Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 4, 2012

Research Article

# ANTIDIABETIC EVALUATION OF AQUEOUS EXTRACT OF AERIAL PARTS OF *MOLLUGO PENTAPHYLLA* L.

**LAXMIDHAR MAHARANA*, DURGA MADHAB KAR, SNIGDHA PATTNAIK**

***School of Pharmaceutical Sciences, Siksha 'O' Anusandhan Deemed to be University, Bhubaneswar, Odisha, India 751003.**
**Email: mantuplus@yahoo.com, dmkar1970@yahoo.com**

*Received: 19 July 2012, Revised and Accepted: 06 Sep 2012*

**ABSTRACT**

The aqueous extract of aerial parts of *Mollugo pentaphylla* Linn.*(AAMP),*commonly known as carpet weed (English), pitta saga (Odia) was studied for its antidiabetic and antioxidant potential in different experimental models. The antidiabetic potential of the plant extract was undertaken in both normoglycemic and alloxan induced hyperglycemic models by comparing different biochemical parameters like blood glucose, plasma-insulin, lipid profiles along with the liver antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and glutathione reductase (GR) and liver oxidants like thiobarbituric acid reacting substances (TBARS), hydroperoxide (HP), malondialdehyde (MDA) and conjugated dienes (CD) were quantified using standard experimental procedures. The antidiabetic activity of the extract was evidenced by significant fall of blood sugar level apart from its ability to increase plasma insulin level along with decreased quantity of liver oxidants and increased quantity of liver antioxidant enzymes, with a positive support of elevated glucose uptake by rat hemi diaphragm. The plant extract was studied in 250 and 500 mg/kg dose levels for a period of thirty days and found by analyzing the tested parameters in support of the activity that, the extract is endowed with potential antidiabetic activity, the potency of which rest on the dose.

**Keywords:** *Mollugo pentaphylla*, Antidiabetic, Antioxidant, Lipid peroxidation.

## INTRODUCTION

Diabetes mellitus (DM) is a major heterogeneous endocrine and metabolic disorder, characterized by altered metabolisms of carbohydrate, lipid and protein, which not only lead to hyperglycaemia but also cause many complications, such as hyperlipidemia, hypertension and atherosclerosis[1,2,3]. The increased glucose level tends to glucose auto oxidation and auto oxidative glycosylation of proteins, which leads to oxidative stress by increasing the reactive oxygen species[4]. The oxidative stress, caused by free radicals induced by hyperglycemia, contributes to the development and progression of diabetes along with secondary complications[5, 6,7]. Antioxidants have been shown to prevent the destruction of β-cells[8, 9] by inhibiting the peroxidation chain reaction and thus, may provide protection against the development of diabetes[10,11,12]. Abnormally high levels of free radicals cause membrane damage due to lipid peroxidation and protein glycation and the simultaneous decline or disturbance of antioxidant defense mechanisms leads to cell and tissue damage[7]. As a new strategy for alleviating the oxidative damage in diabetes, interest has grown in the usage of natural antioxidants. It has been postulated that many of the negative effect of oxidative stress are diminished upon supplementation with certain dietary antioxidants such as vitamin E, C and other non-nutrient antioxidant such as phenolic compounds and flavonoids[5,13]. On the other hand, plants contain natural antioxidants (tannins, flavonoids, vitamins C and E, etc.) that can preserve β-cell function and prevent diabetes induced ROS formation[14] and many plant species are known in folk medicine of different cultures to be used for their hypoglycaemic properties and therefore used for treatment of DM[15,16]. Despite this, few traditionally used antidiabetic plants have received proper scientific screening. The World Health Organization (WHO) has recommended that this area warrants further evaluation[17].

*Mollugo pentaphylla* Linn, commonly known as carpet weed (English), Pitta saga (Oriya) is a perennial herb found throughout India, Ceylon, Malacca, China, Japan, Fiji etc. Roots are creaper and adventitious, leaves are trifoliate small oval shape; flowers are white, pentameric and bisexual. The urban people used this plant medicinally in paste form orally and externally for treatment of skin allergic condition, antimicrobials etc[18,19,20].Highly esteemed by Hindus as a bitter vegetable which they eat occasionally on account of its stomachic, aperient and antiseptic properties[21]. Ethnomedical Information on *Mollugo pentaphylla* cited the folkloric use of the plant as an emmenagogue on female human adult in India[22] and Indonesia[23].Hot $H_2O$ extract of dried entire plant in India used for whooping cough and in cases of atrophy in human[24] and Decoction of dried entire plant used to treat hepatitis in Taiwan[25]. *M. pentaphylla* is a component in an important folk medicine named "Peh-Hue-Juwa-Chi-Cao" in Taiwan, which is used as an anticancer, antitoxic and diuretic agent[26]. Eaten as a pot-herb; it is also used medically for mouth infections. The original scientific studies on the plant reported to possess active antifungal activity[27,28], antibacterial activity[29], spermicidal and spermiostatic effect[30], anti-inflammatory and hepatoprotective activity[31] and antioxidant activity[26]. The plant is reported to contain Flavones such as Apigenin and Mollupentin[32], Mollugogenol A, an antifungal triterpenoid, Mollugogenol B, Mollugogenol D, Oleanolic acid and a steroid–Sitosterol Beta[27,28,33,34].

In our earlier study, it has been reported that the aqueous extract of the said plant possess a significant hypoglycemic and antidiabetic activity in the normoglycemic and alloxan induced and glucose loaded hyperglycemic rat models in single dose administration[35]. In continuation of our earlier work, the objective of the present study aims at extensive evaluation of the anti-hyperglycemic effect of the aqueous extract of the aerial parts of *Mollugo pentaphylla* in the sub-acute diabetic models up to thirty days and to validate the folklore claim of the activity of the plant in a more scientific manner.

