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# ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF A HERBOMINERAL AMINOACID EXTRACT ON DIABETIC RATS OF WISTAR STRAIN

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## ABSTRACT

The objective of this study was to investigate the single dose antioxidant and antidiabetic effect of a herbomineral amino acid extract (HMAA) of Spinacia oleracea on alloxan induced diabetic rats. In the present study, the animals were divided into 6 groups: Group 1 – Normal saline as vehicle –control, Group 2 – Untreated diabetic rats (Alloxan induced), Group 3 – Animals treated with Metformin (0.5 mg/kg) – standard drug, Group 4 – Animals treated with Extract alone, Group 5 – Animals treated with Herbal + Mineral, Group 6 – Animals treated with Herbal + Mineral + Amino Acid. Based on the oxidative stress hypothesis of alloxan action, the role of herbal extract on free radicals in the pathology of diabetes mellitus has been studied in diabetic nephropathy rats. The present study demonstrated that herbal extract in the presence of an aminoacid (L-Arginine, 0.1 mM) and mineral (Zn+, in the form of Zinc Sulphate, 0.5 mM) was found to be a potent antioxidant and on alloxan induced diabetes. Further, the data suggested that the mechanism underlying such protection is mediated via restoration of antioxidant status leading to normalization of the diabetic rats.

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## KEY WORDS

Alloxan, anti-oxidant, diabetic nephropathy, HMAA, Spinacia oleracea,

## INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder, characterized by disturbed glucose metabolism due to an absolute or relative insulin deficiency. As a consequence of the metabolic derangements in diabetes, various complications develop including both macro and micro vascular dysfunction[1]. New biochemical and molecular advances have contributed to a more profound understanding of the pathogenesis of diabetes and its complications[2]. Recently, increased oxidative stress has been proven to play a pivotal role in the etiology and pathogenesis of diabetes mellitus and its complications[3]. The role of oxidative stress in both type I and II diabetic mellitus is currently under intensive scientific investigation[4-6]. It is believed that insulin dependent diabetes mellitus (IDDM) results from the destruction of insulin producing pancreatic $\beta$ cells by multiple factors including viruses, chemical toxins and autoimmune responses[7-9].

It has been found that anti-oxidants, especially $\alpha$-lipoic acid ($\alpha$-LA) improved insulin sensitivity (Maddux et.al., 2001; Rudich et al., 1999; Jacon et. al., 1996). Spinacia oleracea has $\alpha$-lipoic acid as one of its active ingredients (Dr. Duke's Ethnobotanical databases). In view of the above studies and previous data showing $\alpha$-LA partially ameliorate diabetes related alternations in skeletal muscle glucose metabolism and PI-3 kinase /AKT activites (Bitar et al, 2004), it is aimed here to evaluate the hypoglycemic and antioxidant activities of aqueous and methanolic extract of a herbal extract (Spinacia oleracea).

Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones. (Momin, 1987). In traditional methods, the medicinal plants being used, are very often in powder or paste forms of the crude herbs, which contain both the organic and inorganic constituents.

Zinc ion (Zn), an essential trace element with relatively low toxic profile in animals and humans (Life Science 75(2004), 741-751, (Underwood)), has been proven to have a pronounced insulinomimetic activity. For example, Coulston and Dandona reported the insulinomimetic activity of Zn (II) ion $5 \times 10^{-4}$ M in the isolated rat adipocytes in 1980 (Coulston and Dandona, 1980). Following this finding, the invivoantidiabetic effect of Zn (II) ion was reported by Shisheva et al. (1992), Chen et al (1998), and Song et al (2001). However experiments done so far on hypoglycemic herbs were mostly with their organic active principles. The role of some inorganic elements like potassium, zinc, calcium, manganese and traces of chromium in the improvement of impaired glucose tolerance and their indirect role in the management of DM are being increasingly recognized (Gusson and Saner, 1971; Mertz, 1981; Kinlse et. al., 1983; Niewochnn et al., 1986).

Besides, Zn ions are necessary for crystallization of insulin and insulin is stored as zinc complexes. Although many antidiabetic drugs from herbal and mineral origin are used as a single medicine as well as in combination with the other, these compound medicines secure to be more effective than a single drug.

Amino acids - Arginine and Leucine are found to have antidiabetic activity. (Pharmacology, Rang,

Dale, H.M).In view of these, the present preliminary study was undertaken to investigate the single dose effect of herbal extract, a herbomineral extract and a herbominalaminoacid extract of methanolic form.

