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In Vivo Antioxidant Activity of *Bougainvillea Glabra*

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Abstract: The present study was aimed at investigating the in-vivo antioxidant activity of the methanol extract of Bougainvillea glabra. Methanol extract of Bougainvillea glabra was administered to rats at two different doses of 250 and 500 mg/kg for 21 days and to evaluate oxidative stress parameters such as ferric reducing ability of plasma (FRAP), thiobarbituric acid reactive substance (TBARS), reduced glutathione (GSH) and antioxidant enzyme levels [catalase (CAT) & superoxide dismutase (SOD)]. The methanol extracts of Bougainvillea glabra significantly ($p < 0.05$) elevated the ferric FRAP on 7, 14 and 21 days of treatment. Significant ($p<0.05$) decrease of TBARS level along with an increase in the SOD & CAT enzymes level in the liver and kidneys at two different doses of the plant was observed. However, there was no major effect of TBARS, SOD, CAT and GSH levels in heart at two different doses of Bougainvillea glabra. The above results recommend that Bougainvillea glabra have potent antioxidant activity, which were responsible for its reported pharmacological activity.

Keywords: Antioxidant activity, Bougainvillea glabra, FRAP, GSH**,** SOD, TBARS,

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I. INTRODUCTION

In the development of diseases, free radicals have emerged because the major entity causing harm to cells. These cytotoxic metabolites are produced by aerobic metabolism within the cell that sequentially considerably will rise pathological conditions, resulting in free radical mediate denaturation of protein, enzymatic deactivation, base hydroxylation of nucleic acids, cross-linking or strand cutting, mutation or may be death [1]. Nevertheless, the physiological system has a series of defense mechanisms as well as antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), and other free radical scavengers, β-carotene, vitamin C, vitamin E, α-lipoic acid, curcumin, rutin, BHT and glutathione to protect the cell against cytotoxic ROS (reactive oxygen species) [2]. The antioxidants in biological system can be either enzymatic or non-enzymatic. The enzymatic antioxidants comprise catalase, superoxide dismutase, and glutathione which catalyze neutralization of many types of free radicals [3], while the non-enzymatic antioxidants include vitamin C, selenium, vitamin E, carotenoids, and polyphenols. The enzymatic antioxidants catalyze the neutralization of numerous types of free radicals. Though, when the balance between the oxygen species and antioxidants is altered, a state of oxidative stress results, possibly leading to everlasting cellular damage. There is evidence that antioxidants may be useful in avoiding the deleterious consequences of oxidative stress, and there is an increasing interest in the protective biochemical function of natural antioxidants contained in vegetables, fruits and medicinal herbs [4]. Generally, plants, herbs, and spice rich in phenolic compounds like flavonoids, have been confirmed to have anti-inflammatory, antiallergic, antiviral, antiaging and anti-carcinogenic activities which can be accredited to their antioxidant properties [5]. In this respect, flavonoids and polyphenolic compounds have received the greatest attention [6-8].

Bougainvillea glabra is a useful medicinal plant from Nyctaginaceae family finding applications in indigenous systems of medicine. *Bougainvillea glabra* is an ornamental plant with several medicinal properties. It is distributed everywhere, mostly in warm climatic regions. *Bougainvillea glabra* is having colorful flowers and the plant is seen in front of houses and in office and on walls and fences [9]. Leaves juice of *Bougainvillea glabra* is orally taken with rhizome juice of *Curcuma caesia* for a few days to treat helminthiasis in Bangladesh [10]. Various pharmacological activities like antidiabetic [11], antilipidemic [12] and anthelmintic [13] were proved from *Bougainvillea glabra.* The phytochemical analysis of the extract reveals the presence of alkaloids, terpenoids, saponins and cardiac glycosides in *Bougainvillea glabra*. Five flavonoids like vitexin, isovitexin, chrysoeriol, apigenin and luteolin were isolated from *Bougainvillea glabra* [14]. The GC-MS study of

Bougainvillea glabra has shown many phytochemicals which contributes to the medicinal activity of the plant. The present study was aimed to investigate the antioxidant activity of methanol extract of whole plant of *Bougainvillea glabra* by *in vivo* method.

