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In-Vivo Anti-oxidant activity of Methanolic extract of *Sapindus emarginatus* in mono sodium glutamate induced obesity rats

S. Divya Teja Banda[1]*, D. Suneetha[1] and T.N.V.S. Narayana Reddy[2]
Sri Sai Aditya Institute of Pharmaceutical Sciences and Research, East Godavari, (AP) - India

Abstract

The Methanolic extract fine pericarp of flowers of of Sapindus emarginatus leaf were subjected to the evaluation of invivo anti-oxidant in monosodium glutamate induced obesity rats.The anti oxidant activity of Sapindus emarginatus may varies from each part of the plant.The anti oxidant property of the pericarp of the flower may increase with increase in the test dose.The invitro antioxidant property of methanolic extract of leaves of Sapindus emarginatus already revealed[1].Now in the present study of evaluation anti oxidant activity in in-vivo manner we were tested a wide variety of intrinsic anti oxidants called SOD(superoxide dismutase),GSH(Glutathione),Catalase. There are five groups were taken for the in vivo antioxidant screening. Monosodium glutamate solution was administered to induce obesity by 8mg/g body weight to each animal orally daily up to 7 days[2] normally. But here in the present by administering MSG we were subjected to screening antioxidant activity after the 28 days of treatment with test doses.

Key-Words: *Sapindus emarginatus*, Monosodium glutamate, SOD (superoxide dismutase), GSH (Glutathione), Catalase. *In-vivo* anti oxidant activity

Introduction

In the traditional system of medicine there are many plants showing a wide variety of medicinal properties and they have been using in the daily life and treating many diseases and disorders. The major chemical constituents present in the every plant part show the medicinal properties. The medicinal property also varies from each part of plant. The free radicals are reactive molecules which are causing many diseases. Many studies has shown that these radicals also affect the equilibrium between pro-oxidants and antioxidants in biological systems, it leads to modification in genes, proteins, carbohydrates, lipids and lipid peroxidation[3]thus inactivating antioxidant defence. Plants are the major natural source of rich anti oxidants. There are various methods to analyse the anti oxidant property of plants. Among all the methods we have taken the MSG induced model. Actually mono sodium glutamate is the chemical agent which widely used for the screening of anti-obesity property. But we were taken this chemical to test not only anti obesity activity but also in-vivo anti oxidant property. These intrinsic antioxidants protect the organism from oxidative stress and shows first line defence[4].

*** Corresponding Author**
E.mail: tej.pharma01@gmail.com

Material and Methods

Plant and plant material

Sapindus emarginatus whole plants materials were collected from Tirupathi. The plant authentication was done by Department of Botany, Sri Venkateshwara University, Tirupathi dist. Chittoor, Andhra Pradesh, and the voucher was preserved.

Preparation of the extract

The flowers of Sapindus emarginatus were taken and pericarps were collected and those are subjected to shade drying for 2-3 days.After all the pericarps were dried they were powdered in mixture and sieved. The fine powder was taken and weighed. From the powder 75-100gms of powder was suspended in the 150-200ml of methanol and continued for extraction for 24 hours at 72 degrees of temperature. After the extraction the solvent was evaporated by using rotary evaporator and the residue was dried[5].

Animals

Healthy adult albino rats of Wister strain of either sex between the age of 2-3 months and weighing 150-200 grams were used for the present study. The animals were housed individually in polypropylene cages, maintained under standard conditions (12 hours light and12 hours dark cycle, 23±5°C and 40-60% humidity). They were fed with standard rat pellet diet (National Institute for Nutrition, Hyderabad) and provided water ad libitum. All the animals are collected from central animal house SICRA LABS PVT.LTD,

IDA-KUKATPALLY, HYDERABAD and all experiments were conducted according to the ethical norms approved by CPCSEA, Ethical committee IAEC reg. no. (769/2011/CPCSEA).

Preliminary phytochemical screening

The methanolic extract of pericarps of flowers of Sapindus emarginatus contains huge amount of saponins[6].Normally the plant Sapindus emarginatus consists of various phytochemical constituents such as flavonoids, Triterpenoids, glycosides, carbohydrates, fatty acids, phenols, fixed oil, and saponins[7].

