Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(7): 341-349

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Phytopharmacological evaluation of hydro-alcoholic extract of *Mesua ferrea* stamen for their anti-oxidant and antidiabetic activity

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ABSTRACT

Reactive oxygen species (ROS) and oxygen free radicals play important roles, both beneficial and detrimental, in aerobic life. Excess ROS have been implicated in a variety of patho-physiological phenomena, such as inflammation, ageing, diabetes, cancer and atherosclerosis. Considering the importance of this area, hydroalcoholic extracts obtained from Mesua ferrea Stamen was tested for antioxidant and Antidiabetic activity. The In vitro antioxidant activity of the extract was done by DPPH free radical activity, Super oxide radical scavenging activity and Hydroxyl radical scavenging activity. The hydro alcoholic extracts of M. ferrea exhibit 73-83 % scavenging activity at a concentration of 10mg/ml, whereas other extracts showed moderate antioxidant activity. The hydro alcoholic extracts of M. ferrea stamen extract shows 61.30% and 81.35% free radical scavenging activity at a dose of 10 and 20 mg /ml. The Super oxide radical scavenging activity of the extracts MFSE-I and MFSE-II were found as, 20.76% and 66.92% respectively. The hydroxyl radical scavenging activity was shown by the extracts MFSE-I and MFSE-II as, 40.00% and 52.75% respectively. These results indicate that the plant extract scavenged free radicals significantly at the concentration of 20 mg /ml. The finding of this work indicates that the extract effectively reduces the oxidative stress induced by alloxan and produced a dose dependent reduction in blood sugar (hypoglycemia) at a dose of 150 and 300 mg /kg body weight.

Key words: *M. ferrea*, ROS, antioxidant, DPPH free radical activity, Super oxide radical scavenging activity and Hydroxyl radical scavenging activity, Antidiabetic

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INTRODUCTION

Today it is realized that majority of diseases are due to imbalance of pro-oxidant and the antioxidant homeostatic phenomenon in the body. Pro-oxidant condition dominates either due to the increase generation of free radical or due to the excessive oxidative stress of the current life or due to poor scavenging/ quenching in the body[1-3]. Free radical have been implicated in causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes etc. reactive oxygen species (ROS) have been known to cause tissue injury through covalent binding and lipid peroxidation. Lipid peroxidation process has been shown to augment collagen synthesis and fibrosis[4]. Hence antioxidants have a role in inhibiting the fibrotic process induced during the cell damage of liver[5]. As plants produce lot of antioxidants to control the oxidative stress caused by sunbeam and oxygen, they can represent a source of new compounds with antioxidant activity.

Mesua ferrea (Ceylon ironwood, Indian rose chestnut) is a species in the family Clusiaceae. Many parts (Fig: 1,2 ,3) of this plant have medicinal properties. It enhances the complexion. It leads to fragility transparency to the skin. The flowers are acrid, anodyne, digestive, constipating, and stomachache. They are useful in conditions like asthma, leprosy, cough, fever, vomiting and impotency. The seed oil is considered to be very useful in conditions like vata and skin diseases. Phytochemical studies of plant *M. ferrea* revealed that it is rich in many classes of secondary metabolites including phenylcoumarins, xanthones and triterpenoides[5,6,7]. while stamens afforded two novel biflavnones designated as mesuaferrone-A and mesuaferrone-B, mesuanic acid, and b-amyrine, b-sitosterol[8]. Thus present study aims to access the antioxidant and Antidiabetic potential of hydroalcoholic extracts of stamens of *Mesua ferrea* and to evaluated the stamen for different free radical scavenging activity including Hydroxyl Radical Scavenging activity, DPPH method, super Oxide scavenging activity and antidiabetic activity.

Figure-1 Whole tree of *Mesua ferrea*

Figure-2 Flower of *Mesua ferrea*

Figure-3 Stamens of *Mesua ferrea*

EXPERIMENTAL SECTION

Collection of Drugs

Stamens of *Meusa ferrea* were purchased from local market authenticated by Dr. Sriram Sharma, Lecturer, Pandit Madan Mohan Malviya Govt. Aurvedic College, Udaipur, Rajasthan. The collected stamens were dried in the shade and stored in airtight glass container for further studies.