## MATERIALS AND METHODS

***Collection of Plant Material***

Whole plant Spinacia Oleracea plant powder were procured from the Nilgiri hills and were authentified by Botany Research Officer, Survey of Medicinal plants unit-siddha, Government Siddha Medical College Campus, Palayamkottai, Tamilnadu.

***Preparation of leaf extract by methanol using soxhlet apparatus[10]:***

Methanolic extract was prepared by mixing 80gms of wet powder with 90% methanol. The residue was collected and dried on water bath at (60-80°C). The obtained extract was filtered, concentrated and the residue was stored in refrigerator at 2-8°C for use in subsequent experiments.

Male adult (Wistar strain) albino rats (150-200g). They were obtained from Animal House of RMMCH, Annamalai Nagar, Annamalai University. The animals were maintained on standard ratio and provided clean drinking water ad libitum. The animals were kept in air-conditioned room (temperature 20 ±2C) and acclimatized for a period of 7 days. The mineral zinc was taken in the form of salt zinc sulphate 0.5 mM and the amino acid was taken as L-arginin 0.1 mM in the present study.

***Induction of Diabetes:-***

A single dose, generally of 125mg/kg (intraperitonial) of alloxan monohydrate 5% dissolved in normal saline (warmed), was used for diabetes induction. Induction of Diabetes mellitus was confirmed after the 5$^{th}$ day of alloxan treatment of rats, with blood glucose level ≥ 150mg/dl, selected for the study.

***Sample Collection***

Fasting blood samples were collected in fresh vials containing sodium fluoride and sodium oxalate as anticoagulant / anti-glycolytic agents, retro-orbitally every 10$^{th}$ day till the end of experiment (i.e. 50$^{th}$ day) from the inner canthus of the eye under light ether anaesthesia using capillary tubes. Plasma was separated in a T8 electric centrifuge (RemiUdyog, New Delhi) at 2000 rpm for 2 min.

***Study Design:-***

A total of 36 rats (Wistar Strain) were used for the present study and were divided into 6 groups with 6 animals in each group. Group 1 – Normal saline as vehicle –control, Group 2 – Untreated diabetic rats (Alloxan induced), Group 3 – Animals treated with Metformin (.5 mg/kg) – standard drug, Group 4 – Animals treated with Extract alone, Group 5 – Animals treated with Herbal + Mineral, Group 6 – Animals treated with Herbal + Mineral + Amino Acid.

***Glucose Estimation:*** The fasting blood serum glucose between 70-100 mg% or mg/dl was considered as normal. The serum samples were collected from the animal groups of normal control, alloxan included diabetic control, standard drug metformin, herbal extract, herbal + mineral and herbal + mineral + Aminoacid groups respectively. Working enzyme reagent (B), glucose standard (S) and serum / plasma (T) were pipetted into clear dry test tubes. The test tubes were mixed well and incubated at 37°C for 15 minutes. The absorbance of the test

(T) and standard (S) against blank (B) were observed on an autoanalyzer at 505 nm.

***Determination of Serum Lipid Levels, Lipid Peroxidation*:** Serum Total Cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and Triglycerides (TG) concentrations were measured enzymatically and colorimetrically using kits from Sigma Diagnostics India Pvt. Ltd., Baroda. For thiobarbituric acid reactive substances (TBARS), kidney cortex homogenates (5%, w/v) were mixed with an equal volume of ice-cold 10% trichloroacetic acid (TCA). After centrifugation, a volume of the supernatant was added to an equal volume of 0.67% thiobarbituric acid substances (TBA) and the mixture was kept in a boiling water bath for 15 min. Samples were cooled to room temperature and absorbance at 532 nm was recorded. The results were expressed as MDA equivalents using a molar extinction coefficient of $1.56 \times 10^5$ $M^{-1}$ $cm^{-1}$.