Induction of monosodium glutamate to produce obesity

In the experimental procedure the preliminary step is to induce the monosodium glutamate. Five groups of Wistar albino variety of rats of both sexes were taken to evaluate the antioxidant activity. Total 30 rats of weighed varying between 150-200gms were taken and divided in to five groups; each group consists of 6 Rats. Among all five groups except group-I, remaining all the four groups were administered with 8mg/gm of monosodium glutamate orally up to 7 days.

Experimental procedure

The rats were divided into six groups with six rats each:

Group I: Normal Diet Fed or Normal

Group II: Obesity control (8mg/gm monosodium glutamate)

Group III (standard group): Monosodium glutamate + Standard Drug Orlistat (50mg/kg body wt.).

Group IV (test-1-200mg/kg):8mg/gm monosodium glutamate+200mg/kg test drug

Group V (test-2-400mg/kg): 8mg/gm monosodium glutamate+400mg/kg test drug.

All the drugs were given through oral route. After the administration of MSG for 7 days from 8$^{th\ day}$ to 28 days the Rats in group-III, IV and V were treated with standard and test drugs with different drug doses respectively. The day 8th is considered to be day-1 after 7days of MSG induction. From this day 28 days were considered as screening days and drug given through oral route to group-III, IV and V. Group-I animals served as normal control, treated with vehicle (gum acacia 3% solution). Daily body weight and behavioural changes were examined and recorded. After completion of 28 studies the animals were sacrificed and before that the blood is collected from each rat from all the five groups for biochemical estimation. The liver, kidney and spleen were dissected out, washed in ice cold saline and weighed. Liver tissues were homogenized and used for the analysis of hepatic lipids and antioxidants[8].

***In-Vivo* anti-oxidant study**

As mentioned above the total study of antioxidant activity in MSG induced obesity rats was 28 days after 7 days of oral treatment of MSG.After 28 days of drug treatment(standard and two test doses) the 29th day all the animals are sacrificed and blood and liver,kidney,spleen are collected and homogenised. Till the completion of total study the everyday animals' weight and behavioural should be observed. After the scarification livers of rats from each group was isolated and it is subjected to homogenisation. The 10%w/v homogenate was prepared by using 0.15M KCI, centrifuged at 800 g for 10 min at 4°C[9]. The supernatant fluid which is obtained after centrifugation was used for the estimation of GSH, SOD, and Catalase.

Estimation of *In-Vivo* anti oxidant parameters

Three intrinsic anti oxidants called SOD, GSH, Catalse were estimated in liver tissue to assess the oxidative stress.

Estimation of superoxide dismutase

The estimation of SOD was done by oxidation of hydroxylamine at pH 10.2, which was done by the reduction of NBT by Mc cord and fridowich, and the nitrite produced in the presence of EDTA was detected colorimetrically[10].

Estimation of Catalase

To estimate the catalase activity by the method of Aebi[11] by measuring the rate of decomposition of hydrogen peroxide at 240 nm.

Estimation of GSH

The level of GSH was estimated as total non protein sulphydryl group by the method of Moron[12]. Free endogenous-SH was assayed in a total volume of 3 ml by the addition of 2 ml of 0.6 mM 5, 5 dithio-bis (2-nitrobenzoic acid) (DTNB) prepared in 0.2 M phosphate buffer (pH 8.0) to 0.1 ml of the supernatant and the absorbance was recorded at 412 nm using a UV-VIS spectrophotometer. Reduced GSH was used as a standard in the estimation.

Statistical analysis

The results are expressed as the mean ± standard error. The data from biochemical determinations were analyzed using the Student's t- test. Comparisons between the treatment groups and control groups were performed by one-way analysis of variance (ANOVA) followed by Dunnett's test. In all the tests the criterion for statistical significance was $P<0.05$ (95% level). P value<0.05 is considered as significant (*$P<0.05$, **$P<0.01$).

Results and Discussion

After the treatment with the methanolic extract of pericarps of flowers of Sapindus emarginatus of two

test doses that were 200mg/kg,b.w,400mg/kg b.wt. In MSG induced obesity rats shows significant results. After the completion of 28 days the five groups rat's were sacrificed and their livers were collected and the homogenised liver extract was subjected to test for the antioxidant parameters like SOD, GSH, Catalse. The test group-2 has shown the good anti oxidant property when compared with test group-1.The obtained results were compared with that of control group.