II. MATERIALS AND METHODS

2.1 Collection and authentication of plant material

The whole plant of *Bougainvillea glabra* was collected in the month of July from botanical garden, Hanamkonda, Warangal district, Telangana, India. It was shade dried away from sunlight and stored suitably. The plant material was taxonomically identified by Dr. Vatsavaya S Raju, Professor, Plant Systematics Laboratory, Department of Botany, Kakatiya University, Warangal district Telangana, India and a voucher specimen was deposited in the herbarium against accession number 4610 for future reference.

2.2 Chemicals

All chemical substances used in the study stood analytical grade.

2.3 Extraction

The whole plant of *Bougainvillea glabra* was powdered with a mechanical grinder and passed through Sieve no. 40. Powder of *Bougainvillea glabra* was extracted with methanol by continuous Soxhlet extraction method. The excess solvent was removed by rotary vacuum evaporator; the remaining mass of extract was concentrated and dried. The extract was freeze dried and stored in a vacuum desiccator for further *in vivo* antioxidant studies.

2.4 Safety evaluation study

The safety study was carried out using OECD guide lines No. 423. Three female rats of the same age group and weight were taken in a single dose of methanol extract of *Bougainvillea glabra* (MEBG) up to the highest dose of 2000 mg/kg orally. The animals were observed for 1 h continuously and then hourly for 4 h, and finally after every 24 h up to 15 days for any mortality or gross behavioral changes [15]

2.5 In-vivo antioxidant activity

Experimental animals

Albino rats of Wistar strain (200-250gm) were procured from Ghosh enterprises Kolkata, India. All the rats were accommodated in polypropylene cages and preserved in a precise environment (28-32°C) with 12-12 hr of light and dark cycle. Each day all the animals were fed a normal laboratory diet *ad libitum* and had free access to water. The animals were maintained under standard conditions in an animal house as per the guidelines of committee for the purpose of control and supervision on experiments on animals (CPCSEA). Experiments on animals were performed based on the animal ethics guidelines of institutional animal ethics committee (CPCSEA Registration No:1287/PO/Re/S/09/CPCSEA).

2.6 Experimental protocol

Wistar albino rats (200–250gm) were divided into 3 groups of 6 rats each. Group I animals were treated as normal control and received only saline (1ml/kg/day p.o) for 21 days. Group II animals were treated with methanol extract of *Bougainvillea glabra* (250 mg/kg, p.o.) daily for 21 days. Group III animals were treated with methanol extract of *Bougainvillea glabra* (500 mg/kg, p.o.) daily for 21 days. Twenty-four hours after the last treatment, all rats were weighed; blood was collected through direct cardiac puncture and then sacrificed. The organs like heart, liver and kidney were removed, washed in cold saline and stored in liquid nitrogen for further biochemical studies. This *in vivo* antioxidant activity was analyzed by the method described by Rajlakshmi et al. [16].

2.7 Preparation of rat heart, liver and kidney homogenate

Tissue homogeneate was prepared in a ratio of 1gm of wet tissue to 10 times (w/v) 0.05 mol/L ice cold phosphate buffer (pH 7.4) and homogenised using a homogeniser (Tissue homogeniser). A 0.2 mL of sample homogenate was used for assessment of thiobarbituric acid reactive substance (TBARS). The left over part of the homogeneate was separated into two parts. One part was mixed with 10% trichloroacetic acid (1:1), centrifuged at 5000g (4^0C, for 10min) and the supernatant was used for reduced glutathione (GSH) estimation. The second part of the homogeneate was centrifuged at 15000g at 4^0C for 60 min and the supernatant was used for reduced glutathione (GSH) estimation.