***Superoxide Dismutase (SOD) and Catalase:*** The SOD activity was measured according to the method of Beauchamp and Fridovich. An adequate amount of the liver supernatant was mixed with the reaction mixtures, which contained 0.1 mM EDTA, 25 mM NBT, 0.1 mM xanthine, 50 mM sodium carbonate buffer (pH 10.2), and the final volume of the reaction mixture was brought upto 3 ml with distilled water. The reaction was initiated by the addition of 2 mU $ml^{-1}$ xanthine oxidase and maintained under two 40 W lamps at 25°C. After 15 min, the inhibition rate of NBT reduction was spectrophotometrically determined at 560 nm. One unit of SOD is defined as the amount of enzyme required to reduce the NBT by 50%. The specific activity of SOD was expressed as a unit $mg^{-1}$ protein in each supernatant and the values were calculated as a percentage of the control value. Catalase assay was conducted according to the method of Thomson et al. The liver supernatant was mixed with 2.8 ml of 50 mM phosphate buffer (pH 7.4). After equilibration at 30°C for 5 min, the reaction was started by the addition of 200 ml of 100 mM sodium perborate (pH 7.4). The catalase activity, which reduces the sodium perborate as a sustrate, was assessed spectrophotometrically following the consumption of hydrogen peroxide at 220 nm for 2 min. One unit of catalase was defined as the amount of enzyme required to reduce 1 μM of $H_2O_2$ $min^{-1}$. The results were expressed as a unit $mg^{-1}$ protein in each supernatant and the values were calculated as a percentage of the control value.

***Glutathione:*** Cytosolic reduced glutathione (GSH) was measured using Glutathione Assay Kit from Cayman Chemicals according to the manufacturer's instructions. The sample (100-150 μg) was deproteinized using phosphoric acid, and the amount of 5-thio-2-nitrobenzoic acid produced was measured in the supernatant.

***Creatinine and Pancreatic Nitrite Levels:*** The creatinine kit was procured from S.d fine chemicals and was manufactured by Sigma Diagnostic India Pvt. Ltd. Baroda. The preparations were mixed well and allowed to stand at room temperature for 20 minutes and the absorbance was measured for blank, standard and test against distilled water on a autoanalyzer at 520 nm. The serum creatinine values of 0.8-1.3 mg% were considered as normal. Nitrite production was measured as 100 to 150-μg sample using Greiss reagent as described previously (Tanous, Veluthakal, Amin & Kowluri, 2002). The absorbance was measured at 540 nm, and the nitrite

concentration was calculated from a sodium nitrite standard curve.

***Urinary Albumin Levels:*** Albumin was measured by commercially available kits (based on quantitative colorimetric assay)

***Food intake, Body weight and Liquid intake:*** Food and liquid intake was evaluated every day. Weight of individual animals was measured every $10^{th}$ day.

***Triglycerides***

I & IV - $p < 0.01$ , II & IV- $p < 0.01$, II & V- $p < 0.05$, III & IV- $p < 0.001$, III & V-$p < 0.001$, III & VI - $p < 0.001$, V & VI - $p < 0.05$

***Total Cholesterol***

I & III -$p < 0.001$, I & IV- $p < 0.05$, II & III- $p < 0.05$, II & IV- $p < 0.05$, III & IV- $p < 0.001$, III & V- $p < 0.001$, V & VI - $p < 0.01$

***HDL***

Showed a significant difference only in the overall treatment but not on individual groups

***Tissue MDA***

Tissue MDA levels in Group I decreased and was significant ($p < 0.001$) from Group I (normal control) and Group II (diabetic control). No significant difference was observed with herbal extract alone. However, in Group V (H + M) and Group VI (H + M + AA) the results were significant at $P < 0.001$ & $P<0.001$ respectively from normal control and diabetic control groups.

## RESULTS

**Glucose:** The present study seems to support these facts, as the extract was able to decrease the glucose levels in both normal and alloxan induced diabetic rats. The mechanism of action of the extract is unclear but it could be possible that it exerts its hypoglycemic action by either increasing the peripheral utilization of glucose (or by action) similarly to insulin (Table1).

**Table – 1**

***Effect of the H, H + M, H+M+AA extracts from Sp. Oleracea on blood glucose levels in alloxan induced diabetic rats Mean blood glucose conc. ± S.D. (mg / dl)***

| Test samples Dose (mg/kg) | 0 min | 30 min | 60 min | 120 min | 240 min | 360 min |
|---|---|---|---|---|---|---|
| Normal control | 68.3 ± 3.5 | 71.1 ± 2.4 | 69.4 ± 2.9 | 70.6 ± 4.2 | 72.4 ± 1.6 | 69.1 ± 3.4 |
| Diabetic control (Alloxan induced) | 391.6±19.7 | 393.4 ± 11.7 | 390.6 ± 19.2 | 391.4 ± 18.6 | 392.1 ± 12.6 | 391.9 ± 11.1 |
| Metformin 0.5 mg / kg | 383.5±21.8 | 307.3 ± 22* | 273.7 ± 10.9 | 207.1 ± 11.4** | 80.4 ± 11.1*** | 78.2 ± 14** |
| Herbal extract | 380.7±10.7 | 342.7 ± 11.3*** | 294.7 ± 17.8 | 234.6 ± 14.3 | 114.3 ± 16.8 | 109.1 ± 14.3 |
| Herbal + Mineral | 382.5±11.5 | 322.2 ± 17.3** | 283.5 ± 7.9 | 220.7 ± 11.1* | 102.5 ± 9.9 | 81.3 ± 20 |
| Herbal + Mineral + AA | 384.7±14.6 | 302.5 ± 18*** | 277.7 ± 16.4* | 211 ± 12.6 | 91.6 ± 16.3 | 74.6 ± 7.9 |