Generally obesity is the disorder which is associated with a decrease in tissue or plasma antioxidant capacity. GSH constitutes the first line defence mechanism in the liver, against the free radicals.SOD plays a vital role in the catalytic decomposition of superoxide free radical. On treatment with the methanolic extract of pericarps of flowers of Sapindus emarginatus of two test doses significant increase of SOD, GSH, and CAT were observed when compared with the control group to the drug treated group. The test drug significantly acts upon the oxygen free radicals and showed good anti oxidant property.

Conclusion

Due to the presence of active chemical constituents like saponins in the methanolic extract of pericarps of flowers of Sapindus emarginatus, it had shown the significant increase in the intrinsic anti oxidants in the liver tissue when compared with the control group. The obtained results have showed some scientific evidence that the methanolic extract of pericarps of flowers of Sapindus emarginatus was having the property of "anti oxidant".

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References

1. J. Srikanth and P. Muralidharan Evaluation of Antioxidant Properties of Sapindus emarginatus Vahl. *Asian J. Exp. Biol. Sci*., 1 (3) 2010, 693-699.
2. Supriya K, SaritaKotagiri, Vrushabendra Swamy BM, Archana Swamy P, Vishwanath KM, Anti-Obesity Activity of *Shorea robusta* G. Leaves Extract on Monosodium Glutamate Induced Obesity in Albino Rats, *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 3(3), 2012, 555.
3. Romero FJ, Morell FB, Romero MJ, Jareno EJ, Romero B, Marin N. Lipid peroxidation products antioxidants in human disease. *Environ Health Persp*1998; 106: 1229– 1234.
4. Sandhir R,Gill KD,Hepatoprotective effects of Liv-52 on ethanol induced liver damage in rats.Ind.journal of exp.biology,1999,37:762-6.
5. Kokate CK. Handbook of Practical Pharmacognosy. 4th Ed. New Delhi, India: VallabhPrakashan; 1994.

A. Sharma, S.C. Sati, O.P. Sati, D. Maneesha, S.K. Kothiyal, Chemical constituents and Bio Activities of Genus Sapindus. *IJRAP*, 2011, 2(2), 403-409

6. T. Deepa, R. Elamanthi, R. Kavitha, Kamalakannan, S. Sridhar, J. Suresh Kumar, Screening for Physical Phytochemical and Antimicrobial Activities of leaf extracts of Sapindus emarginatus Vahl, *International Journal of PharmTech Research*, 2012, 4(1), 392-397.
7. Atesh K, Karthiga D, Brindha P, Antiobesity effect of aqueous fruit extract of Carica papaya.L in rats fed on high fat cafeteria diet. *Int. Journal of Pharmacy and Pharmaceutical Sciences*, 4(5), 2012, 327-330.
8. S.Divya Teja.Banda, Firasat Ali, D. Suneetha, In-Vivo Anti-Oxidant of "ATH-2K13" in Normal and CCl4 Induced Rats, *IJTPR*, 2013, 5(3), 55-58.
9. Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. *Arch Biochem Biophys*, 1978; 186:189–95.
10. Aebi H, Catalse estimation in methods of enzymatic analysis, (Academic press, New York,) 1974, 673.Adams L.S., Zhang Y., Seeram N.P., Heber D. and Chen S. (2010). Pomegranate ellagitannin-derived compounds exhibit antiproliferative and antiaromatase activity in breast cancer cells *in vitro*. *Cancer Prevention Research*, 3 (1): 108-113.
11. Moron MA, Depierre JW, Mannervick B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver.*Biochem BiophysActa*, 1979; 582:67-78.

