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# ANTIOXIDANT ACTIVITY OF *MELASTOMMA MALABATHRICUM* L. LEAF IN ALLOXAN INDUCED DIABETIC RATS

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## ABSTRACT

Administration of ethanol extract of *Melastomma malabathricum* leaf (100 mg/kg and 200 mg/kg body weight) to alloxan induced diabetic rats for 14 days reduced the elevated level of lipid peroxidation (LPO). The treatment also resulted in significant increase in reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), *glutathione reductase (GRD)* and catalase (CAT) in serum, liver and kidney. The results confirm the antioxidant activity of *Melastomma malabathricum* leaf and suggest that because of its antioxidant effects its administration may be useful in controlling the diabetic complications in experimental diabetic rats.

**Keywords:** Antioxidant, *Melastomma malabathricum*, Oxidative stress.

## INTRODUCTION

Diabetes mellitus (DM) affects a large number of people throughout the world and more so in India. Clinically, the disease is associated with a number of chronic complications including nephropathy, neuropathy, retinopathy and cardiovascular diseases[1]. There is increasing evidence that complications related to diabetes are associated with oxidative stress induced by the generation of free radicals [2]. A free radical is any species capable of independent existence that contains one or more unpaired electrons. Thus, free radicals result in the consumption of antioxidant defenses which may lead to disruption of cellular functions and oxidative damage to membranes and enhance susceptibility to lipid peroxidation. Increased generation of reactive oxygen species (ROS) and lipid peroxidation has been found to be involved in the pathogenesis of many diseases of known and unknown etiology and in the toxic actions of many compounds[3]. Antioxidants thus play an important role to protect the human body against damage caused by reactive oxygen species[4]. The endogenous antioxidant enzymes such as SOD, CAT, GSH and GPx are responsible for the detoxification of deleterious oxygen radicals[5].

WHO has recommended evaluation of plants effective in different diseases. Many plant extracts and plant products have been shown to have significant antioxidant activity [6] 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[7]. Among these herbal resources, *Melastoma malabathricum* leaf is selected for the present study. *Melastoma malabathricum* belongs to the Melastomataceae family. It is also called the Singapore Rhododendron or Sendudok. It is a erect shrub or small tree 1.5 to 5m tall. It was traditionally used to treat diarrhoea, dysentery, leucorrhoea, hemorrhoids, wounds, infection during confinement, toothache, flatulence, sore legs, and thrush and also it is used by the Jah hut people in Malaysia to cure diarrhoea [8].

The present study has been aimed to investigate the effect of ethanol extract of *Melastoma malabathricum* leaf on lipid peroxides and enzymatic antioxidants in serum liver and kidney of alloxan induced diabetic rats.

## MATERIALS AND METHODS

### Plant Material

The leaves of *Melastoma malabathricum* L. were collected from Daudeli,Joide Taluk, Hubli District, North Karnataka. With the help of local flora, a voucher specimen was retained in Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin for further reference.

### Preparation of plant extract for phytochemical screening and antidiabetic studies

The *Melastoma malabathricum* leaf were shade dried at room temperature and the dried leaf were powdered in a Wiley mill. Hundred grams of powdered *Melastoma malabathricum* leaf was packed in a Soxhlet apparatus and extracted with ethanol.The extract were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures[9,10]. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antidiabetic studies.

**Animals**

Normal healthy male Wistar albino rats (180- 240g) were housed under standard environmental conditions at temperature (25±2º C) and light and dark (12: 12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*.

**Acute Toxicity Study**

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study [11]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric incubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100, and 2000 mg/kg body weight.

**Induction of Diabetes in Experimental animal**

Rats were induced diabetes by the administration of simple intraperitoneal dose of alloxan monohydrate (150 mg/kg) [12]. Two days after alloxan injection, rats screened for diabetes having glycosuria and hypoglycemia with blood glucose level of 200-260 mg/100 ml were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

**Experimental Design**

In the present investigation, a total of 30 rats (24 diabetic surviving rats and 6 normal rats) were taken and divided into five groups of 6 rats each.

Group I: Normal untreated rats

Group II: Diabetic control rats

Group III: Diabetic rats given ethanol extract of *Melastoma malabathricum* leaf (100mg/kg body weight)

Group IV: Diabetic rats given ethanol extract of *Melastoma malabathricum* leaf (200mg/kg body weight)

Group V: Diabetic rats given standard drug glibenclamide (600µg/kg body weight).

The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes and serum was stored at -4$^{o}$C until analyses completed. The liver and kidney tissues were excised, rinsed in ice cold saline, cut into small pieces and homogenized with homogenizer in Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 10 min. Supernatant was used for enzyme assays for the estimation of non enzymatic and enzymatic antioxidants such as lipid peroxidation (LPO)[13]; superoxide dismutase (SOD) [14], catalase (CAT)[15], glutathione peroxidase (GPx)[16] , *glutathione reductase (GRD)*[17] and reduced glutathione (GSH) [18].