*Inhibitory effect on blood glucose concentration above 10% shown*

* *P < 0.05 significant from the control animals*

** *P < 0.01 significant from the control animals*

*** *P < 0.01 significant from the control animals*

*H - Herbal extract*

*M - Mineral (Zinc sulphate – 0.5 mM)*

*AA - Amino acid (L – arginine – 0.1 mM)*

**Serum lipids and lipid peroxidation:** The extract caused a significant ($p < 0.05$) decrease in the serum triglyceride, total cholesterol, in the diabetic and non-diabetic rats treated with extract. The hypolipidemic effect of the extract could be an additional advantage of the use of the extract in the management of diabetes. This could also be a potential source of hypolipidemic agents (Table-2).

**Table – 2**

***Serum lipid profile of diabetic and non-diabetic rats; Malondialdehyde (MDA) contents in the kidney of alloxan induced diabetic rat and after their treatment with H, H + M and H+M+AA extracts respectively.***

| Groups | TG (Tryglycerides) mg/dl | Total Cholesterol (mg/dl) | HDL-C (mg/dl) | Tissue MDA Mean ± Sd (n mol / g wet. wt.) | % change |
|---|---|---|---|---|---|
| Normal control | 85.50 ± 4.44 | 88.97 ± 2.79 | 39.60 ± 1.20 | 181.4 ± 13.2 | - |
| Diabetic control | 82.0 ± 2.58 | 83.15 ± 1.51 | 36.8 ± 2.06 | 340.7 ± 6 | +87.8 |
| Metformin | 79.0 ± 4.13 | 67.11 ± 3.48 | 32.8 ± 2.02 | 271.4 ± 12.7 | -20.3 |
| Herbal | 117.0 ± 4.44 | 97.38 ± 1.09 | 41.2 ± 0.40 | 333.9 ± 25.5 | -2.0 |
| H + M | 100.0 ± 4.32 | 86.19 ± 2.29 | 38.4 ± 1.31 | 290.3 ± 8.5 | -14.8 |
| H + M + AA | 80.0 ± 1.64 | 73.68 ± 4.30 | 32.4 ± 3.36 | 272.9 ± 7.8 | -19.9 |

**SOD and Catalase:** To avoid redox imbalance and oxidative damage, aerobic organisms possess an efficient biochemical defense system such as enzymatic (SOD, CAT and GSH), but these antioxidant enzymes do not completely protect from the attack of ROS in conditions of severe oxidative stress. Values differ significantly at $p < 0.05$ (liver) and at $p < 0.01$ (kidney) (Table-3).

**Table – 3**

***Effect of H+M+AA extract on SOD and Catalase***

| Groups | SOD (Unit[A] / mg protein) | | Catalase (unit[B] / mg protein) | |
|---|---|---|---|---|
| | Liver | Kidney | Liver | Kidney |
| Normal control | 6.80 ± 0.25 | 16.9 ± 1.38 | 75.2 ± 1.46 | 33.1 ± 1.64 |

| | | | | |
|---|---|---|---|---|
| Diabetic control | 3.41 ± 0.09* | 10.1 ± 0.91* | 44.9 ± 1.38** | 21.1 ± 5.05** |
| Metformin | 6.48 ± 0.24* | 15.9 ± 1.37* | 65.9 ± 3.10** | 30.1 ± 2.43* |
| Herbal | 5.01 ± 0.34 | 14.6 ± 1.32 | 53.7 ± 2.94 | 26.8 ± 0.94 |
| H + M | 5.94 ± 0.20 | 13.7 ± 1.02 | 41.4 ± 0.95 | 23.1 ± 1.80 |
| H + M + AA | 5.81 ± 0.27 | 13.4 ± 0.47 | 40.3 ± 0.89 | 22.5 ± 1.75 |

** Value differ significantly at p < 0.05, ** Value differ significantly at p < 0.01*

*A – one unit of activity was taken as the enzyme reaction which gave 50% inhibition of nitrobluetetrazolium (NBT) reduction in one minute.*

*B - μ moles of hydrogen peroxide consumed / min.*

**Glutathione:** Duncan's Multiple Range Test was performed. Values differ from each other significantly at p < 0.05 (Table-4).