## MATERIAL AND METHODS

### Plant Materials

Fresh and mature plant of *Mollugo pentaphylla* Linn was collected from Odisha, India and the plant was authenticated by taxonomist, Dr. A. K. Pradhan, Professor, Dept. of Botany, PPD Mahavidyalaya, Tigiria, Cuttack, Odisha, India. A voucher specimen (Regdn.No. SPS/SOAU/2008/005) has been preserved in the institutional herbarium of School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University for future reference. After due authentication,fresh aerial parts were collected in bulk, cleaned thoroughly with distilled water followed by shade drying for 12 days. The shade dried leaves were coarsely powdered and stored in nylon bags in a deep freezer till further use.

### Preparation of the extract

Powdered plant material (750 g) was refluxed with 2000 ml of distilled water for 48h after defatting with petroleum ether (60-80 $^0$C). The solvent with plant residue were filtered and concentrated in a rotary evaporator, a dark brownish viscous residue was obtained (yield: 29.72% w/w with respect to dried plant material).

**Preparation of the test samples**

The measured quantity of aqueous extract of aerial parts of *Mollugo pentaphylla* Linn *(*AAMP*)* and glibenclamide (2.5 mg/kg) was suspended in 25% Tween 20 in distilled water and used as test drug for oral administration.

**Maintenance of Animal and approval of protocol**

Healthy male albino Wistar rats, weighing 150–200 g body weight were collected from the Institutional animal house for the study. The selected animals were housed in acrylic cages in standard environmental conditions (temp: 20–25 $^0$C; relative humidity: 45-55 % under 12 h light/dark cycle), fed with standard rodent diet for one week in order to adapt to the laboratory conditions and water *ad libitum.* The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and as per the experimental protocols duly approved by the Institutional Ethical Committee (IAEC No. 1171/C/08/CPCSEA).

**Determination of blood glucose levels**

Fasting blood glucose level was measured, using a Glucomonitor (Optium make), based on the glucose oxidase method. Blood samples were collected from the tip of tail at the defined time patterns[36,37]. The Screening for antihyperglycaemic activity was performed as per the standard procedures[38].

**Study of blood glucose level on normoglycaemic animals**

The animals were fasted for 12 h, but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), the rats were then divided into four groups of six animals each. Group I served as solvent control and received only vehicle (2 ml/kg) through oral route, Group II received glibenclamide (2.5 mg/kg) and served as reference control. Groups III and IV received the test extract at a dose of 250 and 500 mg/kg, respectively, in a similar manner. The test extract, standard drug and solvent were administered to respective group once daily for 30 days. Blood was withdrawn (0.1 ml) from the tip of the tail of each rat under mild ether anaesthesia. The blood glucose level was measured on 0, 5, 10, 15, 20, 25 and 30$^{th}$ day of treatment.

***Study of blood glucose level on alloxan induced diabetic animals***

The animals were kept fasting for 24 h with water *ad libitum* and injected alloxan monohydrate intraperitoneally at a dose of 150 mg/kg in normal saline. After 1 h, the animals were provided rodent-feed *ad libitum*. The blood glucose level was measured 72 h after administration of alloxan. The animals showing blood glucose level beyond 200 mg/dl, were considered for the study. The diabetic animals were segregated into four groups of six rats each. Group I served as solvent control and received only vehicle (2 ml/kg) through oral route. Group II received glibenclamide (2.5 mg/kg); Groups III and IV received the test extract at doses of 250 and 500 mg/kg respectively in a similar manner, for 30 days. The blood glucose level was measured on 0, 5, 10, 15, 20, 25 and 30$^{th}$ day of treatment.

**Study of glucose utilization on isolated rat hemidiaphragm**

The rats' hemi diaphragms were isolated from the selected healthy albino rats immediately after killing the animals by decapitation. The diaphragms were divided into two halves. The hemi diaphragms were then placed in culture tubes containing 2ml tyrode solution with 2g% glucose and incubated for 30 min at 37 $^0$C in an atmosphere of 95% $O_2$ – 5% $CO_2$ with shaking. Six sets of similar experiments were performed, in which, (I) corresponds to diabetic control (II) reference standard insulin (0.25 IU/ml), (III) AAMP (250 mg/ml), (IV) AAMP (500 mg/ml), (V) insulin (0.25 IU/ml + extract (250 mg/ml)) and (VI) insulin (0.25 IU/ml + extract (500 mg/ml)). Following incubation, the hemi diaphragms were taken out and weighed. The glucose content of the incubated medium was measured. Glucose uptake was calculated as the difference between the initial and final glucose content in the incubation medium[39].

**Study of the test extract on Plasma Insulin levels**

Four groups of rats were taken out of which Group I served as diabetic control, Group II, Group III and Group IV animals received oral daily dose of glibenclamide (2.5 mg/kg), AAMP (250mg/kg) and AAMP (500mg/kg) respectively. Blood was collected at 0, 5, 10, 20 & 30$^{th}$ day and plasma insulin was measured by following the method of Radio Immunoassay (RIA), employing double antibody technique using insulin kit[40]. Insulin values were expressed as µU/ml.

**Estimation of serum lipid profile**

At the end of 30 days of treatment with the test extract, the animals were sacrificed by decapitation under ether anesthesia and blood samples were collected from test, standard and solvent treated groups including normal animal as reference. The serum supernatant was separated out by centrifugation and was subjected for the determination of the lipid profile studies such as total lipids, phospholipids, total cholesterol, triglycerides, HDL, LDL, VLDL and free fatty acids[41].

**Estimation of antioxidant profile**

After sacrificing the animals on 30$^{th}$ day, the liver tissue from various groups of animals were removed carefully followed by washing thoroughly with ice cold saline, 0.5 gms of the wet tissue was weighed exactly and homogenized in 0.1M Tris–HCl buffer, pH 7.4 at 4 $^0$C in a Remi homogenizer with a Teflon pestle rotated at 600 rpm for 30 min. The homogenate was centrifuged at 2500 rpm for 10 min at 4 $^0$C using refrigerated centrifuge. The supernatant was used for the assay of lipid peroxidation products and antioxidant enzymes such as thiobarbituric acid reacting substances (TBARS)[42], hydroperoxides (HP)[43,44], malondialdehyde (MDA)[45], conjugated dienes (CD)[46], reduced glutathione (GSH)[47,48], glutathione peroxidase (GSH-Px)[49], glutathione reductase (GR)[50], superoxide dismutase (SOD)[51], catalase (CAT)[52].

