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Anti-oxidant activity determination of *Clerodendron infortunatum* Linn by *in vivo* method

Mathew George*, Lincy Joseph[1], Leena PN [2]

*Professor, Department of Pharmacology, Pushpagiri College of Pharmacy, Thiruvalla, Kerala, India

[1]Professor, Department of Pharmaceutical Chemistry, Pushpagiri College of Pharmacy, Thiruvalla, Kerala, India

[2]Associate Professor, Department of Pharmacognosy, Pushpagiri College of Pharmacy, Thiruvalla, Kerala, India

Abstract

Objective: To evaluate the anti-oxidant activity determination of *Clerodendron infortunatum* linn by *in vivo* method.

Methods: Serum was analyzed for various biochemical parameters like serum glutamic oxaloacetic (SGOT), serum glutamic pyruvic transaminase (SGPT) activities and alkaline phosphatase. The total protein concentration and total bilirubin were also measured. All the analyses were performed. Estimation of lipid peroxidation (LPO), enzymic (CAT,) non-enzymic (GSH) antioxidant system by the livers were quickly removed, weighed and homogenized in phosphate buffer (0.1 M, pH 7.4) The homogenate was centrifuged at (1000 rpm , 15 min) to remove debris. The supernatant was used to assay the LPO, CAT and GSH activities. The percentage inhibition was calculated using the equation: %Inhibition $=A_0-A_1 /A_0$

Results: Ethanolic extract of *Clerodendron infortunatum* Linn in the dose of 200 mg/kg, p.o.,400mg/kg has improved the glutathione, SOD, catalase and peroxidase levels significantly, which were comparable with standard.

Conclusion: In the present study,plant *Clerodendron infortunatum*, were selected based on their ethnomedical uses. Ethanolic extract of *Clerodendron infortunatum* Linn has improved the glutathione, SOD, catalase and peroxidase levels significantly, which were comparable with standard.

Keywords: Anti-oxidant activity, *Clerodendron infortunatum*, Root, Liv 52, *In vivo* method

Introduction

Medicinal plant life normally comprises combinations of different chemicals which can act individually or additively to enhance fitness. The understanding of natural remedies in conventional cultures developed through trial and error over many centuries. The huge majority of people on earth nonetheless depend on these traditional medicinal vegetation and substances for regular healthcare needs. Quality control is the procedure that ensures safety and efficacy of phytomedicines by carefully checking. A) The correct identity of active ingredients: herbs/extracts (identifying plant parts), anatomical knowledge (identifying characteristic tissues), and phytochemical knowledge (identifying main components of plants).B) The correct (therapeutic) concentration of active ingredients: Standardization of phytomedicines is done to ensure that all batches contain the same specified concentration of active chemical compounds. The concentration is important because herbal medicines contain biologically active substances that produce non-trivial side effects, when taken in excessive doses. Very low dose would have no therapeutic value. For compounds with narrow therapeutic window, chemical entries are used instead of extracts. Example-cardiac glycosides. C) The purity and hygiene: The presence of soil and inorganic materials can be monitored by determining ash value of material (Total ash value fixed at 30% dry weight and acid soluble ash at not more than 1%). Microbiological tests are performed as a matter of routine to test for various microbial contaminants. In case of internal products, *Salmonella* and *Escherichia coli* must be negative while fungi should not exceed 104/105 g or ml respectively.

Oxidative stress is characterized as an imbalance between the production of reactive species and antioxidant defence hobby, and its superior nation has been associated with among the chronic diseases consisting of cancer, diabetes, neurodegenerative and cardiovascular diseases. Based on that, many research corporations have driven efforts to assess the antioxidant homes of natural products. These homes were investigated thru either chemical (*in vitro*) or biological (*in vivo*) techniques, or each. The effects of those researches have led some to indicate that the lengthy-term consumption of meals wealthy in antioxidants can retard or keep away from the incidence of such illnesses .according to Brewer the effectiveness of a huge variety of antioxidant

