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# Study on Antioxidant Potential of *Murraya koenigii* Leaves in Wistar Rats

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**Abstract:** The antioxidant activity of *Murraya koenigii* (L.) Spreng (Family: Rutaceae), leaves was investigated in male wistar rats. Potassium dichromate was used to induce oxidative stress. The traditional medical literature describes its potential role as a source of many vitamins, flavonoids, phenols and domestic remedy for many human disorders. The whole plant is considered to be tonic, antidiarrhoeal, febrifuge, blood purifier and as stomachic. In the present study animals were divided into four groups Group I (Control), Group II (Potassium dichromate), Group III (*Murraya koenigii*+Potassium dichromate) and Group IV (*Murraya koenigii*). *in vivo* antioxidant activity of *Murraya koenigii* inhibited the toxicity of potassium dichromate. The GSH content in liver (1.79±0.019) and kidney (1.967±0.013) of Group IV rats significantly ($p<0.05$) increased whereas, hepatic malondialdehyde content in liver (2.44±0.29) and kidney (2.34±0.057) was significantly ($p<0.05$) reduced as compare to control. However, Chromate significantly ($p<0.05$) decreased the reduced glutathione (GSH) content and increases hepatic malondialdehyde (MDA) content in both liver and kidney as compared to control. Further post treatment with *Murraya koenigii* (Group III) significantly ($p<0.05$) increase the GSH content in liver (1.54±0.013) and kidney (1.27±0.011) as compared to Group II whereas, hepatic malondialdehyde content in liver (4.24±0.71) and kidney (3.86±0.038) was significantly ($p<0.05$) reduced as compare to Group II(Potassium dichromate) These results clearly indicate that *Murraya koenigii* leaves have significant potential as a natural antioxidant agents.

**Key words:** Antioxidant activity, reduced glutathione, potassium dichromate, malondialdehyde

## INTRODUCTION

Medicines derived from natural plants are known as phytomedicines which are clinically safe and effective due to lesser number of side effects and effective therapeutic index. Thus the use of herbal medicine now the days has been increased (Kavimani, 2000). *Murraya koenigii* commonly known as curry leaf plant belong to family Rutaceae is a small aromatic deciduous shrub (up to 6 m) available throughout India up to an altitude of 1400-1500 m. The plant is found in forest and is cultivated for its medicinal aromatic leaves. The plant is used as tonic, carminative and used internally in dysentery and diarrhea (De Britto *et al*., 2012; Vohra and Gupta, 2011). Crushed leaves are used externally to cure burns and paste of leaves is applied externally to treat the bites of poisonous animals (Kesari *et al*., 2005). Fresh juice of the root is taken to relieve pain associated with kidney and are applied externally for skin eruptions and to treat the bites of poisonous animals (Muthumani *et al*., 2009). It is also useful in leucoderma, blood disorders and possesses antioxidant, antibacterial, anticarcinogenic, hypoglycemic, hypolipidemic, antimutagenic, anti-inflammatory properties (Mhaskar *et al*., 2000; Iyer and Devi, 2008). The plant has been reported for antimicrobial and antiulcer activity also (Srinivasan, 2005). Leave part contain constituents like mahamimbicine, icyclomahanimbicine, phebalosin, coumarine as murrayone imperatoxin and triterpenoids alkaloids like cyclomahanimbine, tetrahydromahanimbine, girinimbin, koenine, iso-mahanimbine and koenimbine (Rastogi and Mehrotra, 1980; Kureel *et al*., 1969; Schmidt *et al*., 2012; Rageeb *et al*., 2012).

Free radicals are capable of inducing lipid peroxidation which induces damage in biological membranes. Antioxidants are the molecules which inhibit the generation of free radicals and are protective in disorders like ageing, atherosclerosis, lipofuscinosis, oxygen toxicity and liver injury (Iyer and Devi, 2009; Smerq and Sharma, 2011). Natural plants having constituents like flavanoids and phenols' exhibit antioxidant activity (Kumar *et al*., 2008; Pourmorad *et al*., 2006). *Murraya koenigii* is one of important Plant which has been very frequently used traditionally moreover antioxidant activity of *Murraya koenigii* leaves has not been evaluated yet. Our aim of the present study was to evaluate antioxidant potential (*in vivo*) of *Murraya koenigii* leaves by using wistar rats (Shafiq-Ur-Rehman, 1984; Sedlak and Lindsay, 1968).

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## MATERIALS AND METHODS

**Collection of plant material:** Fresh plant leaves were collected from the vicinity of Palampur, (HP) India. Plant material was shade dried and then powdered (at low temp.) using blender and stored in air tight bottles.

**Preparation of extract:** Powdered drug material was extracted using distilled water in a conical flask and plugged with cotton wool. After 24 h the extract was filtered using muslin cloth and filtrate was collected and finally lyophilized. The crude extract so obtained was stored at 4°C.