**Acute oral toxicity studies**

The method of Seth et al. was followed[53]. Eight groups of ten mice each, of mixed sex fasted overnight were kept under laboratory conditions and allowed free access to water. The aqueous extract at concentration of 100, 500, 1000, 2000, 2500, 3000, 4000 and 5000 mg/kg dissolved in distilled water were administered orally via a gastric catheter. After administration of test sample, the animals were observed critically for first 4 h for any behavioural changes, followed by occasional observation for 6h and finally mortality was recorded after 48 hours. The test result denoted that, the extract was found to slightly toxic in the group treated with 5000 mg/kg registering the death (20%) of only two out of ten animals.

**Statistical analysis**

All the results were analyzed statistically evaluated using one-way analysis of variance followed by Dunnet's t-test. A *p*-value less than 0.05 were considered significant. All the results were expressed as Mean ± S.E.M, for six animals in each group.

**RESULTS**

**Effects of AAMP on normoglycemic rats**

The results of AAMP on blood sugar level of normoglycemic rats are illustrated in Table 1. The test result indicates that, there is a significant reduction ($p<0.001$) in blood glucose level from 15$^{th}$ day onwards, and registered 38.3 and 49.2 % fall, at the end of 30 days, in animals treated with 250 and 500 mg/kg of the test extract. However the standard drug glibenclamide at the same day reduces the blood glucose level up to an extent of 33.9% with $p<0.001$, when compared with solvent control group.

**Effects of AAMP on alloxan induced diabetic rats**

The results depicted in Table 2, of the study reveals that, the extract reduces the blood glucose level to an extent of 51.14% and 65.86% at 250mg/kg and 500mg/kg dose level respectively at the end of the 30 day of the study, where as the standard drug glibenclamide registered 66.79% of reduction at the same day of the study. However the individual data shows a statistical significance ranges

between p<0.01 to p<0.001, throughout the experimental result when compared with solvent control while analysis of variance registered p value less than 0.01.

**Effects of AAMP on glucose uptake by isolated rat hemidiaphragm**

The results of study on glucose uptake by isolated rat hemidiaphragm are shown in Table 3, which reveals that the test extract at 250 mg/ml and 500mg/ml concentration exhibited uptake of 5.34 and 6.81 mg/g/30min respectively, while only insulin showed 6.36mg/g/30min. However, insulin and test extract combination respond to 7.23 and 8.45 mg/g uptake of glucose at the same time. The extent of glucose uptake differ significantly ranges from p<0.05 to p<0.001 when compared with diabetic control group, which showed 3.21 mg/g glucose uptake within 30 min.

**Table 1: Effect of *AAMP* on Blood glucose level in normoglycemic rats**

| Groups & Treatment | Blood Glucose Levels (mg/dl) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0th day | 5th day | 10th day | 15th day | 20th day | 25th day | 30th day | % Change from 0Hr |
| I. Solvent Control (Tween + Water) | 99.5 ± 4.80 | 98.66 ± 4.66 | 96.5 ± 4.77 | 101.16 ± 4.04 | 96.83 ± 4.15 | 97.83 ± 5.60 | 102.16 ± 4.90 | - |
| II. Glibenclamide (2.5mg/kg) | 92.5 ± 2.68 | 86.83 ± 2.75 | 79.83 ± 2.95[a] | 59.5 ± 2.77[c] | 62.33 ± 2.33[c] | 56.16 ± 2.68[c] | 61.16 ± 3.29[c] | 33.88 |
| III. AAMP (250mg/kg) | 92.66 ± 4.13 | 89.83 ± 4.22 | 84.16 ± 4.71 | 71. 16 ± 3.29[c] | 67.5 ± 4.51[c] | 60.33 ± 3.44[c] | 57.16 ± 4.64[c] | 38.31 |
| IV. AAMP (500mg/kg) | 91.5 ± 6.48 | 89.16 ± 6.80 | 82.16 ± 5.67 | 66.66 ± 3.62[c] | 62.66 ± 4.32[c] | 57.66 ± 5.15[c] | 46.5 ± 4.46[c] | 49.18 |
| F (3,20) | 0.60 | 1.15 | 2.57 | 28.00** | 17.61** | 20.70** | 25.43** | - |

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test.

(F-value denotes statistical significance at *p<0.05, **p<0.01) and (t-value denotes statistical significance at [a]p<0.05, [b]p<0.01 and [c]p<0.001 respectively, in comparison to group-I)

**Table 2: Effect of *AAMP* on the Blood glucose level in control and experimental rats**

| Groups & Treatment | Blood Glucose Levels(mg/dl) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0th day | 5th day | 10th day | 15th day | 20th day | 25th day | 30th day | % Change from 0Hr. |
| I. Solvent Control (Tween + Water) | 285.66 ± 12.71 | 279.83 ± 11.81 | 257.66 ±11.67 | 246.83 ±11.80 | 239.33 ±10.39 | 235.83 ± 9.76 | 231.83 ± 6.30 | |
| II. Glibenclamide (2.5mg/kg) | 296.66 ± 13.07 | 199.66 ±10.21[c] | 138.16 ±10.33[c] | 116.83 ± 6.60[c] | 115.16 ± 5.24[c] | 103.83 ± 7.46[c] | 98.5 ± 6.82[c] | 66.79 |
| III. AAMP (250mg/kg) | 269.83 ± 10.70 | 221.66 ± 9.17[b] | 173.83 ±12.17[c] | 151.5 ±10.92[c] | 142.16 ±12.83[c] | 133.33 ±11.26[c] | 131.83 ± 8.71[c] | 51.14 |
| IV. AAMP (500mg/kg) | 275.83 ± 9.95 | 192.83 ± 7.98[c] | 149.5 ±10.25[c] | 128.83 ±7.96[c] | 116.83 ± 5.53[c] | 106.66 ± 7.94[c] | 94.16 ± 4.90[c] | 65.86 |
| F (3,20) | 1.01 | 15.95** | 23.48** | 38.07** | 41.52** | 45.14** | 88.18** | |

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test.