**Table – 4**

***Effect of H+M+AA on reduced GSH – in alloxan induced diabetic rats GSH mg/100 g tissue***

| Group | Liver | Kidney |
|---|---|---|
| Normal | 52.0 ± 4.38* | 33.7 ± 6.02* |
| Diabetic control | 28.0 ± 3.38** | 18.7 ± 1.70** |
| Std. (Metformin) | 46.3 ± 0.98* | 30.6 ± 1.96* |
| Herbal extract | 38.5 ± 4.35 | 24.0 ± 2.26 |
| H + Mineral | 32.6 ± 4.45* | 20.0 ± 2.84 |
| H + M + AA | 37.1 ± 31.4 | 28.3 ± 1.47 |

*Values differ from each other significantly at * p < 0.05 ** p < 0.01 Duncan's Multiple Range Test was performed*

**Serum Creatinine and Pancreatic Nitrite levels:** No significant difference was observed between normal control and diabetic controls or the treated groups for serum creatinine throughout the duration of experiment (Table-5).

**Table – 5**

***Serum Creatinine and Pancreatic Nitrite levels***

| Groups | Serum Creatinine | | Pancreatic Nitrite levels after (time) (in Umol / L) | | |
|---|---|---|---|---|---|
| | 10th day | 31st day | 24 hr | 48 hr | 72 hr |
| Normal control | 41.5 ± 3.08 | 46.6 ± 3.05 | 240 ± 4.3 | 241 ± 3.8 | 240 ± 4.2 |
| Diabetic control | 46.8 ± 4.49 | 50.0 ± 1.67 | 377 ± 5.6 | 382 ± 6.9 | 771 ± 18.64 |
| Metformin | 42.8 ± 3.90 | 51.0 ± 5.36 | 280 ± 6.7 | 259 ± 7.1 | 243 ± 7.4 |
| Herbal (H) | 46.3 ± 4.63 | 42.3 ± 3.92 | 332 ± 4.9 | 344 ± 5.2 | 483 ± 11.46 |
| H + M | 45.3 ± 1.63 | 47.5 ± 3.08 | 309 ± 5.6 | 338 ± 4.7 | 407 ± 10.41 |
| H + M + AA | 46 ± 6.32 | 47.1 ± 7.54 | 298 ± 3.9 | 290 ± 4.4 | 276 ± 5.2 |

*No significant difference observed between normal control and diabetic controls or the treated groups for serum creatinine throughout the duration of experiment.*

**Urinary Albumin Levels:** Herbal extract has a significant ($p < 0.05$) protective action than standard metformin (Table-6).

**Table – 6**

***Urinary Albumin Excretion Levels Urine Albumin level from 0-40 day (μg/24hr)***

| Groups | 0th day | 10th day | 20th day | 30th day | 40th day | Mean rise in UAE levels from 0 - 40 |
|---|---|---|---|---|---|---|
| Normal control | 170.83 ± 21.01 | 168.83 ± 20.02 | 172.14 ± 19.26 | 173.14 ± 23.46 | 172.16 ± 17.42 | 171.41 ± 16.23 |
| Diabetic control | 1055.33 ± 18.6 | 1552.66 ± 48.68 | 1504.8 ± 150.28 | 1488.6 ± 102.27 | 1411.33 ± 22.69 | 387 ± 70.9 |
| Std (Metformin) | 1079.16 ± 52.65 | 1449.83 ± 72.41 | 1410.5 ± 47.60 | 1406.33 ± 15.76 | 1277.5 ± 18.9 | 198.33 ± 63.74 |
| Herbal | 1054.66 ± 20.39 | 1527 ± 36.55 | 1510 ± 59.294 | 1420 ± 82.62 | 1323.66 ± 17.23 | 269.1 ± 10.64 |
| Herbal + Mineral | 1097.16 ± 0.87 | 1465.5 ± 59.19 | 1451.5 ± 74.54 | 1399 ± 54.13 | 1301 ± 90.54 | 204.3 ± 23.16 |
| Herbal + Mineral + Amino acid | 1043.5 ± 29.69 | 1481 ± 51.21 | 1501 ± 60.12 | 1437 ± 79.12 | 1214.5 ± 18.9 | 171.4 ± 18.21 |