**Experimental animals:** Male wistar rats weighing 150-200 g were used in the study. A total of one hundred, rats were procured from IIIM Jammu and were kept in the Experimental Animal House of the Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Palampur (Himachal Pradesh), under standard lab condition. Experiment protocol was approved by Institutional Ethical committee of College of Veterinary and Animal Sciences, CSKHPKV, Palampur, H.P, India.

**Housing and maintenance:** Before arrival of the rats, the experimental animal house was thoroughly disinfected. The cages and water containers were washed with water and potassium permanganate solution and the edges were flamed with blowlamp. The animal house was fumigated with potassium permanganate crystals mixed in formaldehyde. All the rats were examined for any abnormality and ill health. They were acclimatized to the new environment for the first two weeks. Animals were kept under 12 h light/dark cycle and controlled temp. (24±2°C) and fed with feed obtained from Department of Animal Nutrition, COVAS, C.S.K.H.P.K.V.V,Palampur, India.

**Experimental design:** Wistar rats were divided into four groups containing six rats each. The lyophilized extract of *Murraya koenigii* leaves was given orally to the rats at dose rate of 250 mg $kg^{-1}$ b.wt.

**Experimental procedures:** Animals were sacrificed on day 32 and their organs were collected and stored at low temp. for further analysis.

**Preparation of tissue homogenate:** The frozen tissue samples (liver and kidney) were partially thawed and 500 mg of the tissue sample was triturated using 5 mL of ice-cold saline.

**Lipid peroxidation (LPO):** The extent of lipid peroxidation determined by the Thiobarbituric Acid (TBA) method of Shafiq-Ur-Rehman (1984). The homogenate was centrifuged at 3000 rpm for 10 min. The supernatant was used for estimation of lipid peroxidation. One mL of tissue homogenate was incubated at 37°C for 2 h. To each sample, 1 mL of 10% TCA was added. This was mixed thoroughly and centrifuged at 2000 rpm for 10 min. To 1 mL of supernatant, an equal volume of 0.67% TBA was added and kept in boiling water bath for 10 min. The reaction mixture was cooled and diluted with 1 mL of distilled water. The absorbance was read at 535 nm. The amount of lipid peroxidation was expressed as nM Malondialdehyde (MDA) formed per gram of tissue.

**OD total volume of reaction mixture:**

$$\text{LPO (nM MDA gm}^{-1}) = \frac{\text{OD}}{\text{EC}} \times \frac{\text{Total volume of reaction mixture}}{\text{Amount of sample taken}} \times 10^{9} \times \text{DF} \times \text{IT}$$

Where:
OD = Optical density
EC = Extinction coefficient ($1.56\times10^{8}\ M^{-1}cm^{-1}$)
DF = Dilution factor
IT = Incubation time

**Reduced glutathione (GSH):** GSH was assessed by estimating free-SH groups, using DTNB method of Sedlak and Lindsay (1968). 10% of tissue homogenates were used for GSH estimation. One mL of tissue homogenate, comprising 0.8 mL of water and 0.2 mL of 50% TCA was incubated at room temperature for 15 min. The mixture was centrifuged at 3000 rpm for 15 min. From this, 0.4 mL of supernatant was taken and 0.8 mL of 1 M tris buffer was added followed by 0.2 mL DTNB (0.01 M). The absorbance was recorded at 412 nm within 5 min. The sample blank was also run sidewise.

**Calculation:** Results were expressed as mM of GSH $g^{-1}$ of the wet tissue:

$$\text{GSH (mM gm}^{-1}) = \frac{\text{OD}}{\text{EC}} \times \frac{\text{Total volume of reaction mixture}}{\text{Amount of sample taken}} \times 1000 \times \text{DF} \times \text{IT}$$

Where:
OD = Optical density
EC = Extinction coefficient
DF = Dilution factor
IT = Incubation time

## RESULTS

The antioxidant effect of *Murraya koenigii* leaves extract and potassium dichromate on lipid peroxidation

Table 1: Experimental groups to evaluate antioxidant potential of *Murraya koenigii* in rats

| Groups | Treatment | No. of rats | Dose of *Murraya koenigii* (mg $kg^{-1}$ b.wt.) | Potassium dichromate (mg$kg^{-1}$ b.wt.) |
|---|---|---|---|---|
| I | Control | 6 | - | - |
| II | Potassium dichromate | 6 | - | 30 |
| III | *Murraya koenigii* +Potassium dichromate | 6 | 250 | 30 |
| IV | *Murraya koenigii* | 6 | 250 | - |

Table 2: Effect of *Murraya koenigii* leaves extract on liver and kidney lipid peroxidation (nM MDA/g) in rats

| S.No. | Groups | Liver | Kidney |
|---|---|---|---|
| I | Control | 3.19±0.17$^{a***}$ | 3.24±0.043$^{a***}$ |
| II | Potassium dichromate | 4.37±0.34 | 4.96±0.045 |
| III | *Murraya koenigii* +potassium dichromate | 4.24±0.71$^{a**}$ | 3.86±0.038$^{a**}$ |
| IV | *Murraya koenigii* | 2.44±0.29$^{b***}$ | 2.34±0.057$^{b***}$ |