(F-value denotes statistical significance at *p<0.05, **p<0.01) and (t-value denotes statistical significance at [a]p<0.05, [b]p<0.01 and [c]p<0.001 respectively, in comparison to group-I)

**Table 3: Effect of *AAMP* on Peripheral glucose uptake by isolated rat hemi-diaphragm**

| *Incubation medium* | | Glucose uptake (mg/g/30 min) |
|---|---|---|
| I. | Tyrode solution with Glucose (2 g%) – Diabetic Control | 3.21 ± 0.18 |
| II. | Tyrode solution with Glucose (2 g%) + Insulin (0.25 IU/ml) | 6.36± 0.62[c] |
| III. | Tyrode solution with Glucose (2 g%) + AAMP (250 mg/ml) | 5.34 ± 0.45[a] |
| IV. | Tyrode solution with Glucose (2 g%) + AAMP (500 mg/ml) | 6.81 ± 0.30[c] |
| V. | Tyrode solution with Glucose (2 g%) + Insulin (0.25 IU/ml + AAMP (250 mg/ml) | 7.23 ± 0.39[c] |
| VI. | Tyrode solution with Glucose (2 g%) + Insulin (0.25 IU/ml + AAMP (500 mg/ml) | 8.45 ± 0.45[c] |
| F (5,30) | | 39.25** |

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test.

(F-value denotes statistical significance at *p<0.05, **p<0.01) and (t-value denotes statistical significance at [a]p<0.05, [b]p<0.01 and [c]p<0.001 respectively, in comparison to group-I)

**Effects of AAMP on Plasma Insulin Level**

The results of the test depicted in Table 4, demonstrated that, the extract at both the tested dose levels significantly ($p$<0.001) progressively increase plasma insulin concentrations in diabetic extract treated rats when compared with diabetic control. The extract at the dose level of 250 and 500 mg/kg dose levels, recorded a maximum increase in insulin concentration of 126.5 and 156.16 µU/ml respectively on 20th day. On the other hand, glibenclamide showed maximum plasma insulin concentration of 181.83 µU/ml at the end of 20th day.

**Effects of AAMP on serum lipid profile**

The Table 5 illustrate the levels of serum lipid profile such as total lipids, total cholesterol, phospholipids, triglycerides, HDL, LDL, VLDL

and free fatty acids at the end of 30th day of the study. The diabetic rats showed significant ($p<0.001$) increase level of all tested lipid profiles except HDL, which showed decrease value in a significant ($p<0.05$) extent. The extract at both the dose levels showed a dose dependent and significant ($p<0.05$ to $p<0.001$) reduction in total lipids, triglycerides, LDL, VLDL and free fatty acids, however a marked decrease in the levels of total cholesterol and phospholipids were also been recorded, when compared to diabetic control group, while the HDL levels were approaching almost normal values when compared to without treatment normal control group.

**Table 4: Effect of *AAMP on* Plasma Insulin Level in control and diabetic rats**

| Group and Treatment | Plasma Insulin (µU/ml) | | | | |
|---|---|---|---|---|---|
| | 0th day | 5th day | 10th day | 20th day | 30th day |
| I. Diabetic Control | 21.66 ± 3.75 | 23.33 ± 4.79 | 21.25 ± 2.80 | 26.66 ± 3.44 | 23.83 ± 2.73 |
| II. Glibenclamide (2.5mg/kg) | 33.83 ± 3.87[a] | 74.16 ± 7.00[c] | 169.83 ± 11.71[c] | 181.83 ± 9.28[c] | 119.66 ± 7.64[c] |
| III. AAMP (250mg/kg) | 24.16 ± 3.43 | 56.83 ± 4.97[b] | 89.66 ± 7.56[c] | 126.5 ± 9.12[c] | 113.33 ± 9.42[c] |
| IV. AAMP (500mg/kg) | 31.66 ± 2.45 | 79.83 ± 6.74[c] | 144.5 ± 12.43[c] | 156.16± 10.15[c] | 117.83 ± 6.21[c] |
| F (3,20) | 3.59* | 24.71** | 43.24** | 72.19** | 86.12** |

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test

(F-value denotes statistical significance at *p<0.05, **p<0.01) and (t-value denotes statistical significance at [a]p<0.05, [b]p<0.01 and [c]p<0.001 respectively, in comparison to '0' hr result.

**Table 5: Effect of *AAMP* on serum lipid profile in control and experimental diabetic rats on 30th day**

| Group and Treatment | Serum Lipid profile | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Total Lipids (mg/dl) | Total Cholesterol (mg/dl) | Phospholipids (mg/dl) | Triglycerides (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) | Free Fatty Acids (mg/dl) |
| I. Normal | 113.66 ± 7.76[c] | 75.65 ± 5.90[c] | 105.5 ± 8.10[c] | 62.08 ± 6.93[c] | 51.5 ± 4.68[a] | 27.5 ± 6.86[b] | 16.33 ± 1.40[c] | 412.41 ± 77.47[c] |
| II. Solvent Control (Tween + Water) | 393.16 ± 23.54 | 188.16 ± 16.15 | 212.91 ± 15.42 | 189.08 ± 20.19 | 32.66 ± 3.43 | 63.73 ± 6.21 | 45.06 ± 1.94 | 1324.58 ± 137.14 |
| III. Glibenclamide (2.5mg/kg) | 141.83 ± 17.37[c] | 96.75 ± 10.31[b] | 127.75 ± 13.40[b] | 78.33 ± 6.44[c] | 54.11 ± 6.11[a] | 35.08 ± 3.35[b] | 18.75 ± 1.84[c] | 675.41 ± 80.69[c] |
| IV. AAMP V. (250mg/kg) | 186.33 ± 24.93[c] | 153.75 ± 23.06 | 157.58 ± 24.60[a] | 95.5 ± 10.99[c] | 46.5 ± 5.35 | 45.75 ± 5.87 | 27.5 ± 1.80[c] | 892.25 ± 58.01[b] |
| VI. AAMP VII. (500mg/kg) | 159.83 ± 17.82[c] | 117.33 ± 13.85[b] | 136.5 ± 14.93[b] | 67.58 ± 7.64[c] | 56.41 ± 4.81[b] | 39.25 ± 5.95[a] | 22.08 ± 2.50[c] | 763.08 ± 69.39[c] |
| F (4, 25) | 33.63** | 8.99** | 6.34** | 20.14** | 3.63* | 5.62** | 35.25** | 14.23** |

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test.

