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FREE RADICAL SCAVENGING ACTIVITY OF *RAUWOLFIA SERPENTINA* RHIZOME AGAINST CCl_4 INDUCED LIVER INJURY

Ajay Kumar Gupta [*1], R. Irchhaiya [2], H. R. Chitme [3] and Neelam Misra [4]

University Institute of Pharmacy [1], C.S.J.M. University, Kanpur - 208024, Uttar Pradesh, India.
Institute of Pharmacy [2], Bundelkhand University, Jhansi - 284128, Uttar Pradesh, India
Oman Medical College [3], Bowshar campus - Muscat, Sultanate of Oman.
Department of Chemical Sciences [4], College of Natural Sciences, Crescent University, Abeokuta, Nigeria.

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Correspondence to Author:
Dr. Ajay Kumar Gupta

University Institute of Pharmacy, C.S.J.M. University, Kanpur - 208024, Uttar Pradesh, India.

E-mail: ajaympgupta@yahoo.com

ABSTRACT: *Rauwolfia serpentina* has a great reputation as a miracle medicinal plant, reported in various texts of the indigenous system of medicine. As, free radicals and diseases have great correlation; therefore, in the present study, free radical scavenging activity of methanolic extract (MET) of rhizomes of mature *Rauwolfia serpentina* was investigated by using CCl_4 induced hepatotoxicity model in albino rats. The MET exhibited significant free radical scavenging activity by showing increased levels glutathione peroxidase (GPX), glutathione-S-transferase (GST), glutathione reductase (GRD), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and decreased the level of lipid peroxidation (LPO). The MET, at a dose level of 400 mg kg^{-1} showed significant free radical scavenging activity against CCl_4 induced liver injury in rats. MET of *R. serpentine* had been shown prominent antioxidant activity and CCl_4 intoxicated liver recovery towards normalcy.

INTRODUCTION: Free radicals are found to be responsible for various pathological conditions and to overcome from such deleterious effects of free radicals, an effective natural product with excellent antioxidant potential may be the one of the solutions. Ingested food, stress, drugs, environmental toxicants, etc. are the recognized sources of these free radicals affecting mainly the liver indirectly through free radical chain reactions and direct injury [1].

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Biotransformation of carbon tetrachloride produces hepatotoxic trichloromethyl radical and peroxy radical similar to the pathophysiology of many chronic diseases [2]. Therefore, in this study CCl_4 has been used for the evaluation of free radical scavenging activity of *Rauwolfia serpentina*.

Rauwolfia serpentine commonly known as *Rauwolfia*, *Rauvlfia*, Indian Snakeroot, Sarpgandha belonging to the family Apocynaceae is an erect, evergreen shrub. It has been used in India for at least 3000 years and reported in various texts of the indigenous system of medicine like Ayurveda, Siddha, and Unani. It has been long used in for treating many diseases and disorders including mental illness, as an antidote to the bites of poisonous reptiles like snakes, *etc* [3]. Monograph published by WHO reports that it contains more

than 60 indole alkaloids [4]. Many chemical constituents especially isolated alkaloids including Ajmalicine, ajmaline, isoajmalium ajmalicine, isoajmaline, chandrine, rouwolfine, renoxidine, rescinnamine, reserpine, rescinnamine, reserpine, deserpidine, sarpagine, serpentine, serpentinine, tetraphyllicine, yohimbine, rauwolscine, 3-epi-α-yohimbine are identified along with their structural illustration for major diseases and disorders [1].

It is being pronounced as the "Wonder drug of India" in 1949 when the British Heart Journal reported the plant to be "clinically effective in treating high blood pressure."

The purpose of present work was to explore the possibilities *in-vivo* antioxidative and hepato-protective *in-vivo* mechanism of methanolic extract of rhizomes of *Rauwolfia serpentina* using biochemical parameters in CCl_4 induced hepatotoxicity models in rats.