Preparation of Extract

200g of air dried and cleaned plant drug was ground in the grinder and seived in 60 mesh seive and macerated with 1 liter of 70% ethanol for 72 hours with occasional stirring. The obtained extracts were filtered through muslin cloth and were further concentrated using vacuum evaporator. The percent extractive values were calculated and stored in airtight glass containers. Extracts obtained from *Meusa ferrea* was subjected to preliminary phytochemical screening for identification of various chemical constituents.

Experimental Animals

Healthy, 8-12 weeks young Albino (Rates norvegicus) rats (150-180 g) of either sex were used for present study and obtained from B.N. College of Pharmacy, Udaipur. The animals were maintained as per CPCSEA regulations and the studies were approved by institutional Animal Ethical Committee (IAEC) at Bhupal Nobles' College of Pharmacy, Udaipur (Rajasthan) with approval number-33/ACR/BNCP-08/IAEC. The animals were housed in polypropylene cages individually at 22^0C (± 3^0C), relative humidity 30%, lighting sequence being 12 hours light and 12 hours dark cycle. The animals were housed individually for 5 days in cages. For feeding, conventional rodent laboratory diets were used with unlimited supply of drinking water.

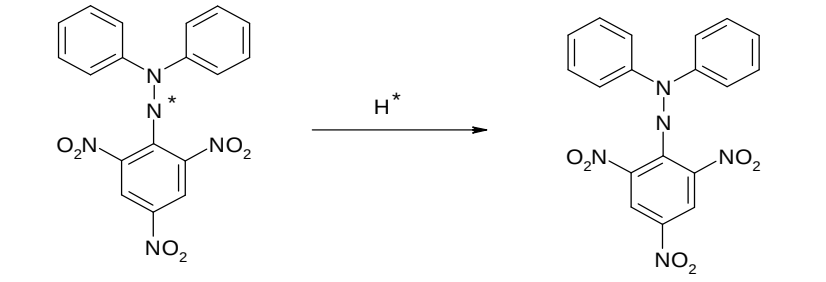
Acute toxicity Study

The acute toxicity of the extracts obtained from *Meusa ferrea* was determined by up and down procedure of OECD guideline 425. One animal was dosed with 2000mg/kg of test drug solution. The animal survived, therefore main test was conducted to determine LD_{50} .Then four additional animals were dosed sequentially and total five animals were tested. After dosing, animals were observed for 48 hours, no animal was died.

In-Vitro Antioxidant Activity

DPPH Scavenging Activity

Scavenging free radical potentialities were evaluated against a methanolic solution of 1, 1-Di Phenyl-2-Picryl Hydrazyl (DPPH), a stable free radical. Antioxidants react with DPPH and convert it to Diphenyl-2picryl hydrazine and the degree of discoloration indicates the scavenging activity of drugs(Fig:4). The change in absorbance produced at 517 nm, has been used as a measure of antioxidant activity[4].



1-1-diphenyl-2-picryl-hydrazyl 1-1-diphenyl-2-picryl-hydrazine

Figure-4 Reaction of DPPH

The Methanolic solution of DPPH (100µM), was prepared by dissolving 39.4 mg of DPPH in one liter of analytical grade methanol. Test extracts was prepared by dissolving 1 g of plant extract in 100 ml of methanol and filtered, the filtrate was used for the experiment. The standard drug solution (1% Ascorbic acid solution) was prepared by dissolving 100mg of ascorbic acid in 10 ml of distilled water.

100µL of 1% extract solution and standard were taken in different test tubes. To this 5 ml of methanolic solution of DPPH was added, shaken well and mixture was allowed to stand for room temperature for 20 min. A blank was also prepared in similar manner and the absorbance was measured at 517 nm using Shimadzu 1601 UV

Spectrophotometer.. The change of sample absorbance was calculated with blank absorbance as percentage scavenging activity[9,10]. Scavenging activity was expressed as the percent inhibition and calculated using the following formula. Each experiment was carried out in triplicate and the deviation of absorbance value was less than 10%. The results are tabulated in Table 1 and Figure 5.