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agents is generally proportional to the number of hydroxyl (OH) companies present in their aromatic ring(s). Based on that, the herbal compounds might seem to have higher antioxidant pastime than the currently used synthetic antioxidants, making them a mainly attractive component for business meals. Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular harm despite the fact that the antioxidant defences are exclusive from species to species; the presence of the antioxidant defence is standard. Antioxidants exists each in enzymatic and non-enzymatic bureaucracy inside the intracellular and extracellular surroundings. Regular biochemical reactions, extended exposure to the surroundings, and better tiers of nutritional xenobiotics result in the era of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are answerable for the oxidative strain in first rate patho physiological situations.3 mobile additives of our body are altered in oxidative pressure conditions, resulting in several ailment states. The oxidative stress can be successfully neutralized with the aid of improving mobile defences inside the form of antioxidants. Certain compounds act as in vivo antioxidants by means of elevating the degrees of endogenous antioxidant defences. Expression of genes encoding the enzymes consisting of SOD, CAT, GSHPx will increase the extent of endogenous antioxidants.

Antioxidants may be labelled in a couple of approaches. Primarily based on their hobby, they'll be categorised as enzymatic and non-enzymatic antioxidants. The antioxidant enzymes convert dangerous oxidative products to hydrogen peroxide (H_2O_2) and then to water, in a multi-step system in presence of cofactors together with copper, zinc, manganese, and iron. Non-enzymatic antioxidants paintings by way of manner of interrupting loose radical chain reactions. Few examples of the non-enzymatic antioxidants are nutrition C, nutrients E, plant polyphenol, carotenoids, and glutathione. The alternative manner of categorizing the antioxidants is based on their solubility in the water or lipids. The antioxidants may be classified as water-soluble and lipid-soluble antioxidants. The water-soluble antioxidants (e.g. weight loss plan C) are gift in the cell fluids which encompass cytosol, or cytoplasmic matrix. The lipid-soluble antioxidants (e.g. nutrition E, carotenoids, and lipoic acid) are predominantly positioned in cell membranes. The antioxidants additionally may be labelled in step with their length, the small-molecule antioxidants and massive-molecule antioxidants. The small-molecule antioxidants neutralize the ROS in a system called radical scavenging and convey them away. The principle antioxidants on this category are nutrition C, vitamins E, carotenoids and glutathione (GSH). The massive-molecule antioxidants are enzymes (SOD, CAT, and GSHPx) and sacrificial proteins (albumin) that soak up ROS and save you them from attacking special essential proteins. To apprehend the mechanism of motion of antioxidants, it's far crucial to recognize the technology of free radicals and their destructive reactions. This examine elaborates the era and damages that free radicals create, mechanism of motion of the natural antioxidant compounds and assays for the evaluation of their antioxidant [1, 2, 4].

The response mechanisms of the antioxidant assays are mentioned

$$2O_2 + NADPH \xrightarrow{(oxidase)} 2O_2^{\cdot -} + NADP^+ + H^+$$

The O_2^- is then rapidly converted to H_2O_2

$$2O_2^{\cdot -} + 2H^+ \xrightarrow{(SOD)} H_2O_2 + O_2$$

H_2O_2 is converted to hypochlorous (HOCl), a potent oxidant and antimicrobial agent

$$Cl^- + H_2O_2 + H^+ \xrightarrow{(MPO)} HOCl + H_2O$$

Materials and methods

Collection and authentication of plant material

The root of *Clerodendron infortunatum* Linn, belongs to the family Verbenaceae was collected from Pathanamthitta District of Kerala. Taxonomical identification of the plants was done by Dr. Thomas Mathew, Marthoma College Thiruvalla and the voucher herbarium specimen was deposited in college museum as VSCI-13.

Preparation of the extract

Clerodendron infortunatum Linn, roots washed separately using water and extracted with 90% ethanol as solvent. The solvents were allowed to evaporate to obtain the dried extracts. It was then weighed and percentage yield was calculated. The extracts were stored properly in a desiccator for further studies. .