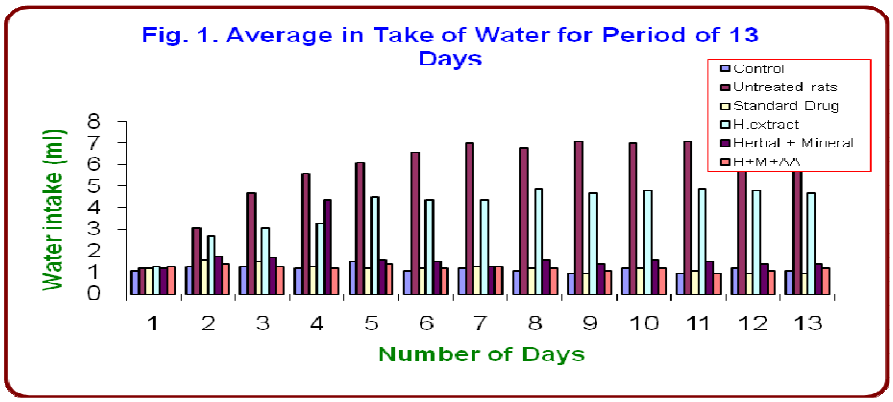
* Herbal extract has better nephropathic protective action than standard metformin.
UAE – Urinary Albumin Excretion
H - Herbal extract,
M - Mineral (Zinc sulphate – 0.5 mM)
AA - Amino acid (L – arginine – 0.1 mM)

**Water Intake:**

In non-diabetic control animals, water intake (mean ± S.E) was 1.0 ± 0.06 on day 1 and remained at that level during the next 13 days. Diabetes caused the water intake to increase significantly from 1.3 ± 0.2 on day 1 to 7.5 ± 0.4 on day 14. Treatment with metformin and the combination of H + M extract reversed their effect and brought water consumption to control level.

Treatment with herbal extract reduced the water intake significantly in diabetic animals, although not to control levels.

In the animals, water intake decrease significantly from day 4 onwards (3.6 ± 0.4) till the end of the treatment period (5.2 ± 0.3), when compared to diabetic rats ($p < 0.05$) Fig-1

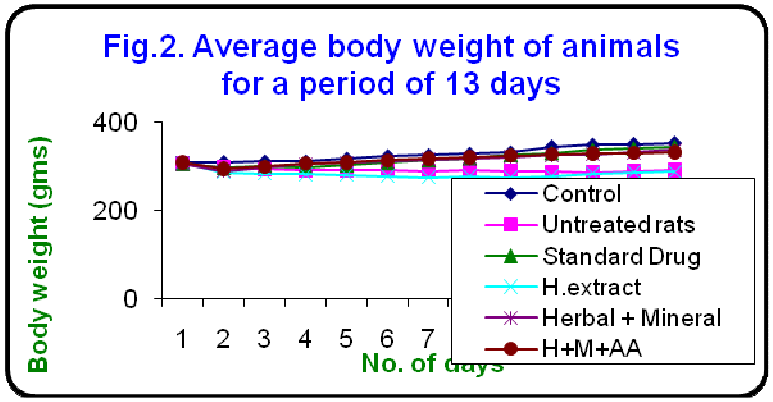



**Body Weight:**

There were no significant differences in body weights between different groups at the beginning of the treatment. Body weight (mean ± S.E) of non-diabetic control animals on day 0 was 309.4 ± 4.6 and increased significantly to 351.2 ± 6.4 on day 14. On the other hand, body weight of diabetic animals was 298.1 ± 8.1 on day 1 and remained at that level during the rest of the period of observation.

Treatment of diabetic animal with std. drug metformin (0.5 mg / kg b.wt), increased their body weight from 297.7 ± 4.3 on day to 345.5 ± 1.5 on day 14 and was not different from the control group. Treatment with both herbal and mineral and even the H + M + AA increased the body weight of diabetic animal from day 1 to day 14 ($p < 0.05$).