(F-value denotes statistical significance at *p<0.05, **p<0.01) and (t-value denotes statistical significance at [a]p<0.05, [b]p<0.01 and [c]p<0.001 respectively, in comparison to group-II).

**Table 6: Effect of *AAMP* on in vivo antioxidant profiles in the 30-days treated diabetic rat liver**

| Group and Treatment | TBARS (µM/100g wet tissue) | HP (µM/100g wet tissue) | MDA (µM/100g wet tissue) | CD (µM/100g wet tissue) | GSH (µM/g wet tissue) | GSH-Px (µM/g wet tissue) | GR (µM/g wet tissue) | SOD (Units/mg protein) | CAT (Units/mg protein) |
|---|---|---|---|---|---|---|---|---|---|
| I. Normal Control | 4.41 ± 0.71[c] | 15.66 ± 0.84[c] | 0.84 ± 0.05[c] | 55.41 ± 2.55[c] | 21.66 ± 1.13[c] | 0.47 ± 0.04[c] | 5.41 ± 0.10[c] | 9.11 ± 0.13[c] | 8.38 ± 0.23[c] |
| II. Diabetic Control (Tween+Water) | 26.5 ± 2.06 | 29.25 ± 1.04 | 1.41 ± 0.05 | 94.83 ± 3.01 | 12.58 ± 0.89 | 0.29 ± 0.02 | 3.43 ± 0.09 | 5.31 ± 0.17 | 4.76 ± 0.18 |
| III. Glibencamide (2.5 mg/kg/day) | 9.41 ± 1.16[c] | 19.26 ± 0.94[c] | 0.91 ± 0.04[c] | 59.08 ± 2.54[c] | 18.58 ± 0.86[c] | 0.42 ± 0.01[b] | 5.08 ± 0.20[c] | 8.71 ± 0.34[c] | 7.81 ± 0.36[c] |
| IV. AAMP (250mg/kg/day) | 12.33 ± 0.55[c] | 17.61 ± 0.73[c] | 0.99 ± 0.04[c] | 69.81 ± 3.03[b] | 15.63 ± 0.77[b] | 0.38 ± 0.01[b] | 3.96 ± 0.19[b] | 6.58 ± 0.19[b] | 5.47 ± 0.37[a] |
| V. AAMP (500mg/kg/day) | 8.16 ± 0.70[c] | 14.91 ± 0.61[c] | 0.87 ± 0.10[c] | 58.25 ± 3.43[c] | 19.78 ± 0.61[c] | 0.45 ± 0.01[b] | 4.68 ± 0.16[c] | 8.18 ± 0.24[c] | 6.86 ± 0.30[b] |
| F (4, 25) | 48.45** | 38.39** | 13.35** | 32.32** | 14.99** | 6.91** | 23.67** | 46.82** | 15.92** |

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test.

(F-value denotes statistical significance at *p<0.05, **p<0.01) and (t-value denotes statistical significance at [a]p<0.05, [b]p<0.01 and [c]p<0.001 respectively, in comparison to group-II.)

**Effects of AAMP on serum lipid peroxidation products and reduced glutathione contents**

The results of the study are depicted in Table 6. The estimated concentrations of liver TBARS, HP, MDA and CD on 30th day of the study of both test and standard drug, indicated that, the TBARS, HP, MDA and CD levels are declined with an extent of 53.47, 39.79, 29.78, 26.38% respectively in case of 250mg/kg dose, while in 500mg/kg body weight dose level, the % decrease become 69.20, 49.02, 38.29, 38.57 respectively, with statistical significance of $p<0.001$.In a similar way the standard drug also showed a reduced

value of 64.49, 34.15, 35.46, 37.69 in percentage wise, with statistical significance in the same experiment.

The enzymes like GSH, GSH-Px, GR, SOD and CAT values are lowered significantly ($p < 0.001$) in diabetic rats as compared with normal control rats. The test extract showed an elevated value of these enzymes to an extent of 24.24, 31.03, 15.45, 23.91, 14.91 % in 250mg/kg dose level, while in 500mg/kg dose level, the respective enzyme percentage become 57.23, 55.17, 36.44, 54.04, 44.11, with statistical significance ($p < 0.001$). However the standard drug glibenclamide at the same time registered an increased % of 47.69, 44.82, 48.10, 64.03, and 64.07 respects to the above enzymes with statistical significance ($p < 0.001$).

## DISCUSSION

Many traditional plant treatments for diabetes mellitus are used throughout the world. Management of diabetes without any side effect is still a challenge to the medical system. This has led to an increasing demand for natural products with antidiabetic activity and fever side effects. From literature review it was found that many herbs and plant products have been shown to have hypoglycemic action. Flavonoids are known to be bioactive antidiabetic principles[54].

In our earlier work, we have reported hypoglycemic and antidiabetic activity of aqueous extract of *Mollugo pentaphylla* aerial parts (AAMP) in normoglycemic and alloxan induced hyperglycemic rats by single dose treatment only[35]. In continuation of our earlier work, the present study aims at extensive evaluation of antidiabetic activity of aqueous extract of aerial parts of Mollugo pentaphylla up to an extensive period of study of 30 days with expected mechanism of action of such activity.

The conclusion derived from the experimental results revealed a defined role of aqueous extract in normoglycemic, and alloxan-induced diabetic rats, AAMP found to possess dose dependent suppression of glucose level, the effect of which correspond like that of glibenclamide. All these glucose lowering effect of the extract may possibly due to the insulinotropic and free radical scavenging potentiality of the plant extract. The insulinotropic effect of the extract is evidenced by the increased plasma insulin levels which are comparable with that of standard drug glibenclamide and all these parameters used in the study are widely accepted as markers of insulinotropic effect[40]. The glucose up take by peripheral tissues in presence of insulin is evidenced in the experiment by the property of the extract to increase the glucose uptake using isolated rat hemi diaphragm, suggest that the extract may contribute to the insulinotropic effect or direct insulin like activity and extra pancreatic effect[39].