In vivo method

Animals

Wistar albino rats weighed about 150-200g were divided into seven groups of six rats each. Animals were housed at a temperature of 23 ± 2 ºC and relative humidity of 30–70%. A 12:12 light: day cycle was followed. All animals were allowed free access to water and fed with standard commercial rat chaw pellets. The study was approved by the Institutional Ethical Committee, which follows the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (KMCP/IAEC/279/2012-2013).

Experimental Design

Group I: Control group received normal saline in a dose of 10 ml/kg

Group II: Toxic control group received CCl_4 in a single of 1.5 ml/kg intraperitoneally

Group III: Standard group received Liv 52 in a dose of 25 mg/kg /day, p.o

Group IV: Received with EECi (200 mg/kg /day; p.o) + CCl_4 1.5 ml/kg intraperitoneally

Mathew George, E-mail:- leena09narayanan@gmail.com

Group V: Received with EECi (400 mg/kg /day; p.o) + CCl_4 1.5 ml/kg intraperitoneally

The animals of Group IV-V were given single dose of CCl_4, 1.5 ml/kg intraperitoneally, 6 h after the last treatment. On day 8 the rats were sacrificed by carotid bleeding and liver was rapidly excised, rinsed in ice-cold saline and a 10% w/v homogenate was prepared using 0.15 M KCl, centrifuged at 800 rpm for 10 min at 4 ºC. The supernatant obtained was used for the estimation of catalase, peroxidase, and other enzymes. Further, the homogenate was centrifuged at 1000 rpm for 20 min at 4 ºC and the supernatant was used for biochemical estimation.

Figure 1: Morphology of *Clerodendron infortunatum* Linn plant

Table 1: Physical evaluation of the extracts

Extract Description	*Clerodendron infortunatum*
Colour	Light yellow
Odour	Pleasant odour
Consistency	Semisolid mass
Method of extraction	Cold maceration
% Yield	65.07% w/w (Ethanol), 15.11% w/w (Pet ether), 65.14% w/w (Ethyl acetate), 64.17% w/w (Water)

Biochemical estimation

Serum was analyzed for various biochemical parameters like serum glutamic oxaloacetic (SGOT), serum glutamic pyruvic transaminase (SGPT) activities and alkaline phosphatase. The total protein concentration and total bilirubin were also measured. All the analyses were performed by using commercially available kits from Nice chemicals Ltd.

Estimation of lipid peroxidation (LPO), enzymic (CAT,) non-enzymic (GSH) antioxidant system

Tissue supernatant preparation for LPO, CAT, GSH assay

The livers were quickly removed, weighed and homogenized in phosphate buffer (0.1 M, pH 7.4). The homogenate was centrifuged at (1000 rpm, 15 min) to remove debris. The supernatant was used to assay the LPO, CAT, and GSH activities.

Determination of lipid peroxidation

Lipid peroxidation was estimated by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA). To 1 mL of supernatant, 0.5 mL of 30% trichloroacetic acid (TCA) was added followed by, 0.5 mL of 0.8% TBA. The tubes were kept in a shaking water bath for 30 min at 80 °C. After 30 min of incubation the tubes were taken out and kept in ice-cold water for 10 min. These were then centrifuged at 800 g for 15 min. The amount of MDA was assessed by measuring the absorbance of supernatant at 540 nm at room temperature against an appropriate blank. The percentage inhibition of Lipid peroxidation was calculated using the equation:

$\%Inhibition=A_0-A_1 /A_0$

Where, A_0 is the absorbance of the control without extract and A_1 is the absorbance of the sample extract.

Determination of catalase activity

To estimate the CAT activity by the reaction mixture consisted of 1.95 mL phosphate buffer (0.1 M, pH 7.4), 1.0 mL hydrogen peroxide (H_2O_2) (0.019 M), and 0.05 mL of supernatant in a final volume of 3 mL. Changes in absorbance were recorded at 240 nm. . The percentage inhibition of free radicals by CAT was calculated using the equation

$\%Inhibition=A_0-A_1 /A_0$

Where, A_0 is the absorbance of the control without extract and A_1 is the absorbance of the sample extract.