Calculation

Percent anti radical activity is calculated by following formula

$$\% \text{ Anti radical activity} = \frac{\text{Control}_{Abs} - \text{Sample}_{Abs}}{\text{Control}_{Abs}} \times 100$$

Super Oxide Scavenging Activity

Super oxide radicals are the most important among the reactive oxygen species and produced at relatively high rate by cells during normal metabolism. Auto oxidation of hydroxyl amine at pH 10.2 generates super oxide anions which reduce the yellow dye nitroblue tetrazolium to blue coloured formazon. Drugs possessing super oxide scavenging activity will decrease the reduction of NBT to blue formazon, which is a measure of super oxide anion scavenging activity[11].

Sodium carbonate solution (500mM) was prepared by dissolving 1.06 g of sodium carbonate in 100 ml of distilled water. Nitroblue tetrazolium AR (NBT) (24mM) was prepared by dissolving 10 mg of NBT in 100 ml of distilled water. Ethylene diamine tetra acetic acid (EDTA,0.1 nM)) solution was prepared by dissolving 10 mg of EDTA in 100 ml of distilled water. Hydroxyl amine hydrochloride (1nM) solution was prepared by dissolving 16.5 mg of hydroxyl amine hydrochloride in 10ml of distilled water. The test extract was prepared by dissolving 1 g of plant extract in 100 ml of methanol and filtered, the filtrate was used for the experiment.

100 microlitres of 1% alcoholic solution of the extracts were taken in different test tubes and evaporated on water bath. 1 ml of 50 mM sodium carbonate, 0.4 ml of 24nM NBT, 0.2 ml of 0.1 mM EDTA were added and zero minute reading was taken at 560 nm. The reaction was initiated by addition of 0.4 ml of 1m M hydroxyl amine hydrochloride to the reaction mixtures. After incubating for 15 min at 25^0C, the reduction of NBT was measured at 560 nm. using Shimadzu 1601 UV Spectrophotometer.

Percentage scavenging of super oxide anions was calculated by comparison of the results of test compounds with blank. The necessary corrections were made for absorbance of test compounds. Super oxide scavenging activity was calculated by using formula mentioned below. Each experiment was carried out in triplicate and the deviation of absorbance value was less than 10%. The results are shown in Table 2 and Figure 6.

Calculation

Percent super oxide scavenging activity is calculated by following formula

$$\% \text{ Super oxide scavenging activity} = 1 - \frac{\text{Difference in sample absorbance}}{\text{Difference in blank absorption}} \times 100$$

Hydroxyl Radical Scavenging Activity

Hydroxyl radicals have been implicated as highly damaging species in free radical pathology. This radical has the capacity to attack nucleotide in DNA, causes strand breakage, which contributes to carcinogenesis, mutagenesis, and cytotoxicity, In addition, this species is considered to be one of the initiators of lipid peroxidation process. Hence in the present study, Hydroxyl radical scavenging activity of the selected plants was assessed by generating hydroxyl radicals using ascorbic acid-Iron EDTA as the model. The hydroxyl radicals are formed by the oxidation reacting with dimethyl sulphoxide (DMSO) to yield formaldehyde. The formaldehyde production from DMSO provides a convenient method to detect hydroxyl radicals formed during the oxidation of DMSO by using Fe^{3+}/ ascorbic acid system.

100 µL of 1% alcoholic solution were taken in different test tubes and evaporated on a water bath. To these, 1 ml of Iron – EDTA solution, 0.5 ml of EDTA and 1 ml of DMSO were added and the reaction was initiated by adding 0.5 ml of ascorbic acid to each of the test tubes. Test tubes were capped tightly and heated on a water bath at 80 – 90^0C for 15 min. Then, the reaction was terminated by the addition of 1 ml of ice cold TCA (17.5%w/v) to all test tubes. All test tubes were allowed to stand for 2 min and formaldehyde formed was determined by adding 2 ml of Nash

reagent and left undisturbed for 15 min for colour development. The intensity of yellow colour formed was measured using Shimadzu 1601 UV Spectrophotometer at 412 nm against reagent blank.

Percentage scavenging of hydroxyl radicals was calculated by comparison of the results of test compounds with that of blank. The necessary corrections were made for the absorbance of the test compounds. Each experiment was carried out in triplicate and deviation of absorbance was less than 10% and % scavenging activities are shown in Table 3 and Figure 8.