2.8 Ferric reducing ability of plasma assay

Total plasma antioxidant capacity was restrained conferring to the ferric reducing ability of plasma (FRAP) method [17]. The blood samples were collected from the rat retro-orbital venous plexus into heparinised glass tubes at 0, 7, 14 and 21 days of treatment. Briefly, 3mL of freshly prepared and warm (37°C) FRAP reagent (1mL of 10 mmol/L TPTZ [2,4,6 tripyridyl-s-triazine] solution in 40 mmol/L HCl, 1mL 20 mM/L $FeCl_2.6H_2O$, 10mL of 0.3 mM/L acetate buffer [pH 3.6]) was mixed with 0.375mL distilled water and 0.025mL of test samples. The absorbance of developed colour in the organic layer was measured at 593nm. The temperature was maintained at 37°C. The readings at 180 sec were selected for the calculation of FRAP values. Ferrous sulphate ($FeSO_4.7H_2O$) was used as a standard for calibration and the data expressed as nmol Fe^{2+}/L.

2.9 Superoxide dismutase assay

Superoxide dismutase (SOD) activity was analysed by the method described by Rai et al., [18]. Assay mixture contain 0.1mL of supernatant, 1.2mL of sodium pyrophosphate buffer (pH 8.3; 0.052M), 0.1mL of phenazine methosulfate (186 mM), 0.3mL of nitroblue tetrazolium (300 mM), and 0.2mL of NADH (750 mM). Reaction was started by the addition of NADH. After Incubation at 30^{o}C for 90s, the reaction was stopped by the addition of 0.1mL of glacial acetic acid. Reaction mixture was stirred vigorously with four mL of n-butanol. Color intensity of the chromogen in the butanol was measured spectrophotometrically at 560nm and the concentration of superoxide dismutase (SOD) was expressed as units/mg of protein.

2.10 Catalase assay

Catalase activity (CAT) was measured by the method of Aebi [19]. 0.1mL of supernatant was added to cuvette containing 1.9mL of 50mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0mL of freshly prepared 30mM H_2O_2. The rate of the decomposition of H_2O_2 was measured spectrophotometrically at 240 nm. Activity of catalase (CAT) was expressed as units/mg of protein.

2.11 Estimation of thiobarbituric acid reactive substance (TBARS)

Lipid peroxidation (LPO) was measured by the method of Liu et al., [20]. The solution [acetic acid 1.5mL (20%; pH 3.5), 1.5 of TBA (0.8%), and 0.2mL of sodium dodecyl sulfate (8.1%)] was added to 0.1ml of supernatant and heated at 100^{o}C for 60 min. Mixture was cooled to room temperature. Further, 5mL of n-butanol: pyridine mixture and 1mL of distilled water were added and vortexed vigorously. After centrifugation at 1200g for 10min, the organic layer was separated and the absorbance was measured at 532nm using a spectrophotometer. Malonyldialdehyde (MDA) was an end product of LPO, which reacts with TBA to form pink chromogen–TBA reactive substance. It was calculated using a molar extinction coefficient of $1.56 \times 10^5 M^{-1} cm^{-1}$ and it was expressed as nM/g wet wt.

2.12 Estimation of reduced glutathione (GSH)

Reduced glutathione was measured according to the method of Ellman [21]. An equal quantity of homogeneate was mixed with 10% trichloroacetic acid and it was centrifuged to separate the proteins. From this, 0.01mL of the supernatant was mixed with 2mL of phosphate buffer (pH 8.4), 0.5mL of 5'5-dithio, bis (2-nitrobenzoic acid) and 0.4mL double distilled water. The mixture was vortexed and the absorbance was read at 412nm within 15 min. The concentration of reduced glutathione was expressed as µg/g tissue.

2.13 Statistical analysis

All experimental data were expressed as mean ± standard error of the mean (SEM). The statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnet-t-test with the SPSS statistical software for comparison to the control group. $p<0.05$ was considered as statistically significant.

III. RESULTS

3.1 Safety evaluation study

Rats when fed with methanol extract of *Bougainvillea glabra* up to 2000 mg/kg, p.o. exhibited no mortality or any sign of gross behavioral changes when observed initially for 72 h, and finally up to 15 days.