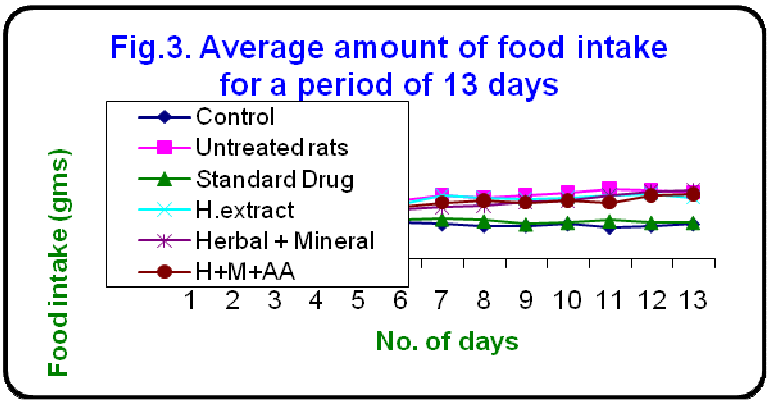
In contrast, treatment with herbal extract alone did not produce any significant changes in body weight compared to the weight on day 1 (287.2 ± 5.7). The body weight of diabetic animals decreased significantly on the second day and remained at that level for the rest of the observation period (Fig-2).




**Food intake:**

The daily food intake (mean ± S.E; g) of non-diabetic control animal on day 0 was 23gms and remained at that level during the entire period of observation. In contrast, induction of diabetes caused the food intake to increase from 14 ± 0.4g on day 1 to 40 ± .07 g on day 14.Food intake of Metformin treated animals was similar

to that seen in control animals. Treatment of diabetic animals with H + M + AA also maintained the food intake around control levels. However, herbal extract treatment alone attenuated diabetes induced hyperphagia, although not to control levels. After day 5, food intake in herbal-extract treated animals decreased significantly compared to diabetic animals until the end of the treatment period, although still higher than the control and metformin – treated animals ($p < 0.05$) (Fig3).




## DISCUSSION

The exact cellular mechanism of β-cell destruction remains unclear.

It has been established that locally produced Reactive Oxygen Species (ROS) and Nitric Oxide (NO) induced after cytokine stimulation are in involved the destruction of β-cells[11,12]. Recent studies by Kaneto et al.[13] and Matsuoko et al[14] have proven that ROS lead to damage of β-cell through the induction of apoptosis and suppression of insulin biosynthesis. However, the exact cellular mechanism of β-cell destruction remains unclear.

Recent studies by Kaneto et al.[13] and Matsuoko et al[14] have proven that ROS lead to damage of β-cell through the induction of apoptosis and suppression of insulin biosynthesis.

Similarly, the development of type II diabetes has been associated with pancreatic β-cell dysfunction, and once hyperglycemia becomes apparent, β-cell function progressively deteriorates[15].

Previous studies, have shown that sustained hyperglycemia, a characteristic of diabetes, increases intracellular ROS in pancreatic β-cell which leads to cellular dysfunction[16,17]. Pancreatic β-cells are particularly susceptible to the deleterious effects of ROS because of their low expression of the antioxidant enzymes genes as compared to other tissues[18,19]. Hence, the cellular antioxidant status is an important determinant of its susceptibility to oxidative damage.

Glutathione (GSH) is an endogenous antioxidant that acts as a first line defense system against pro-oxidant status[20]. Anathan et al[21] showed a significant reduction in plasma GSH levels in experimental diabetic rats. Similarly, depleted GSH levels have been repeatedly reported in several tissues of experimental diabetic animals, including eye, aorta, kidney as well as small intestine[22, 24]. Furthermore, significant decreases in plasma as well as erythrocyte GSH levels have been documented in diabetic patients[25,26].

Zinc is an essential trace element necessary for normal protein metabolism, for the function of more

than 200 metalloenzymes and for a host of physiologic functions.

. A poor zinc status is common in both liver cirrhosis and diabetes mellitus. Many of the clinical features of liver cirrhosis and diabetes mellitus have been linked to zinc deficiency. Kurt Grangreiff, Dirk Seinhold have reported earlier that zinc supplementation improved in patients with liver cirrhosis and hepatic encephalopathy with and without diabetes mellitus, neurological symptoms and signs of malnutrition. They also reported that zinc supplementation increased glucose disposal.

The data presented revealed a marked protective effect of herbal extract on alloxan-induced elevation of total nitrate/ nitrite level in pancreatic tissue, whereby, concurrent treatment with H+M+AA normalized the pancreatic NO levels.

Diabetic animals treated with extract showed a significant reduction in the pancreatic nitrate / nitrite level. Such effect was obvious at both doses used following 48 h as well as 72 h.

The present study substantiated the earlier reports of an increase in hepatic and renal MDA concentration in alloxan induced diabetic rats when compared with the normal rats.