Values expressed as Mean±SEM for six rats in each group, a: p<0.05 vs chromate, b: p<0.05 vs control, Where *represents p<0.05, **represents p<0.01, ***represents p<0.001

Table 3: Effect of *Murraya koenigii* leaves extract on liver and kidney reduced glutathione (nM/g) in rats

| S. No. | Treatment group | Liver | Kidney |
|---|---|---|---|
| I | Control | 1.72±0.074$^{a***}$ | 1.31±0.022$^{a**}$ |
| II | Potassium dichromate | 1.47±0.031 | 1.20±0.012 |
| III | *Murraya koenigii* +potassium dichromate | 1.54±0.013$^{a**}$ | 1.27±0.011$^{a*}$ |
| IV | *Murraya koenigii* | 1.79±0.02$^{b***}$ | 1.96±0.012$^{b***}$ |

Values expressed as Mean±SEM for six rats in each group. a: p<0.05 vs chromate, b: p<0.05 vs control where *represents p<0.05, **represents p<0.01, ***represents p<0.001

and glutathione (GSH) was evaluated in liver and kidney of rats. The experimental groups and its dose (mg $kg^{-1}$ b.wt.) to evaluate antioxidant potential (*in vivo*) in rats were shown in Table 1. In case of lipid peroxidation the hepatic malondialdehyde content in liver (2.44±0.29) and kidney (2.34±0.057) of Group IV rats was significantly (p<0.05) reduced whereas, chromate increases MDA content in both liver and kidney on the comparison of both the groups with control (Group I). Further post treatment with *Murraya koenigii*, the hepatic malondialdehyde content in liver (4.24±0.71) and kidney (3.86±0.038) was significantly (p<0.05) reduced as compare to Group II (Potassium dichromate) as shown in Table 2. *In vivo* antioxidant activity of *Murraya koenigii* inhibited the toxicity of potassium dichromate.

In case of glutathione (GSH) the content of GSH in liver (1.79±0.019) and kidney (1.967±0.013) of Group IV rats significantly (p<0.05) increased whereas, hepatic malondialdehyde content in liver (2.44±0.29) and kidney (2.34±0.057) was significantly (p<0.05) reduced as compare to control. However, Chromate significantly (p<0.05) decreased the GSH content and increase MDA content in both liver and kidney as compared to control. Further post treatment with *Murraya koenigii* (Group III) significantly (p<0.05) increase the GSH content in liver (1.54±0.013) and kidney (1.27±0.011) as compared to Group II. whereas, hepatic malondialdehyde content in liver (4.24±0.71) and kidney (3.86±0.038) was significantly (p<0.05) reduced as compare to Group II (Potassium dichromate) as shown in Table 3.

## DISCUSSION

Research was conducted over last few years to promote the antioxidant nutritional medicine in the nutraceutical field (Gill *et al*., 2010). The antioxidant activity of various extracts of *Murraya koenigii* has been already described but little was known on the potent *in vivo* antioxidant activity of aqueous *Murraya koenigii* extract. Present work demonstrated that the aqueous extract possesses significant antioxidant properties as revealed by lipid peroxidation and reduced glutathione methods. Normally free radicals are generated because of heat, cold, ionizing radiations, drugs, pollutants which cause oxidative damage (Milan *et al*., 2011). The living system therefore constantly quenches leaked and free oxidative species through various mechanisms such as GSH, LPO, SOD etc (Sharma *et al*., 2012).

Recently, free radical induced lipid peroxidation has gained much importance because of its involvement in several pathologies such as ageing, wound healing, oxygen toxicity, liver disorders, inflammation etc. Many natural and synthetic antioxidants are in use to prevent lipid peroxidation (Gupta and Sharma, 2010).

GSH is an important factor in this defence system. The mechanism of action by which GSH system work is scavenging of free radicals, donation of hydrogen molecules to restore damaged molecules and peroxide reduction (Bump and Brown, 1990). It is known that GSH, together with GSHPx, is involved in a cellular defence system against peroxidation. The increase in GSH content may be due to an exchange reaction between the protector and protein bound GSH and that of protein-bound GSH might play a role in the protection of cells against radiation (Rotruck *et al*., 1973).

Iyer and Devi (2009) reported that MK extract increased the GSH and cause a reduction in radiation induced lipid peroxidation in the liver of mice. Smerq and Sharma (2011) reported that transfer of electrons, metal chelating activity and other various factors are responsible for antioxidant activity of *Murraya koenigii*.

## CONCLUSION

In the present *in vivo* study on male wistar rats clearly indicate that *Murraya koenigii* leaves has potent

antioxidant potential. Further studies has to be required in future to evaluate various activities from the isolated compounds of *Murraya koenigii* by using experimental animal models.

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