MATERIALS AND METHOD:

Plant Material and Extract: Shade-dried coarsely powdered (1 kg) rhizomes of *Rauwolfia serpentina* were extracted with methanol by using soxhlet extractor. After drying the methanolic extract under reduced pressure using a rotary evaporator and kept under refrigeration. The extract was administered to the animals as a suspension in propylene glycol.

Animals*:* Six male albino rats (150-200 g) in each group were used in all sets of experiments, and animal experiments were performed according to rules and regulations of the Animal Ethical Committee, Government of India, in the animal house of Institute of Pharmacy, Bundelkhand University, Jhansi; where controlled conditions of light (12 / 12 h light / dark cycle), temperature (25 ± 1°C) and relative humidity were maintained. Food pellets (DRDE, Gwalior) and tap water were provided *ad libitum*. For the experiment, animals were kept fasting overnight but were allowed free access to water.

Acute Oral Toxicity: Acute oral toxicity of *Rauwolfia serpentina* was performed as per OECD/OCDE/420/423. LD_{50} of *Rauwolfia* is ranging from 690 to 2000 mg/kg, depending upon the species, environment, and place of cultivation[5]. At a limit dose of 2000 mg/kg of MET of *R. serpentina*, lethality was observed, one animal died while one extra animal was seriously affected, survived animals showed some behavioral changes and decreased locomotion. Therefore, the next lower dose was tried and cut off dose of 1000 mg/kg was fixed.

Treatments: The adult albino rats (male) were divided into four groups of six animals each. Group, I served as control received only propylene glycol (2ml kg^{-1}, i.p., 10 days). Group II intoxicated with CCl_4 (CCl_4 in olive oil 1:9 v/v, final concentration 2 ml kg^{-1} i.p., on alternate days). Group III received a methanolic extract of rhizomes of mature *Rauwolfia serpentina* (400 mg kg^{-1} p.o., 10 days) and CCl_4 on 10^{th} day. Group IV served as standard and received silymarin (25 mg kg^{-1} p.o., 10 days) and CCl_4 on the 10^{th} day. All the animals were sacrificed by cervical dislocation under light ether anesthesia on the eleventh day.

Assay of Antioxidant Enzymes: Freshly collected livers were washed in cold saline, weighed and homogenates were prepared in 0.1 M Tris-HCl buffer (pH 7.5) containing 10mM EDTA, by using motor-driven Teflon-pestle. The supernatant was used for the assay of marker enzymes – GPX, GST, GRD. The absorbance was read at 340 nm [6-8].

SOD and CAT Activities: The activities of superoxide dismutase (SOD) and catalase (CAT) were determined by the modified method at 240 nm (decrease in absorbance) and 560 nm (decrease in optical density), respectively [9-10].

Lipid Peroxidation and Glutathione: Malondialdehyde (MDA), the most abundant aldehyde obtained after lipid peroxidation is a measure of LPO and it was estimated with slight modifications of the previous method. Absorbance read at 532 nm, and 2.5N mole MDA was used as the standard to calculate the amount of lipid peroxide in the samples and results were expressed as n mole of MDA/ml plasma. GSH contents were measured by the method of Beutler *et al*., 1963. Optical density was measured at 412 nm [11-12].

RESULTS: The concentrations of glutathione peroxidase (GPX), glutathione–S–transferase (GST), glutathione reductase (GRD), superoxide dismutase (SOD) and catalase (CAT) and glutathione (GSH) in animals treated with carbon tetrachloride were significantly reduced ($p<0.05$) in

homogenates of liver as compared with control animals. While thiobarbituric acid reactive substances of CCl_4 treated animals was significantly higher ($p<0.05$) than the control animals. Administration of 400 mg/kg MET of *R. serpentine*, significantly increased ($p<0.05$) the concentrations of GPX, GST, GRD, and GSH; liver SOD and CAT activities were also significantly increased ($p<0.05$) when compared to CCl_4 treated group. Similar increased antioxidant activities were also observed with silymarin treated group **Table 1**. On the other hand, the increased level of liver thiobarbituric acid reactive substances of CCl_4 treated animals was significantly reduced ($p<0.05$) in groups of animals receiving both MET of *Rauwolfia* and CCl_4 as well as in silymarin treated group **Table 1**.