Calculation

Percent hydroxyl radical scavenging activity is calculated by using following formula.

$$\% \text{ Hydroxyl Radical scavenging activity} = 1 - \frac{\text{Difference in sample absorbance}}{\text{Difference in blank absorption}} \times 100$$

ANTI DIABETIC ACTIVITY

Diabetes was induced in 12 hours fasted rats by intraperitoneal injection of alloxan monohydrate (120mg/kg) freshly dissolved in sterile normal saline immediately before use. The diabetic state was assessed by measuring fasting blood glucose concentration 72 hours after alloxan treatment. The rats with a blood glucose level above 200mg/dl were selected for the experiment. The rats were divided in to five groups of six animals in each group[12].

Group-I Animal served as normal and received 1% CMC solution, p.o., Group-II- animal were treated with single dose of Alloxan (120 mg/kg i.p.), Group-III treated as Standard reference drug Glibenclamide (10 mg/kg, p.o.) + Alloxan (120 mg/kg i.p.) , Group-IV have MFSE – I (150 mg/kg, p.o.) + Alloxan (120 mg/kg, i.p) and Group-V have MFSE – II (300 mg/kg, p.o.) + Alloxan (120 mg/kg, i.p). Blood samples were collected on 1, 7, 14, 21 days after treatment from individual rat by orbital sinus under ether anesthesia and were analyzed by using the glucometer (Abbott Healthcare Pvt. Ltd. Mumbai)

RESULTS AND DISCUSSION

Antioxidant activity of the extracts was measured by evaluating free radical scavenging activity (Table 1), Super oxide radical scavenging activity (Table 2) and Hydroxyl radical scavenging activity (Table 3) The hydro alcoholic extracts of *M. ferrea* exhibit 73-83 % scavenging activity at a concentration of 10mg/ml, whereas some extracts showed moderate antioxidant activity.

The hydro alcoholic extracts of *M. ferrea* shows 61.30% and 81.35% free radical scavenging activity, 20.76% and 66.92% Super oxide radical scavenging activity and 40.00% and 52.75% Hydroxyl radical scavenging activity at the concentration of 10 and 20 mg /ml respectively. These results indicate that *M. ferrea* extracts has potent antioxidant activity, achived by scavenging ability observed in present study. Thus the ethanobotanical claims of the plants being used in diabetes may be in part due to the antioxidant activity.

Hydroalcholic extract of *M. ferrea* produced a dose dependent hypoglycemia in alloxan-induced diabetic rats. They produced significant reduction in blood glucose with dose of 150 and 300 mg /kg body weight when compared to control. Blood glucose level was observed on 7^{th}, 14^{th} and 21^{st} days of study in alloxan treated diabetic model. The maximum reduction of blood glucose level produced by Gilbenclmide, MFSE-I and MFSE-II were measured as 57.48, 33.89 and 42.53% respectively on 21^{st} day of study. The Values are 123.16, 191.33 and 175.21 mg/dl respectively for Gilbenclmide, MFSE-I and MFSE-II.

These results indicate that MFSE-II possess significant hypoglycemic activity (175.21mg/dl i.e. 42.53% at 21^{st} day). Results are summarized in Table 4 and Figure 8.

Table 1 Antioxidant activity of *M. Ferrea* stamen extract by DPPH method

S. No.	Sample	Concentration	Absorbance	% Scavenging activity
1.	Blank	--	0.858	--
2.	Ascorbic acid	10mg/ml	0.035	95.92%
3.	MFSE-I	10mg/ml	0.332	61.30%
4.	MFSE-II	20mg/ml	0.160	81.35%