3.2 Ferric reducing ability of plasma assay

The ferric reducing ability of plasma level of rats after administration of methanol extracts of *Bougainvillea glabra* over a period of 21 days was presented in figure 1. In the control group, there was no significant change in ferric reducing ability of plasma (FRAP) value on days 7 (762 nM Fe^{2+}/L), 14 (784 nM Fe^{2+}/L) and 21 (808 nM Fe^{2+}/L). But, in group II and III on days 7 (892 and 1024 nM Fe^{2+}/L, respectively), 14 (1108 and 1184 nM Fe^{2+}/L, respectively) and 21 (1122 and 1288 nM Fe^{2+}/L, respectively) there was a significant ($p < 0.05$, p and $p < 0.01$) increase in ferric reducing ability of plasma (FRAP) value. Maximum

enhancement of ferric reducing ability of plasma (FRAP) level was found in group III, which resemble to animals administered with 500 mg/kg body weight of the methanol extract of *Bougainvillea glabra*.

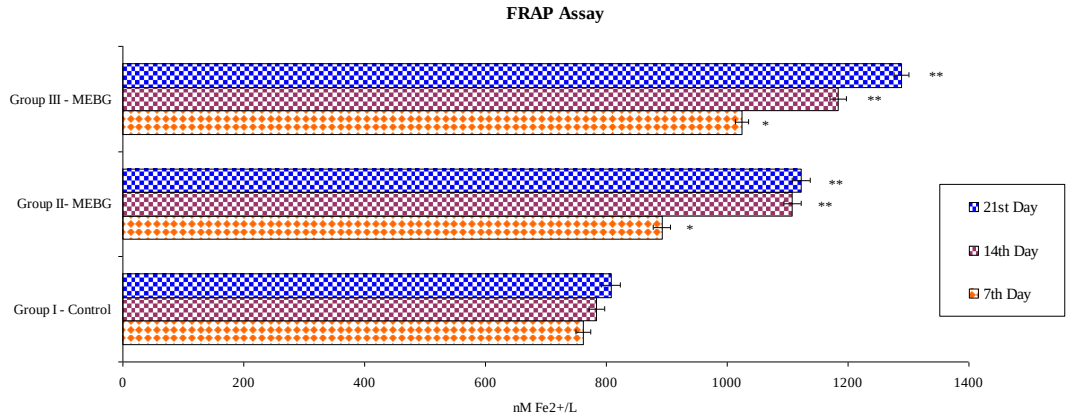

Figure 1: Changes of total antioxidant capacity of ferric reducing ability of plasma (FRAP) in rat, Measured by Fe^{2+} Equivalent after administration of methanol extract of *Bougainvillea glabra*. Values were mean ± s.d (n=6). Group II and III [methanol extract of *Bougainvillea glabra* (MEBG) treated rats] compared to Group I (control rats). $p < 0.05$* and $p < 0.01$**.

3.3 Estimation of superoxide dismutase

The treatment of methanol extract of *Bougainvillea glabra* caused minor significant ($p < 0.05$, and $p < 0.001$) increase at 250 mg/kg (508 units/mg of protein) and 500 mg/kg (512 units/mg of protein) bodyweight in the level of superoxide dismutase in the heart compared with the control (506 units/mg of protein). However, the level of superoxide dismutase in the kidney and liver of the plant treated rats was not dose related and was found to be significantly increased ($p < 0.05$ and $p < 0.01$) at the two different doses of 250 mg/kg (206 and 428 units/mg of protein, respectively) and 500 mg/kg (218 and 432 units/mg of protein, respectively) compared with the control (group-I) (198 and 406 units/mg of protein, respectively). The result was shown in figure 2.