**RESULTS AND DISCUSSION**

The phytochemical screening of ethanol extract of *Melastoma malabathricum* leaf revealed the presence of alkaloid, catechin, coumanin, flavonoid, tannin, saponin, steroid, phenol, glycoside, terpenoid and xanthoprotein. Acute toxicity study revealed the non-toxic nature of the ethanol extract of *M.malabathricum* leaf. The results (Table 1, 2 &3) showed increased lipid peroxidation (LPO) in serum, liver and kidney of alloxan induced diabetic rats. Earlier studies have reported that there was an increased lipid peroxidation in liver, kidney and brain of diabetic rats [19,20]. This may be because the tissues contain relatively high concentration of early peroxidizable fatty acids. In the present study, an increase in the levels of LPO was found and there levels were significantly reduced after the supplementation of the ethanol extract of *M.malabathricum* and glibenclamide (Table 1,2&3). This indicate that plant extract inhibit oxidative damage due to the antiperoxidative effect of ingredients present in ethanol extract of *M.malabathricum.* This should be correlated with previous study reported that *Cassia auriculata* flower, *Syzigium cumini, Tinospora cordifolia, Scoparia dulcis* and *Nigella sativa* [21,22,23,24] has antiperoxidative and antihyperlipidaemic effect of diabetic animals. Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the lipid metabolism. Insulin is a potent inhibitor of lipolysis, since it inhibits the activity of hormone sensitive lipase in adipose tissue and suppresses the release of free fatty acids [25].

The levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), *glutathione reductase (GRD)* and reduced glutathione (GSH) (Table 1, 2&3) were significantly reduced in serum, liver and kidney of alloxan induced diabetic rats. These adverse changes were reversed to near normal values in ethanol extract of *Melastoma malabathricum* leaf treated. It is well known that CAT, SOD and GPx play an important role as protective enzymes against free radical formation of tissues[26]. SOD has been postulated as one of the most important enzymes in the enzymatic antioxidant defense system which catalyses the dismutation of superoxide radicals to produce $H_2O_2$ and molecular oxygen[27], hence diminishing the toxic effects caused by their radical. The observed decrease in SOD activity could result from inactivation by $H_2O_2$ or by glycation of enzymes[28]. The superoxide anion has been known to inactivate CAT, which involved in the detoxification of hydrogen peroxide[29]. Thus, the increase in SOD activity may indirectly play an important role in the activity of catalase.

**TABLE 1: EFFECT OF MELASTOMMA MALABATHRICUM LEAF EXTRACTS ON SERUM LPO,SOD, CAT, GPx, GRD AND GSH IN THE NORMAL, DIABETIC AND DRUG TREATED RATS.**

| Groups | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | LPO (n mol/ml) | SOD (u/gm Hb) | CAT (k/gm Hb) | GPx (U/L) | GRD (U/L) | GSH (n mol/ml) |
| I | 1.69 ±0.21 | 493.16 ±15.26 | 91.33 ±3.88 | 779.16 ±5.16 | 21.16 ±1.04 | 35.84 ±1.13 |
| II | 2.94 ±0.05* | 243.84 ±11.24** | 47.16 ±1.26* | 239.12 ±3.86** | 11.84 ±1.35* | 14.67 ±1.12* |
| III | 2.21 ±0.14[a] | 396.48 ±10.14[a] | 64.46 ±1.16[a] | 368.54 ±2.46[a] | 14.36 ±1.56[a] | 20.56 ±1.36[a] |
| IV | 1.73 ±0.27 | 416.16 ±13.50[ac] | 78.17 ±1.56[a] | 659.16 ±3.42[aa] | 18.23 ±1.33[a] | 28.15 ±0.94[a] |
| V | 1.66 ±0.24[a] | 465.16 ±5.27[ab] | 84.11 ±1.54[a] | 716.18 ±5.31[aa] | 20.18 ±1.09[a] | 30.65 ±1.06[a] |

Alphabets (a,b,c) indicate the result of ANOVA test. The alloxan treated group was compared with normal. Whereas, drug treated group (MML) with the alloxan treated and normal group. Significant at * $p < 0.05$; ** $p<0.01$ levels ., a-< 0.05; aa- $p<0.01$; a- Diabetic vs drug treated ; b-MML treated known drug treated c-MML and known drug treated groups.

Catalase (CAT) is a heme protein which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals[30]. The decrease in CAT

activity could result from inactivation by glycation of enzyme[31]. Reduced activity of SOD and CAT in the serum, liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxides[32]. The reductions of hepatic SOD and CAT activities in alloxan induced diabetic rats when compared with normal rats were reported[33]. Whereas, the extract treated groups showed a significant increase in the hepatic SOD and CAT activities of the diabetic rats. This means that the extracts can reduce the potential glycation of enzymes or they may reduce reactive oxygen free radicals and improve the activities of antioxidant enzymes.