Alloxan, a beta-cytotoxin, induces “chemical diabetes” by pancreatic cell damage mediated through generation of cytotoxic oxygen free radicals. The primary target of these radicals is the DNA of pancreatic cells causing DNA fragmentation[55]. This damages a large number of β-cells, resulting in decrease in endogenous insulin release, which leads to decreased utilization of glucose by the tissue[56]. Insulin plays a key role in glucose homeostasis along the side of a counter regulatory hormone, glucagon, which raises serum glucose. Carrier proteins (GLUT 1- 5) are essential for glucose uptake into cells. The mode of action of the active compound(s) of the plant material is probably mediated through enhanced secretion of insulin from the β-cells of Langerhans or through extra pancreatic mechanism[57].

The results depicted in this study suggest that AAMP possess hypoglycemic and insulintropic properties, which may be the contributing factor for the biological response. The possible mechanism, by which the plant extract mediates its antidiabetic action, is potentiation of pancreatic secretion of insulin from existing residual β-cell of islets and due to enhanced utilization of blood glucose by peripheral tissues as well.

It has been reported that the increase in glucose levels in alloxan-induced diabetic rats is associated with dislipidemia characterized by elevated serum triglycerides and total cholesterol levels. The improvement of blood glucose levels caused by most hypoglycaemic agents is associated with a reduction of serum triglycerides and total cholesterol[58,59]. The significant reduction in the levels of LDL, VLDL, TC, TG, FFA, phospholipids & total lipids and increase in the levels of HDL demonstrates that, the extract may have property to enhance the transcription of lipoprotein lipase similar to that of insulin.

Free radicals e.g. superoxide radical, hydroxyl radical, peroxy radical and singlet oxygen radicals have been implicated in many disease conditions. Many plants possess dynamic antioxidant properties owing to their phenolic and flavonoid contents[60]. In our previous study, the said plant extract is found to possess the phenolic content (75.16 µg of pyrocatechol equivalent /500mg) and flavonoid content (9.58 mg equivalent of quercetin /gm), which may be the contributing factor for its antioxidant activity[35].

Hyperglycemia induces the generation of free radicals which can affect antioxidant defenses thus leading to the disruption of cellular functions, oxidative damage to membranes and increased susceptibility to lipid peroxidation[61]. Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycaemia, which thereby depletes the activity of antioxidative defense system and thus promotes de novo free radicals generation[1]. Reactions of oxygen free radicals with all biological substances especially with polyunsaturated fatty acids lead to increased lipid peroxidation (LPO) [62] resulting in impairment of membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors[63]. The present investigation reports a significant increase in lipid peroxidation products such as TBARS, CD, MDA and HP in liver of solvent control diabetic rats which suggest that peroxidative injury may be involved in the development of diabetes. The extract treated diabetic animals, showed a significant reduction in liver lipid peroxidation products, which indicate that AAMP is having, potential to inhibit the oxidative damage of liver tissues. Moreover, GSH is mainly involved in the formation of correct disulphide bonds in several proteins and prevents the oxidation of -SH group of the proteins to -SS- group, which is essential for the protein function including that of the enzymes [64]. However, the toxic amount of peroxide and free radicals produced in the cell are scavenged by GSH-Px and provide protection against reactive oxygen compounds[65]. A marked decrease in liver GSH is observed in diabetic rats, which contributes a factor in the pathogenesis of diabetes. In the present study the test extract showed a significant increase of liver GSH levels, which may be one more contributing property of the extract towards its antidiabetic potential.

Enzymatic antioxidant such as SOD and CAT are considered primary enzymes since they are involved in the direct elimination of ROS [63]. SOD is one of the most important enzymes and scavenges $O_2-$ anion (which is the first product of $O_2$ radicals) to form $H_2O_2$ in the enzymatic antioxidant defense system and hence abolishes the toxic effects due to this radical or other free radicals derived from secondary reactions[65]. The $O_2-$ anion is reported to inactivate CAT and GSH-Px[66]. Catalase has been recognized as a major determinant of hepatic and cardiac antioxidant status[67] and is known to be involved in detoxification of $H_2O_2$ concentrations[68], whereas GSH-Px is sensitive to lower concentrations of $H_2O_2$. In Diabetes, the alloxan-generated ROS causes non-enzymatic glycosylation and oxidation resulting in the inactivation and inhibition of antioxidant enzymes such as SOD and CAT[13]. In the present study, it was observed that long term treatment with the extract reverse the activities of these enzymatic antioxidants (SOD, CAT, GSH-Px and GR), by significantly increasing the activity of such enzymes. Bioactive molecules present in aqueous extract of aerial parts of *Mollugo pentaphylla* may probably possess insulin- like effect or stimulate the β cells of the pancreas to produce insulin which in turn lowers the blood glucose level.

## CONCLUSION

This study clearly demonstrates that the aqueous extract of aerial parts of *Mollugo pentaphylla*, exhibited strong antihyperglycaemic activity which could be attributed to its possible action on pancreatic and extra-pancreatic site of glucose and lipid metabolism as evidenced by insulinotropic and antioxidant defense properties. Currently, we are carrying out studies to isolate, and characterize

bioactive compounds to evaluate the antihyperglycaemic properties of *M. pentaphylla.* This may further clarify the specific properties of the plant.

**ACKNOWLEDGEMENTS**

The authors are grateful to Hon'ble President and Vice-chancellor, SOA University, Bhubaneswar for providing necessary facilities to carry out the research work in the faculty of pharmacy, SOA University.