In diabetes, hypoinoulinsulinemia increases the activity of the enzyme, fatty acyl coenzyme of oxides, which initiates $\beta$-oxidation of fatty acids, resulting in lipid peroxidation. Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity and changing the activity of membrane bound enzymes and reception.

Evidence is provided for the first time demonstrating that institution of herbal extract soon after induction of diabetes in rats can prevent proteinuria, kidney hypertrophy and increases oxidative stress and nitrative stress. In support of the results presented here, microalbuminuria in diabetic patients is shown to disappear and the risk of developing nephropathy is reduced by herbal extract.

Some studies elsewhere have indicated that plant extracts may contribute to body weight loss in animals (Bwitti et al., 2001). However, results of the current study suggest that oral administration of Spinacea oleracea leaf extract (along with mineral $ZnSO_4$ and Amino acid L-arginine) did not significantly contribute to the changes in body weights, since the loss in body weight of control alloxan-diabetic rats and treated diabetic rats did not significantly differ.

Body weight losses in diabetic rats as well as in type-1 diabetic patients have been previously reported (Bwilli et al., 2001) (McDrmott, et al., 2003).

Recently it was also reported that diabetes mellitus, atherosclerosis and carcinogenesis could be caused by oxidation of membrane lipid mediated radicals such as superoxide or hydroxyl radical in humans. (Teacham R, Witztum J.L The oxidation hypothesis of atherosclerosis, Lancet 1994, 344 : 793-5).

Therefore, many scientists have tried to obtain bioactive substances having the cytoprotective ability against cellular oxidative damage, as well as an enhancing ability of antioxidant enzyme activities (Jilal, Fuller et al 1993).

In these contexts, it was investigated whether HMAA can enhance the activities of SOD, CAT and GSH in alloxan induced diabetic rat.

The mean rise in Urinary Albumin Excretion (UAE) levels from day 0 to 40 in diabetic control was 387 $\pm$ 70.9 mg / 24 hr and it was significantly more in comparison to std, herbal, H + M, H + M + AA treated groups. Both herbal and metformin decreased the UAE levels significantly. However, the herbal extract treated group has shown a marked decrease in the mean UAE when compared to standard drug metformin.

In contrast, the H + M and H + M + AA treated groups brought down the UAE levels similar to that of standard metformin treated groups. Evidence is provided for the first time demonstrating that institution of HMAA soon after induction of diabetes in rats can prevent proteinuria, and increases oxidative and nitrate stress.

Following the effects of food intake, diabetes also caused an increase in water intake starting from day 2. As expected, treatment with metformin completely reversed water intake. Once again, the water intake in animals treated with herbal extract alone did not drop down to the level seen in the control or insulin – treated animals.

This is another indicator that the dose of herbal extract used was not sufficient to bring the water intake down to non-diabetic levels. The mechanism by which diabetes causes polydipia is unclear. Regulation of fluid balance in the body is a highly complicated process.

One of the possible neurochemicals that may have an important effect on this function is arginine varopressin. The cell bodies that secrete this neuro hormone are located in the paraveutricular nucleus of hypothalamus. (H.W. Schwartz, D.P. Figlewicz, O.G. Baskin, S.C. Woods, D.P. Porte, JR., Insulin and the cultural regulation of energy balance; update. Endocr. Rev. Monogr. 2 (1994) 109-113.

## CONCLUSION

Based on the oxidative stress hypothesis of alloxan action, it is considered as an adequate model for investigating the role of free radicals in the pathology of diabetes mellitus. The present study demonstrates that herbal extract, a potent antioxidant, can exert anti-diabetic effects by preserving pancreatic β-cell function. The data presented provide additional benefits of NO administration and may offer a promising natural and safe new trend for the prevention or delay of diabetic complications. In summary, the results of the present study indicate that herbal extract possess potent protective effect on the induction of diabetes by alloxan. The data provided suggest that the mechanism underlying such protection is mediated via prevention and restoration of antoxidant defense systems. Restoration of antioxidant status led to normalization of the release of insulin, hence maintaining glucose serum levels within the normal range. Diabetes produced changes in body weight, food intake, and water intake but also marked effect on the oxidative enzyme. These effects were completely reversed by standard drug metformin (except for the nephropathic condition), while treatment with Herbal + Mineral + AA produced partial to complete reversal. It is unclear at the present time if Herbal + M + AA treatment could provide for long-term benefits. This will be the focus of future studies.

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