Determination of GSH activity

Reduced glutathione was determined by an aliquot of 1.0 ml of liver tissue supernatant was treated with 0.5 ml of Elman's reagent (19.8 mg of DTNB in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was measured at 412 nm. The percentage inhibition of free radicals by GSH was calculated using the equation

$\%Inhibition=A_0-A_1 /A_0$

Where, A_0 is the absorbance of the control without extract and A_1 is the absorbance of the sample extract [3, 5-7].

Results and discussion

Pharmacognostical studies play an important role in the standardization of plant material. In the present study, plant *Clerodendron infortunatum* Linn root were selected based on their ethnomedical uses and was authenticated. Their morphological and microscopical characters were determined. A detailed study of microscopic characters of the two plants along with the photographs serves in identifying the special characters of the plants.

The plant *C. infortunatum*, was collected from Pathanamthitta district of Kerala and identified by Thomas Mathew, HOD of Botany, Marthoma College Tiruvalla, Kerala. Voucher no. VSCI-13 was deposited in the Pharmacognosy department, Pushpagiri College of pharmacy, Tiruvalla. The root portion of

Mathew George, E-mail:- leena09narayanan@gmail.com

the plant was selected and washed with running water to remove soil and other matter and dried in shade for 20 d, powdered, extracted 1 kg with ethanol 90% by cold maceration extraction to yield the respective extracts. The extracts were reduced to molten mass by rotary vacuum evaporator and the yield was 65.07% w/w.

Biochemical parameters (SGOT, SGPT, total protein, total bilirubin) are shown in Table 3. The level of SGPT, SGOT, total protein, total bilirubin was restored towards the normal value in *Clerodendron infortunatum* Linn extract and isolated compound treated carbon tetrachloride intoxicated rats. Per oxidative degradation of cellular membrane due to CCl_4 induction causes functional morphological changes in it resulting in cellular leakage loss of functional integrity of the membrane. It was found from table 3 that in CCl_4 toxic control group by the substantial increase in the level of serum marker enzymes (SGOT, SGPT, and total bilirubin). The reduction of the level of total proteins in CCl_4 challenged animals is attributed to the damage produced localized in the endoplasmic reticulum which results in the loss of P450 leading to its functional failure with a decrease in protein synthesis. The rise in protein levels in the treated groups suggests the stabilization of endoplasmic reticulum leading to protein synthesis [8, 9].

Table 2: Preliminary phytochemical screening of petroleum ether, ethanol, ethyl acetate, water extract of *Clerodendron infortunatum* Linn

Chemical test	Petroleum ether	Ethanol	Ethyl acetate	Water
Carbohydrate	+	+++	++	++
Proteins	+	+	+	+
Amino acids	+	+	+	+
Fats and oils	+	+	+	+
Steroids	-	-	-	-
Cardiac glycosides	-	-	-	-
Anthraquinone glycoside	-	-	-	-
Saponins	+	+++	+++	+++
Flavonoids	+	+++	+++	+++
Alkaloids	-	-	-	-
Tannins and phenolic compounds	+	+++	+++	++

(**+++:** strong intensity reaction, ++: medium intensity reaction, +: weak intensity reaction, - : non detected)

Table 3 Effect of EECi extract on serum enzyme levels, total bilirubin and total protein of CCl_4 intoxicated rats

Groups	Treatment	SGPT (µ/ml)	SGOT (µ/ml)	Serum bilirubin (mg/ml)	Total protein (mg/ml)
I	Control	128.40 ± 0.0577	30.15± 0.0516	2.065 ± 0.0073	4.570± 0.0070
II	CCl_4 (0.7 ml/kg) i.p	249.2 ± 0.0365	58.33±0.0040	4.925± 0.0033	3.010± 0.0025
III	Standard Liv 52 (100 mg) + CCl_4	145.30±0.0166 ***	35.41± 0.0040 ***	2.310 ± 0.0036 ***	4.825 ± 0.0155 ***
IV	EECi (200 mg/kg) + CCl_4	135.55±0.0033 *	23.46± 0.0049 *	3.110 ± 0.0047 **	2.415± 0.0042 **
V	EECi (400 mg/kg)+ CCl_4	131.15± 0.3003**	21.53± 0.0055 **	3.970 ± 0.003 **	2.175 ± 0.0033 **