**REFERENCES**

1. Bakırel T, Bakırel U, Keles OU, Ulgen SG, Yardibi H. In vivo assessment of antidiabetic and antioxidant activities of rosemary (*Rosmarinus officinalis*) in alloxan-diabetic rabbits. J Ethnopharmacol 2008; 116: 64–73.
2. Sepici A, Gurbuz I, Cevik C, Yesilada E. Hypoglycaemic effects of myrtle oil in normal and alloxan-diabetic rabbits. J Ethnopharmacol 2004; 93: 311–318.
3. Luo Q, Cai Y, Yan J, Sun M, Corke H. Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from *Lycium barbarum*. Life Sci 2004; 76: 137–149.
4. Baynes JW. Role of oxidative stress in the development of complications in diabetes. Diabetes 1991; 40: 405–412.
5. Ceriello A. New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy. Diabetes Care 2003; 26: 1589–1596.
6. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the antioxidants in the management of diabetes and its complications. Biomed Pharmacother 2005; 59: 365–373.
7. Tang L, Wei W, Chen L, Liu S. Effects of berberine on diabetes induced by alloxan and a high-fat/high-cholesterol diet in rats. J Ethnopharmacol 2006; 108: 109–115.
8. Slonim AE, Surber ML, Page DL, Sharp RA, Burr IM. Modification of chemically induced diabetes in rats by vitamin E, Supplementation minimizes and depletion enhances development of diabetes. J Clin Invest 1983; 71: 1282–1288.
9. Murthy VK, Shipp JC, Hanson C, Shipp DM.. Delayed onset and decreased incidence of diabetes in BB rats fed free radical scavengers. Diab Res Clin Prac 1992; 18: 11–16.
10. Halliwell B, Gutteridge J. Free Radicals in Biology and Medicine. Oxford University Press, New York, 1989; 177–178.
11. Gordon M. Dietary antioxidants in disease prevention. Nat Prod Rep 1996; 13: 265–273.
12. Montonen J. Plant foods in the prevention of type 2 diabetes mellitus with emphasis on dietary fiber and antioxidant vitamins publications of the National Public Health Institute, Helsinki MS Thesis, Department of Public Health, University of Helsinki, 2005;18 (Dissertation).
13. Al-Azzawie H, Alhamdani MSS. Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. Life Sci 2006; 78: 1371–1377.
14. National Nutrition Council. Finnish Nutrition Recommendations, Committee report 1998, 7; Ministry of Agriculture and Forestry. Helsinki, Finland, 1999; 9.
15. Abdel-Barry JA, Abdel-Hassan IA, Al-Hakiem MHH. Hypoglycaemic and antihyperglycaemic effects of *Trigonella foenum-graecum* leaf in normal and alloxan induced diabetic rats. J. Ethnopharmacol 1997; 58: 149–155.
16. Pushparaj P, Tan CH, Tan BKH. Effects of *Averrhoa Bilimbi* leaf extract on blood glucose and lipids in streptozotocin-diabetic rats. J Ethnopharmacol 2000; 72: 69–76.
17. WHO Expert Committee on Diabetes mellitus, Technical Report Series 646, Second Report. World Health Organization, Geneva, 1980.
18. Rama RAV, Gurjar MK. Drugs from plant resources: an overview. Pharmatimes 1990; 22:19-27.
19. Evans WC, Evans T. Pharmacognosy Aspects of Asian medicine and its practice in the west. 15th edn., Edinburgh. Elsevier science limited, 2002; 687.
20. Chopra RN, Chopra IC. Glossary of Indian Medicinal Plants. CSIR Publication. 1956; 121-148.
21. Kirtikar KR, Basu BD. Indian Medicinal Plants, International Book Publisher, Dehradun. vol-II: 1999; 1185.
22. Quisumbing E. Medicinal plants of the Philippines, Tech Bull 16, Rep Philippines, Dept. of Agriculture and Natural Resources, Manilla. 1951; 1.
23. Douvier. The abortive plants of New Caledonia. Bull Ass Med. New Caledonia 1951; 14: 39-41.
24. Singh VP. Medicinal plants from Ujjain district Madhya Pradesh - Part II. Ind Drugs Pharm Ind 1980; 5: 7-12.
25. Lin CC, Kan WS. Medicinal plants used for the treatment of hepatitis in Taiwan. Amer J Chinese Med 1990; 18 (1/2): 35-43.
26. Lin CC, Ng LT, Yang JJ. Antioxidant activity of extracts of peh-hue-juwa-chi-cao in a cell free system. Amer J Chin Med 2004; 32(3):339-349.
27. Hamburger M, Dudan G, Ramachandran Nair AG, Jayaprakasam R, Hostettmann K. An antifungal triterpenoid from *Mollugo pentaphylla*. Phytochemistry 1989; 28 (6): 1767-1768.
28. Nene YL, Thapliyal PN, Kumar K. Screening of some plant extracts for antifungal properties. Labdev J Sci Tech B 1968; 6 (4): 226-228.
29. Sharma S, Sharma MC. Studies of antibacterial activity ethanolic plant extract of *Mollugo pentaphylla* Linn. Arch Appl Sci Res 2010; 2(1):242-246.
30. Jha OP, Ghosh PK, Singh BP. Chemical investigation of *Mollugo pentaphylla*. J Ind Chem Soc 1984; 61 (1): 93-94.
31. Lin CC, Ng LT, Yang JJ, Hsu YF. Anti-inflammatory and hepatoprotective activity of peh-hue-juwa-chi-cao in male rats. Amer J Chin Med 2002; 30(2-3): 225-34.
32. Chopin J, Besson E, Dellamonica G, et al. Structure of a 6, 8-di-c-pentosylapigenin from Mollugo pentaphylla. Phytochemistry 1982; 21: 2367-2369.
33. Rajasekaran M, Nair AGR, Gellstrom WJG, Sikka SC. Spermicidal activity of an antifungal saponin obtained from the tropical herb *Mollugo pentaphylla*. Contraception 1993; 47: 401-412.
34. Salt TA, Xu S, Patterson GW, Adler JH. Diversity of sterol biosynthetic capacity in the caryophyllidae, Lipids 1991; 26 (8): 604-613.
35. Maharana L, Pattnaik S, Kar DM, Sahu PK, Si SC. Study of hypoglycemic potential of aqueous extract of aerial parts of *Mollugo pentaphylla* Linn. Annal Biol Res. 2010; 1 (2):155-165.
36. Aslan M, Deliorman Orhan D, Orhan N, Sezik E, Yesilada E. In vivo antidiabetic and antioxidant potential of *Helichrysum plicatum* ssp. *Plicatum capitulums* in streptozotocin-induced diabetic rats. J Ethnopharmacol. 2007a; 109: 54–59.
37. Aslan M, Deliorman Orhan D, Orhan N, Sezik E, Yesilada E. A study of antidiabetic and antioxidant effects of *Helichrysum graveolens capitulums* in streptozotocin-induced diabetic rats. J Med Foods. 2007b; 10: 396–400.
38. Dash GK, Suresh P, Ganapaty S. Studies on hypoglycaemic and wound healing activities of *Lantana camara* Linn. J Nat Remed 2001; 1: 105–110.
39. Chattopadhyay RR, Sarkar SK, Ganguli S, Banerjee RN, Basu TK. Effect of extract of leaves of *Vinca rosea* Linn. on glucose utilization and glycogen deposition by isolated rat hemidiaphragm. Ind J Physiol Pharmacol 1992; 36: 137–138.
40. Saxena AM, Murthy PSR, Mukherjee SK. Mode of action of three structurally different hypoglycemic agents: A comparative study. Ind J Exp Biol 1996; 34 (4): 351-355.
41. Sood R. Clinical chemistry, in: Medical laboratory technology, methods and interpretations, (5th ed.), Jaypee brothers' medical publishers, India. 1999; 173-404.
42. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxidation on animal tissue by thiobarbituric acid reaction. Anal Biochem 1979; 18: 909.
43. Jaing ZY, Hunt JV, Wolff PS. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroxide in low density lipoprotein. Anal Biochem 1992; 202: 384–389.
44. Helen A, Vijayammal PL. Vitamin C supplementation I hepatic oxidative stress induced cigarette smoke. J Appl Toxicol 1997; 17: 289–295.
45. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978; 86: 271–278.
46. Recknagel RO, Glende EA. Spectrophotometric detection of lipid conjugated dienes. Methods in Enzymol 1984; 105: 331-337.