**TABLE 2: EFFECT OF MELASTOMMA MALABATHRICUM LEAF EXTRACT ON LIVER LPO,SOD, CAT, GPx, GRD AND GSH IN THE NORMAL, DIABETIC AND DRUG TREATED RATS.**

| Groups | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | LPO (n mol/mg protein) | SOD (u/mg protein) | CAT (k/sec/mg protein) | GPx (U/mg protein) | GRD (U/mg protein) | GSH ( n mol/ml) |
| I | 0.084 ±0.13 | 21.54 ±1.69 | 12.54 ±1.36 | 52.84 ±1.34 | 36.84 ±1.93 | 20.69 ±1.84 |
| II | 0.363 ±0.26* | 8.55 ±1.25** | 3.29 ±0.51** | 24.09 ±0.94** | 19.25 ±0.84* | 12.92 ±0.62* |
| III | 0.246 ±0.16[a] | 14.39 ±1.16[a] | 5.78 ±0.48[a] | 38.14 ±0.98[a] | 22.16 ±1.30[a] | 15.66 ±0.84[a] |
| IV | 0.196 ±0.16* | 17.94 ±1.22[a] | 8.46 ±0.34[a] | 46.27 ±1.13[a] | 26.88 ±1.16ns | 19.11 ±0.54[a] |
| V | 0.091 ±0.021[a] | 19.84 ±0.94[a] | 10.27 ±0.26[a] | 49.38 ±1.56[a] | 32.66 ±2.95[a] | 18.16 ±0.81ns |

Alphabets (a,b,c) indicate the result of ANOVA test. The alloxan treated group was compared with normal. Whereas, drug treated group (MML) with the alloxan treated and normal group. Significant at * $p < 0.05$; ** $p<0.01$ levels ., a-< 0.05; aa- $p<0.01$; a- Diabetic vs drug treated ; b-MML treated known drug treated c-MML and known drug treated groups. ns- Not significant.

GSH is a major non-protein thiol in living organisms which plays a central role in co-ordinating the body's antioxidant defense processes. Perturbation of GSH status of a biological system can lead to serious consequences. GPx catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphate (GSSG) and the

reduction product of the hydroperoxide. In the present study, decline in the activities of these enzymes in alloxan induced rats and attainment of normally in *Melastoma malabathricum* leaf extract treated rats indicate that oxidative stress elicited by alloxan was significantly reduced by this extract.

**TABLE 3: EFFECT OF MELASTOMMA MALABATHRICUM LEAF EXTRACTS ON KIDNEY LPO, SOD, CAT, GPx, GRD AND GSH IN THE NORMAL, DIABETIC AND DRUG TREATED RATS.**

| Groups | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | LPO (n mol/mg protein) | SOD (u/mg protein) | CAT (u/mg protein) | GPx (U/mg protein) | GRD (U/mg protein) | GSH (n mol/ml) |
| I | 0.043 ±0.014 | 16.39 ±0.52 | 32.07 ±1.63 | 6.94 ±0.13 | 19.74 ±0.41 | 31.68 ±1.20 |
| II | 0.189 ±0.06* | 6.21 ±0.27** | 18.16 ±1.31* | 3.06 ±0.31* | 9.46 ±0.18** | 13.46 ±1.17* |
| III | 0.143 ±0.01[a] | 11.14 ±0.34[a] | 19.56 ±1.41[a] | 3.98 ±0.24[a] | 15.26 ±1.14[a] | 22.46 ±0.16[a] |
| IV | 0.093 ±0.02 | 13.28 ±0.26 [b] | 22.61 ±1.93[abc] | 4.34 ±0.54 | 17.33 ±1.01[a] | 26.93 ±0.12[a] |
| V | 0.058 ±0.01[ab] | 18.39 ±0.71[b] | 38.33 ±1.91[a] | 5.34 ±0.13[a] | 18.03 ±0.63[a] | 28.67 ±1.08[a] |

Alphabets (a,b,c) indicate the result of ANOVA test. The alloxan treated group was compared with normal. Whereas, drug treated group (MML) with the alloxan treated and normal group. Significant at * $p < 0.05$; ** $p<0.01$ levels, a-< 0.05; aa- $p<0.01$; a- Diabetic vs drug treated ; b-MML treated known drug treated c-MML and known drug treated groups.

The present study reveals that the *Melastoma malabathricum* leaf extract had antioxidant activity. The bioactive components, responsible for the observed activities are not precisely known but it may be one or more of the phytochemical constituents established to be present in the leaf extracts. In the present study, phytochemical screening reported that the presence of phenolics and flavonoids in extracts which might be the constituents responsible for the antioxidant activities. Further identification and isolation of three compounds may be fruitful.

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