Table 1: Effect of methanolic extract of pericarp of flowers of *Sapindus emarginatus* on body weight in MSG induced obesity rats

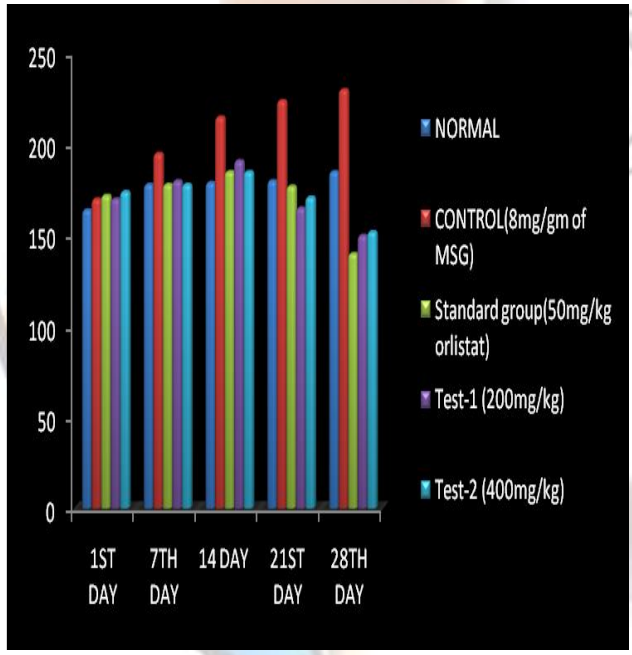
S/No.	GROUP	1ST DAY	7TH DAY	14 DAY	21ST DAY	28TH DAY
1.	Normal	164±4.2	178±3.1	179±2.5	180±3.0	185±3.2
2.	Control (8mg/gm of MSG)	170±3.1	195±2.5	215±3.3	224±1.8	230±1.4
3.	Standard group (50mg/kg orlistat)	172±3.3	178±2.0	185±4.1	177±2.4	140±3.0**
4.	Test-1 (200mg/kg)	170±2.9	180±3.3	191±2.6	165±2.6	150±2.8***
5.	Test-2 (400mg/kg)	174±3.6	178±3.7	185±2.4	171±2.8	152±1.5***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01 and * P<0.05.

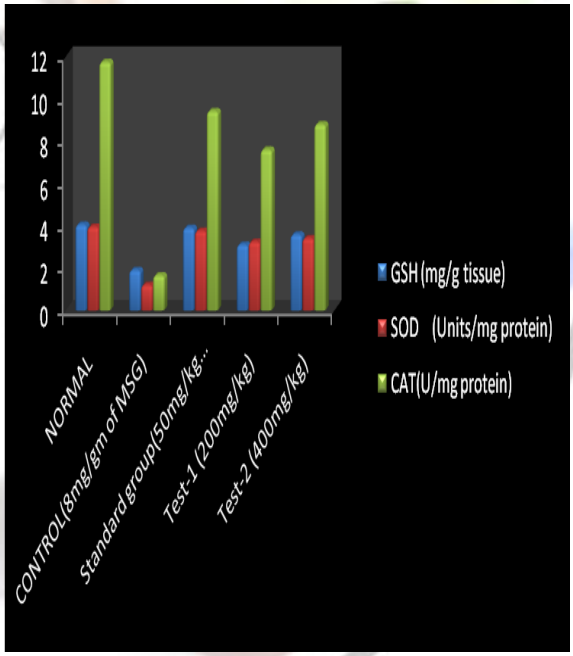
Table 2: Effect of methanolic extract of pericarps of flowers of Sapindus emarginatus on hepatic antioxidants

S/No.	GROUP	GSH (mg/g tissue)	SOD (Units/mg protein)	CAT (U/mg protein)
1.	Normal	4.05±1.1	3.95±0.75	11.75
2.	Control (8mg/gm of msg)	1.9±0.54	1.2±0.76	1.65±0.9
3.	Standard group(50mg/kg orlistat)	3.9±1.22	3.76±1.04	9.42±.0.64
4.	Test-1 (200mg/kg)	3.1±1.42**	3.24±0.8**	7.6±0.9*
5.	Test-2 (400mg/kg)	3.58±1.21**	3.41±1.3**	8.8±0.15*

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01 and * P<0.05.



Graph 1: Effect of methanolic extract of pericarps of flowers of *Sapindus emarginatus* on body weight in MSG induced obesity rats



Graph 2: Effect of methanolic extract of pericarps of flowers of *Sapindus emarginatus* on hepatic antioxidants