47. Sedlak J, Lindsay RH. Estimation of total protein-band and nonprotein sulfhydryl group in tissue with Ellman's reagent. Anal Biochem 1968; 25: 192–205.
48. Ellaman GL. Tissue sulfhdryl group. Arch Biochem Biophysics. 1959; 82:70–72.
49. Robak J, Gryglwsk IR. Flavanoids as scavenger of superoxide anions. Biochem Pharmacol 1988; 37: 837–841.
50. Pinto BE, Bartley S. The effect of age and sex on glutathione reductase and glutathione peroxidase activity on aerobic glutathione oxidation in rat liver homogenate. Biochem Journal 1969; 112: 109– 115.
51. Richard E, Cabbat H, Felicitas. A sensitive assay for superoxide dismutase based on the autoxidation of 6 hydroxydopamine. Anal Biochem 1976; 75: 356–362.
52. Aebi H. Catalase in-vitro. Methods in Enzymol 1984; 105: 121–126.
53. Seth UK, Dadkar NK, Kamat UG. Selected Topics in Experimental Pharmacology. The Kothari Book Depot, Bombay, 1972; 126.
54. Jadhav R, Puchchakayala G. Hypoglycemic and antidiabetic activity of flavonoids: boswellic acid, ellagic acid, quercetin, rutin on Streptozotocin-nicotinamide induced type 2 diabetic rats. Int. J. Pharmacy and Pharmaceutical Sciences. 2012; 4 (2): 251-256.
55. Shankar MB, Parikh JR, Geetha M, Mehta RS, Saluja AK. Anti-diabetic activity of novel androstane derivatives from *Syzygium cuminii* Linn. J Nat Rem 2007; 7: 214–219.
56. Saravanan R, Pari L. Antihyperlipidemic and antiperoxidative effect of diasulin, a polyherbal formulation in alloxan induced hyperglycemic rats. BMC Complem Altern Med 2005; 5: 1–10.
57. Bhuvaneswari P, Krishnakumari S. Antihyperglycemic potential of *Sesamum indicum* (linn) seeds in Streptozotocin induced diabetic rats. Int. J. Pharmacy and Pharmaceutical Sciences. 2012; 4 (1): 527-531
58. Dhanabal SP, Kokate CK, Ramanathan M, Kumar EP, Suresh B. Hypoglycaemic activity of *Pterocarpus marsupium* Roxb. Phytother Res 2006; 20: 4–8.
59. Saravanan R, Pari L. Effect of a novel insulinotropic agent, succinic acid monoethylester, on lipids and liproteins levels in rats with streptozotocin–micotinamide-induced type 2 diabetes. J Biosciences 2006; 31: 581–587.
60. Larson RA. The antioxidants of higher plants. Phytochemistry 1988; 27: 969-978.
61. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care 1996; 19: 257–267.
62. Memisogullari R, Bakan E. Levels of ceruloplasmin, transferin, and lipid peroxidation in the serum of patients with type 2 diabetes mellitus. J Diabetes Complicat 2004; 18: 193–197.
63. Arulselvan P, Subramanian SP. Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultra structural changes of pancreatic β-cells in experimental diabetes in rats. Chem Biol Interact 2007; 165: 155–164.
64. Satyanarayana U. Biochemistry, 2nd edition. 2005; 70.
65. Manonmani G, Bhavapriya V, Kalpana S, Govindasamy S, Apparanantham T. Antioxidant activity of *Cassia fistula* (Linn.) flowers in alloxan induced diabetic rats. J Ethnopharmacol 2005; 97: 39–42.
66. Halliwell B, Gutteridge JMC. Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. Lancet 1984; I: 1396–1397.
67. Wohaieb SA, Godin DV. Alterations in free radical tissue-defense mechanisms in streptotozcin-induced diabetes in rat: effects of insulin treatment. Diabetes 1987; 36: 169–173.
68. Yoshikawa T, Naho Y, Kishi A, Tomil Kaneko T, Inuma S, Ichikawa H, Yasudha M, Takahshi S, Kondo M. Role of active oxygen, lipid peroxidation and antioxidants in the pathogenesis gastric mucosal injury induced by indomethacin in rats. Gut 1993; 34: 732–737.