Data expressed as Mean±S.D, n=6 in each group. **P value<0.001, compared with toxic group. SGOT=Serum glutamate oxalo transaminase, SGOT=Serum glutamate pyruvate transaminase, TBL=Ttotal bilirubin levels, TPL= Total protein levels where the significance was performed by One way ANOVA followed by Dunnett's test.

Conclusion

Clerodendron species possesses multiple pharmacological and therapeutical activities such as anti microbial, anti inflammatory, anti malarial, anti diabetic, anti cancer, analgesic. However, there are limited numbers of studies describing the bioactivities of Clerodendron and this opens up new horizon for researchers working in this filed to explore this raw material as potent nutraceutical and pharmaceutical product.

The genus *Clerodendron L.* (Family: Verbenaceae) is very widely distributed in tropical and subtropical regions of the world. More than five hundred species of the genus are identified till now, which includes small trees, shrubs and herbs. Ethno-medical importance of various species of Clerodendron genus has been reported in various indigenous systems of medicines and as folk medicines. The genus is being used as medicines specifically in Indian, Chinese, Thai, Korean, Japanese systems of medicine for the treatment of various life threatening diseases such as syphilis, typhoid, cancer, jaundice and hypertension. Along with biological

Mathew George, E-mail:- leena09narayanan@gmail.com

studies, isolation and identification studies of chemical constituents and its correlation with the biological activities of the genus has also been studied. The major chemical components reported from the genus are phenolic, steroids, di- and tri terpenes, flavonoids, volatile oils, etc. From the study the antioxidant activity of *Clerodendron infortunatum* Linn root extract had significant effect when compared to the standard Liv 52.

References

1. Auudy B, Ferreira F, Blasina L, Lafon F, Arredondo F, Dajas R, Tripathi, P.C. Screening of antioxidant activity of three Indian medicinal plants traditionally used for the management of neurodegenerative diseases. J Ethanopharmacol 2003; 84: 131-138.
2. Cook NC, Samman S. Flavonoids-chemistry, metabolism, cardioprotective effects and dietary sources. Nutr Biochem 1996; 7: 66-76.
3. Conforti F, Loizzo MR, Statti GA, Menichini F. Comparative radical scavenging and anti diabetic activities of methanolic extract and fractions from *Achillea ligustica*. All Biol Pharm Bull 2005; 28: 1791-1794.
4. Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of *Spondias pinnata*. BMC Complement Alt Med 2008; 8: 63-75.
5. Karmakar I, Dolai N, Saha P, Sarkar N, Bala A, Haldar PK. Scavenging activity of *Curcuma caesia* rhizome against reactive oxygen and nitrogen species. Orient Pharm Exp Med 2011; 11: 221-228.
6. Ou B. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. J Agric Food Chem 2002; 50: 3122-3128.
7. Paz´dzioch-Czochra M, Widen Ska A. Spectrofluorimetric determination of hydrogen peroxide scavenging activity, Analytica Chimica Acta 2002; 452: 177-184.
8. Selvakumar K, Madhan R, Srinivasan G, Baskar V. Antioxidant assays in pharmacological research. Asian J Pharm Tech 2011; 1: 99-103
9. Warokar AS, Ghante MH, Duragkar NJ, Bhusari KP. Anti-inflammatory and antioxidant activities of methanolic extract of *Buchanania lanzan* Kernel. Indian J Pharm Educ Res 2010; 44: 363-368.

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Mathew George, E-mail:- leena09narayanan@gmail.com