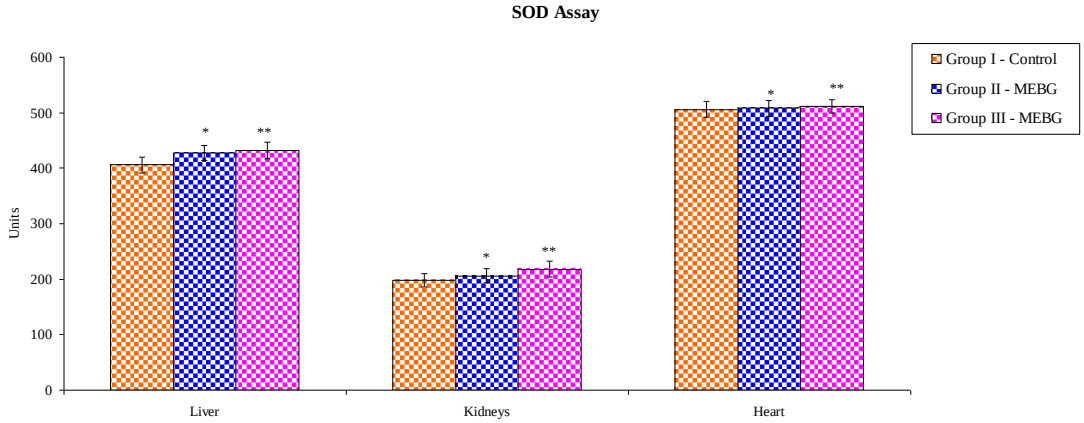

Figure 2. Changes of superoxide dismutase (SOD; 10^{-1} U/ mg protein) following oral administration of methanol extract of *Bougainvillea glabra* in rat liver, kidney and heart. Values are mean ± s.d. (n=6). Groups II and III [methanol extract of *Bougainvillea glabra* (MEBG) treated rats] compared with Group I (control rats). $^{**}P< 0.01$, $^{*}P< 0.05$

3.4 Estimation of catalase

The administration of methanol extract of *Bougainvillea glabra* to normal rats for 21 days induced a dose dependent increase in the level of catalase in liver and kidney, but a decrease in the heart (figure 3). Methanol extract of plant was significantly ($p < 0.01$) increased at 500 mg/kg bodyweight dose of the treatment for liver (349 units/mg of protein) and kidneys (408 units/mg of protein) compared with the control group of liver and kidneys (278 units/mg of protein and 318 units/mg of protein, respectively). Similarly, at a dose of 250 mg/kg a significant ($p < 0.05$) increase in the catalase (CAT) levels were observed for liver (289 units/mg of

protein) and kidney (374 units/mg of protein) compared with the control group. However, there was a little change in the endogenous antioxidant levels in heart tissue.

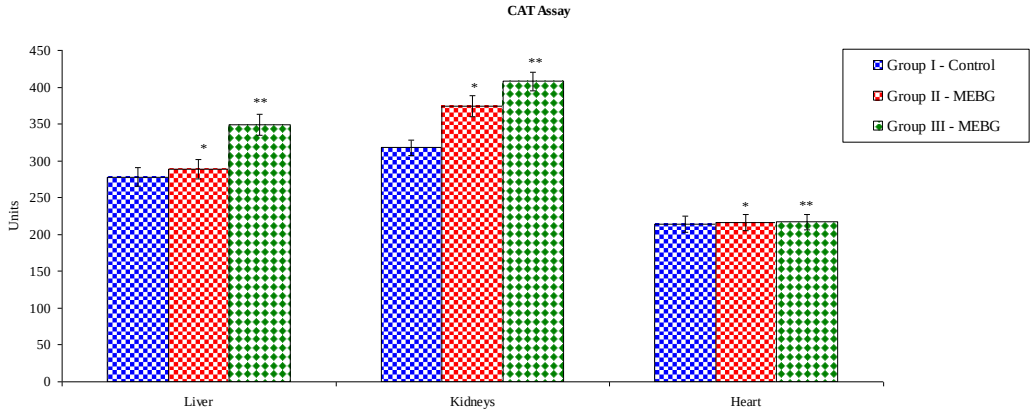

Figure 3. Changes of catalase (CAT;10^{-1} U/mg protein) following oral administration of methanol extract of *Bougainvillea glabra* in rat liver, kidney and heart. Values are mean ± s.d. (n=6). Groups II and III [methanol extract of *Bougainvillea glabra* (MEBG) treated rats] compared with Group I (control rats). $^{**}P< 0.01$, $^{*}P< 0.05$