All values are in mean of triplicate value. MFSE- M. Ferrea stamen extract

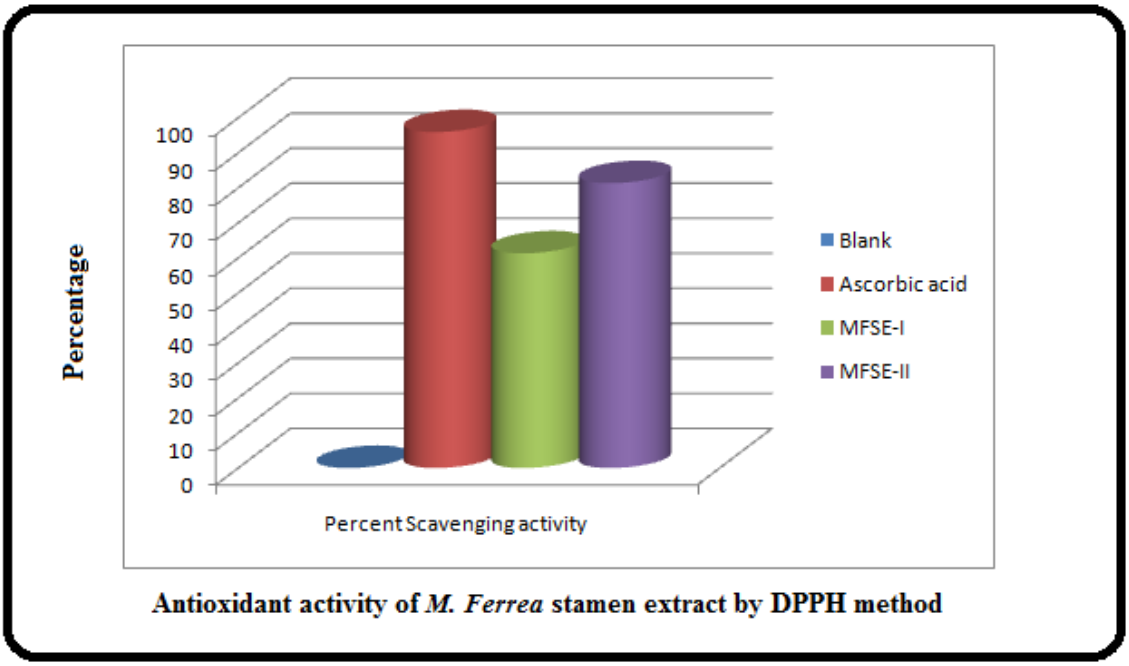


Figure 5 Antioxidant activity of *M. Ferrea* stamen extract by DPPH method

Table 2 Super Oxide Scavenging activity of *M. Ferrea* stamen extract

S. No.	Sample	Concentration	Absorbance	%Scavenging activity
1.	Blank	--	0.260	--
2.	MFSE-I	10mg/ml	0.206	20.76%
3.	MFSE-II	20mg/ml	0.086	66.92%

All values are in mean of triplicate value. MFSE- M. Ferrea stamen extract

Table 3: Hydroxyl Radical Scavenging activity of *M. Ferrea* stamen extract

S. No.	Sample	Concentration	Absorbance	% Scavenging activity
1.	Blank	--	0.580	--
2.	MFSE-I	10mg/ml	0.348	40.00%
3.	MFSE-II	20mg/ml	0.274	52.75%

All values are in mean of triplicate value. MFSE- M. Ferrea stamen extract

Table 4 Effect of *M. Ferrea* stamen extract in alloxan induced diabetic rats

Group	Treatment	Dose mg/kg	Blood glucose level in mg/dl ± S.E.				Percent change in glucose level
			Zero Day	7th Day	14th Day	21st Day	
I	Control	1% CMC	84.66±1.25	82.66±1.11	81.83±0.654	78.33±0.802	-
II	Alloxan	120	306.83±1.32	299.33±0.84	294.00±1.03	291.33±0.42	-
III	Gilbenclamide	10	289.66±3.63	218.66±2.81	152.66±0.84	123.16±0.98	57.48%
IV	MFSE-I	150	297.00±2.51	277.33±2.61	257.50±2.75	191.33±2.23*	33.89%
V	MFSE-II	300	289.26±1.87	267.50±1.67	228.38±2.34*	175.21±2.04**	42.53%

*All values are represented as Mean ±SE (n=6) *** < 0.001; ** < 0.01; * <0.05 when compared with alloxan treated model; MFSE- M. Ferrea stamen extract*