TABLE 1: ANTIOXIDANT ACTIVITY OF MET OF RHIZOMES OF MATURE *RAUWOLFIA SERPENTINE* AGAINST CCL_4 INDUCED LIVER INJURY

Biochemical Parameters	Control	CCl_4 treated Group	MET of *R. serpentina* (400 mg/kg) + CCl_4	Silymarin (25 mg/kg) + CCl_4
GPX (nmole of GSH oxidized/min/mg protein)	440.06±4.68	160.15±2.87***	399.56±3.91***	406.56±2.63***
GST (nmole of CDND conjugate form/min/mg/ protein)	306.81±2.66	132.93±2.40***	292.13±2.58***	294.55±1.89***
GRD (nmole of GSSG utilized/ min/mg protein)	34.71±1.46	7.31±0.59***	29.80±1.39***	30.75±1.26***
SOD (units/mg protein)	69.33±1.45	22.53±1.34***	58.38±1.55***	69.40±1.90***
CAT (nmole of H_2O_2 decomposed/min/mg protein)	145.78±3.02	30.82±1.56***	137.05±0.83***	139.08±0.98***
LPO (nmole of MDA/mg protein)	4.47±0.47	15.18±0.85***	5.18±0.46***	4.87±0.26***
GSH (µmoles/mg protein)	8.16±0.46	1.45±0.39***	7.06±0.47***	7.35±0.32***

All data are expressed as mean ± SEM, n=6 (Results were analyzed by ANOVA test followed by Dunnett's t-test to confirm). Results are considered statistically significant when $p<0.05$

DISCUSSION AND CONCLUSION: Results of our study indicates that the levels of antioxidant enzymes were decreased along with GPX, GST, GRD, SOD, CAT, and GSH. The level of liver thiobarbituric acid reactive substance levels was increased in rats treated with CCl_4 due to trichloro-methyl radical (CCl_3O^*) and trichloromethyl peroxy radical (CCl_3OO^*) produced as a result of the metabolic conversion of CCl_4 in the liver and direct hepatocellular damage [13].

There is clear evidence that MET of *Rauwolfia serpentina* rhizome afforded protection from CCl_4 induced liver damage. Possible mechanisms that may be responsible for the protection of CCl_4 induced liver injury by rhizome extract possibly through; first, itself could act as a free radical scavenger intercepting those radicals involved in CCl_4 metabolism by microsomal enzymes. Its ability to inhibit rat hepatic microsomal membrane lipid peroxidation and to scavenge free radicals. Thus, by trapping oxygen-related free radicals rhizome extract could hinder their interaction with polyunsaturated fatty acids and would abolish the entrancement of lipids peroxidative processes leading to MDA formation [14].

Second; a significantly higher content GSH would afford the tissue better protection against oxidative stress, thus contributing to the abolishment of CCl_4 induced hepatotoxicity.[15] Third; its promising free radical scavenging activity may be due to its antioxidant and normalization of impaired membrane function activity. It is recently reported *in-vivo* antioxidant activity of methanolic crude extract of leaf of *Rauvolfia serpentine* [16], and aqueous ethanolic root extracts of *R. vomitoria* [17] also in support of present findings. Fourth; Its synergistic combination effect in preventing the process of initiation and progress of liver damage.[18] Therefore, our study results substantiate the *in-vitro* antioxidant results of previous studies [19-22].

For the efficacy and to ensure the safety of *Rauwolfia* for hepatoprotective and antioxidant activity, we need a uniform system of conducting pragmatic clinical trials with proper pharmacovigilance.

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CONFLICT OF INTEREST: Nil

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