3.5 Estimation of thiobarbituric acid reactive substance

The effect of two different doses of methanol extract of *Bougainvillea glabra* on the lipid peroxidation and endogenous antioxidants of liver, heart and kidney of rats was shown in figure 4. A significant ($p< 0.05$ and $p< 0.01$) decrease in TBARS concentration was observed in liver (277 nM/g and 274 nM/g wet wt tissues) and kidney (242 nM/g and 240 nM/g wet wt tissue tissues) for group II and group III when compared to control group (317 nM/g and 248 nM/g wet wt tissue). There was no changes of TBARS levels of heart tissue in the methanol extract of *Bougainvillea glabra* treated groups II (301 nM/g) and III (299 nM/g) respectively, when compared to the control group (298 nM/g wet wt tissue).

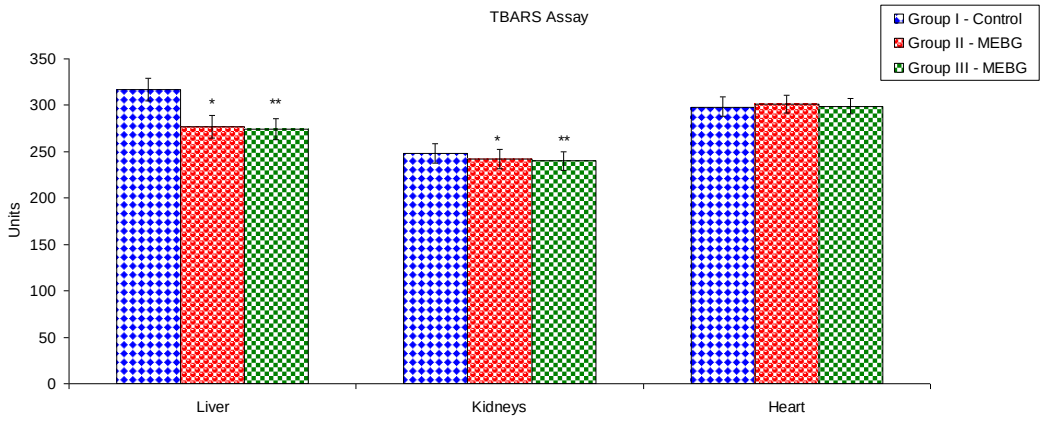

Figure 4. Changes of thiobarbituric acid reactive substance (TBARS; nM/g wet weight [wt]) following oral administration of methanol extract of *Bougainvillea glabra* in rat liver, kidney and heart. Values are mean ± s.d. (n=6). Groups II and III [methanol extract of *Bougainvillea glabra* (MEBG) treated rats] compared with Group I (control rats). $^{**}P< 0.01$, $^{*}P< 0.05$

3.6 Estimation of reduced glutathione

For groups 2 and 3, there was no significant change in GSH level in liver (1.140, and 1.108 mg/g wet wt tissue, respectively), heart (364, and 359 µg/g wet wt tissue, respectively) and kidney (12.3, and 12.0 µg/g wet wt tissue, respectively) as compared to the control group of liver, heart and kidney (1.150 mg/g, 362 µg/g and 12.2 µg/g wet wt of tissues, respectively). The result of reduced glutathione was mentioned in figure 5.

IV. DISCUSSION

Excessive production of reactive oxygen species (ROS) plays a very important role within the pathologic process and progression of many diseases together with completely different organs [22] Visioli. The principle for the utilization of antioxidants is well established in interference and treatment of chronic diseases

wherever aerobic stress plays a serious aetiopathological role. Varied population studies support that consumption of natural sources of fruits and vegetables, rich in antioxidant compounds, are related to a lower incidence of stress evoked diseases [23].

