[b]* |
| Diabetic+MEt (100mg/kg body wt) | 155.91± 2.36[c]* | 154.11± 2.59[c]* | 73.71± 4.87[c]* | 105.11± 4.59[c]* | 117.05± 1.44[c]* | 43.0± 2.16[c]* | 28.9± 3.2[c]* | 30.2± 3.7[c]* | 29.8± 2.9[c]* |
| Diabetic+glibenclamide 600µg/kg body wt) | 155.13± 2.46[d]* | 157.05± 2.18[d]* | 74.68± 2.86[d]* | 109.05± 2.18[d]* | 114.15± 2.43[d]* | 39.85± 0.91[d]* | 30.3± 5.3[d]* | 29.4± 4.2[d]* | 28.7± 3.6[d]* |

Values are given as mean ± SD of 6 rats from each group; * Values are statistically significant * P<0.05; a) Normal + MEt rats were compared with normal rats. b) Diabetic rats were compared with normal rats. c) MEt treated diabetic rats were compared with diabetic rats and glibenclamide treated diabetic rats. d) Glibenclamide treated diabetic rats were compared with diabetic rats.

increase in catalase and SOD in serum, liver and kidney of diabetic rats were compared to normal control animals. Oral administration of MEt and glibenclamide to diabetic animals revert back to normal concentrations. Table 4 indicates the amount of GPx, GSH and LPO in serum, liver and kidney of control and experimental rats in each group. The level of Gpx was significantly increased in serum and it was decreased in liver and kidney of diabetic control rats. The GSH and LPO level was significantly raised in serum, liver and kidney of diabetic control rats. However, oral administration of MEt and glibenclamide to diabetic rats revert back to normal concentrations.

## DISCUSSION

Streptozotocin in addition to hyperglycemia induces degenerative changes in the tissue, along with other complications like cardiomyopathy and nephropathy. These are also common complications of insulin dependent DM. this pathogenicity is believed to be due to oxidative damage of the tissues by OFRs (26, 27). In the present study, a marked hike in the level of fasting blood glucose in diabetic control, however continuous treatment of diabetic animals with cocculus hirsutus leaf extract for 30 days caused a significant reduction in FBG level. This antihyperglycemic action may be due to insulin potentiating effect via stimulation of the

undamaged or residual pancreatic islets to release insulin. Moreover significant reduction in blood glucose level in glibeclamide treated group strengthens the above explanation, since it also exerts its hypoglycemic effect by increasing insulin secretion (28). Oxidative stress is the imbalance between production and removal of reactive oxygen species (ROS). Increased oxidative stress, which contributes substantially to the pathogenesis of diabetic complications, is the consequences of either enhanced ROS production or attenuated ROS scavenging capacity. Several reports have shown the alterations in the antioxidant enzymes during diabetic conditions (29, 30). The antioxidative defense system like SOD and catalase showed lower activities in brain during diabetes and the results agree well with the earlier published data (31). The decreased activities of SOD and catalase may be a response to increased production of H2O2 and O2 by the auto oxidation of excess glucose and non enzymatic glycation of proteins (32). Hodgson and Fridovich (33) and Pigiolet et al., (34) have reported the partial inactivation of these enzyme activities by hydroxyl radicals and hydrogen peroxide. The decreased activity of SOD and catalase could also be due to their decreased protein expression levels in the diabetic conditions as reported recently in liver (35). I n the present study elucidate that the decreased catalase and SOD content in serum, liver and kidney of alloxan induced diabetic rats. MEt administration to diabetic rats significantly increases the level of SOD and catalase.

GPx plays an important role in minimizing oxidative damage (36). GPx catalyzes the reduction of H2O2 to H2O and O2 at the expense of GSH. GPx activity is also reduced in diabetic condition. This may due to inactivation of the enzyme involved in disposal of O2 species and also insufficient availability of GSH (37). In this context, the significant increase in GSH content, GSH dependent enzymes and GPx in diabetic rats treated with MEt indicates an adaptive mechanism in response to oxidative stress. The decreased activity of antioxidant molecules along with elevated LPO levels in diabetic rats could probably be associated with decreased antioxidant defense potential. The reversal in their content treatment with piper nigrum and Vinca rosea may be due to decreased oxidative load (38). In this context LPO levels were decreased with the treatment of cocculus hirsutus on alloxan induced diabetic rats. The higher level of AST and ALT plays the role of providing new supplies of glucose from other sources such as amino acids. Following intraperitoneal administration of different plant fractions, AST and ALT levels were significantly reduced (40).

**Conclusion**

In the present study there was significant reduction in the level of glucose, AST, ALT, catalase, GPx, GSH, LPO and increase in the level of insulin in streptozotocin induced diabetic rats. These changes could have resulted from the methanolic extract of diacure a poly herbal formulation. These results strongly suggest that the diacure a poly herbal formulation have a potent antioxidant effect.

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