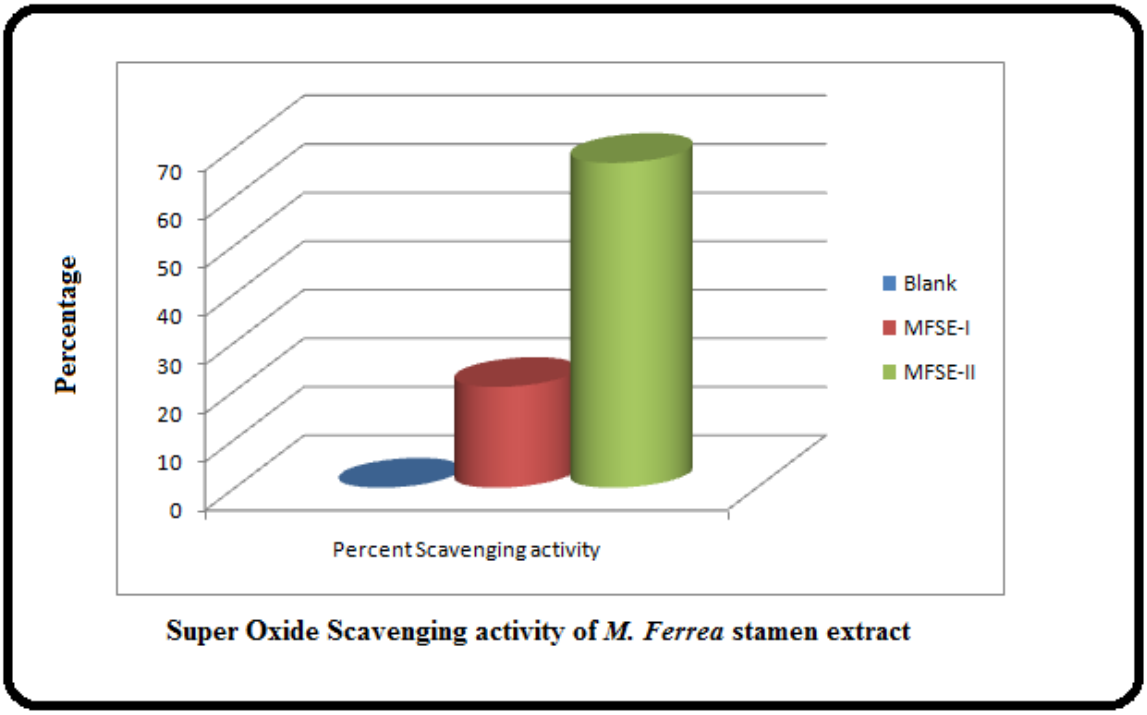


Figure 6 Super Oxide Scavenging activity of *M. Ferrea* stamen extract

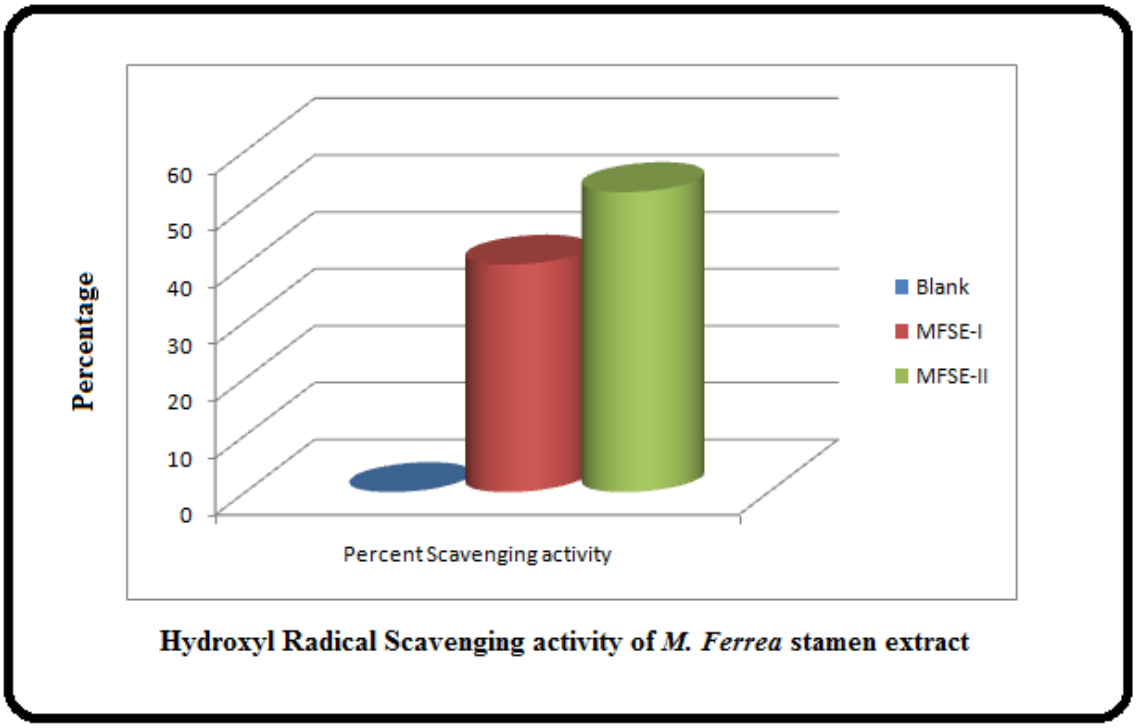


Figure 7 Hydroxyl Radical Scavenging activity of *M. Ferrea* stamen extract

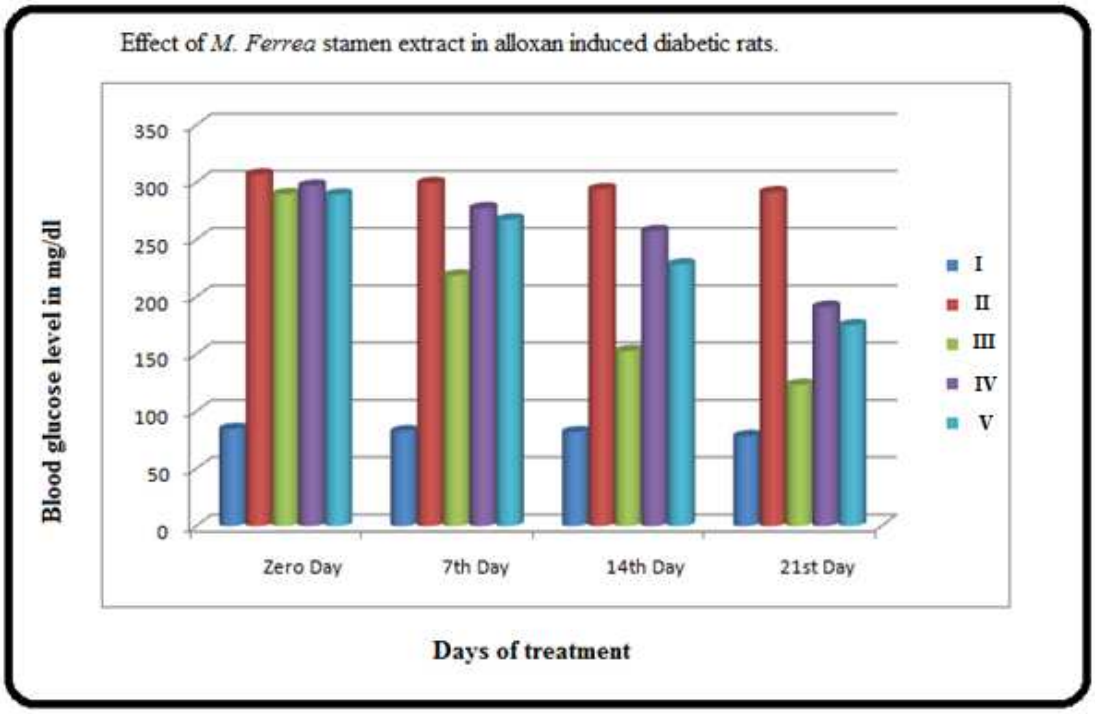


Figure 8 Effect of *M. Ferrea* stamen extract in alloxan induced diabetic rats

CONCLUSION

Many diseases caused primarily due to imbalance between pro oxidant and antioxidant homeostasis [13]. Increasing evidences in both experimental and clinical studies suggested that oxidative stress plays a major role in development and progression of type-I and type-II diabetes mellitus[16]. Antioxidant principles from natural resources provide enormous scope in correcting the imbalance. Free radical scavenging activity has been ascribed extensively to two classes of natural compounds i.e. flavonoids and polyphenolics[14] The major constituents present in *M. ferrea* are flavnone and flavanol glycosides [15] which fulfills structural criteria for being good antioxidant. The ability of extracts to quench free radicals seems to be directly related to the prevention of diabetes .These extracts seem to a good scavenger of ROS, thus reducing the rate of chain reaction.

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