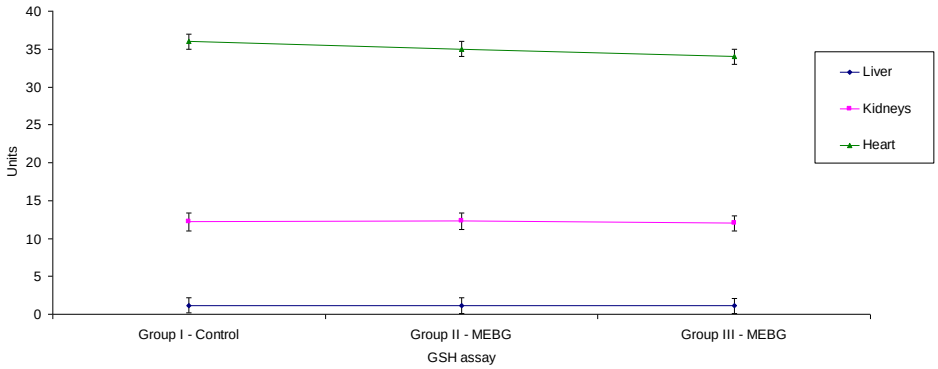

Figure 5. Changes of reduced glutathione (GSH; 10^{-1} µg/g wet wt) following oral administration of methanol extract of *Bougainvillea glabra* in rat liver, kidney and heart. Values are mean ± s.d. (n=6). Groups II and III [methanol extract of *Bougainvillea glabra* (MEBG) treated rats] compared with Group I (control rats).

In the present investigation, the ferric reducing ability of plasma test measured total antioxidant capacity determined by non-enzymatic antioxidants. Numerous approaches have been established to assess the total antioxidant capacity of plasma or serum because of the difficulty in measuring each antioxidant component separately in the serum or plasma [24]. One of these is the ferric reducing ability of plasma, which measures the reduction of Fe^{3+} to Fe^{2+} in the presence of water soluble exogenous antioxidants. The significant increase in ferric reducing ability of plasma level after oral administration of methanol extract of *Bougainvillea glabra* indicates the presence of bio-available antioxidants in these plants.

Reactions with the cell membrane constituents lead to lipid peroxidation (LPO) [25]. Increased LPO impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptor [26]. Malondialdehyde is an endogenous genotoxic product of enzymatic and ROS-induced LPO whose adducts are known to exist in DNA isolated from healthy human being [27]. In our study, the level of thiobarbituric acid reactive substance in the extract treated groups decreased in a dose dependent manner when compared to control. The present study showed the depletion in the lipid peroxidation as observed by significant decrease in the thiobarbituric acid reactive substance level of the liver and kidney in the plant extracts treated groups, but there was no change of thiobarbituric acid reactive substance level in heart as compared to control.

Superoxide dismutase is one more reactive oxygen species defense enzyme existing entirely in the mitochondrial matrix and defends cells against the deleterious actions of super oxide anion resulting from the peroxidative development in tissues. Catalase is a abundant enzyme that catalyzes the decomposition of hydrogen peroxide, a reactive oxygen species, which is a toxic product of both normal aerobic metabolism and pathogenic ROS production [28]. The administration of methanol extract of *Bougainvillea glabra* at 500 mg/kg body weight significantly increased the level of superoxide dismutase and catalase in liver and kidney. This shows the antioxidant nature of the plant extract. Generally, results for the kidney have shown few variations in antioxidant activity compared to liver [29]. Nevertheless, reduction in the level of superoxide dismutase and catalase was observed in the heart, which could define the present opinion.

Reduced glutathione is a protective molecule against chemical induced cytotoxicity [29]. Glutathione is involved in many imperative cellular functions, extending from the control of physicochemical belongings of cellular proteins and peptides to the detoxification of free radicals [30]. Though, long period administration of the methanol extract of *Bougainvillea glabra* did not show significant results in reduced glutathione levels of liver, heart and kidney indicating a protective antioxidant effect.

V. CONCLUSION

It can be concluded that, the methanol extract of *Bougainvillea glabra* had significant *in vivo* antioxidant activity. The methanol extract of *Bougainvillea glabra* was proved to be efficient in reducing the lipid peroxidation in tissues of liver, heart and kidney. The antioxidant activity of methanol extract of the plant may be accredited to the presence of known bioactive compounds (flavonoids), which bring maximum conjugation with radical species, thus reducing the number of free radicals available as well as oxidative stress

related ailments of major organs such as liver, kidney and heart. In future, further studies on active ingredient in the extracts are responsible for antioxidant effect should also be assessed.

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