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Evaluation of Methanolic Extract of *Colocasia Esculenta Linn* Hepatoprotective Activity in Wistar Rats

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ABSTRACT

***Objective**: The main aim of study was investigate the hepatoprotective activity of methanolic extract of Colcosia esculanta Linn in CCl_4 and cisplatin induced in rats.*
***Methods:** Methanolic extract of aerial part of Colcasia esculenta plant was studied for its hepatoprotective activity in experimental models. Hepatoxicity was induced by CCl_4. The standard drug was taken cisplatin 100 mg/kg. Test drugs were given extract of Colocasia esculanta Linn 500 mg/kg and 1000 mg/kg body weight.*
***Results:** In hepatoprotective activity, positive control group was provided with CCl_4 and increased SGPT, SGOT, ALP level compare to negative control group whereas test 2 group was provided with methanolic extract of Colocasia esculanta Linn 100 mg/kg decreased SGPT, SGOT, ALP level compare to standard group.*
***Conclusion:** On evaluating biochemical parameters it was found the methanolic extract of Colocasia esculanta Linn 100mg/kg showed hepatoprotective activity in wistar rats.*

***Keywords**: SGOT, SGPT, ALP, Hepatoprotective.*

INTRODUCTION

The liver is among the most complex and important organism in human body it lays below the diaphragm in the abdominal pelvic region of the abdomen. It is reddish brown organ with unequal shape and size and with four lobes and it is the largest internal organ and largest gland in human body in two largest vessels it is connected in which one known as hepatic artery and other one is called portal vein. It constitutes about 2.5% of an adult's body weight. Liver play a role in regulating various physiological processes. It is also involved in several vital functions, such as metabolism secretion and storage. It has a capacity to synthesize and detoxicate useful principle it helps to maintains regulating, maintenance and performance of homeostasis. It involved with all biochemical pathways to growth, nutrient supply, fight against disease, reproduction and energy provision. The liver injury that cause by chemicals is known as hepatotoxins. Certain medicinal agents is taken in overdose sometimes when produced in therapeutic ranges may injure chemical agent in laboratories (*e.g.* paracetamol carbon tetrachloride) and in industries like (*e.g.* lead, arsenic), natural chemicals (*e.g.* microcystins, aflatoxins) and (IFN), and herbal remedies (*Cascara sagrada*) induce hepatotoxicity. This chemicals agent are coverting in reactive metabolites in liver, it have ability to interconnect the cellular micro- molecules namely lipids, nucleic acid and protein, leading to dysfunction of protein, lipid dysfunction, peroxidation of lipid, damage DNA, and oxidative stress. This cellular function damage can dismiss in cell death and failure in liver [1]. The strategy in modern treatment having some of limitation. Silymarin is associated with

vomiting, headache and nausea. Hence, we found a good rationale beyond probing the hepatoprotective activity in our drug that is *Colocasia esculenta.*

MATERIALS AND METHOD

Animals Husbandry and Statutory Approval

The healthy male and female rats (*Wistar albino*) of 4-8 weeks old were selected after physical and behavioral veterinary examination from Institutional Animal House of Gupta College of Technological Sciences. The range fall within ±20% of body weight mean for each sex at the time of initiation and treatments. The Animals handling experiment complies with standard ethical standard of handling of animals and approved by Institutional Animal ethics committee (Approval number GCTS/IAEC/2019-Jan/01). All the animals were selected under acclimatized on the same day minimum five days will acclimatize before dosing initiation of dosing. In polpypropylene cage of stainless steel, top grill in a groups of six rats per cage. Paddy husk in clean autoclaved was used as bedding. At least thrice a week the paddy husk was changed. The clean environment was maintained for animals with 12 hour light dark circles The air was maintained at 55-65% with 100% exhaust and relative humidity at 22±3°C Librium was provided as standard pellet throughout the study, expect the overnight fasting prior to collection of blood after completion of all the animals feed was offered immediately. Drinking water was provided libitum in polypropylene bottles with stainless steel sipper tube throughout study period
Collection of plant;

COLLECTION OF PLANT

Rhizomes of *C. esculenta* were collected from Durgapur (W.B) India in September 2018 and were authenticated by the Head of Botany Department of Government College, Durgapur, West Bengal, India.

AUTHENTICATION

A herbarium sheet was prepared and it was send to head of Botany Department of Government College, Durgapur, West Bengal, India for authentication no of study plant is “Ref.No. Dgp/BOT/2018/35”.

EXTRACTION

It means treatment of animals or plants tissue with solvent, and the constituent that dissolve known as (menstrum), and most of the inert matter remains undissolved (marc). Leaves extraction depends largely on solubility, and functional group consideration.

SOXHLET EXTRACTION

This extraction was used in small volume in hot menstrum in drug time to dissolve the active constituent out until it exhausted this process is called soxihlation. This apparatus required for the hot percolation was made from a very high grade of glass and consists three parts:

a) The menstrum which was boiled in the flask.
b) Side tube and siphon in which drug was filled in the extracting chamber
c) A condenser. The drug to be extracted, in suitably comminuted from was unusual packed in a ‘thimble’ made of filter paper which was then placed into the wider part of extractor.

Thimble was used to prevent chocking of the lower part of extraction by drug particles. Thus, menstrum in the small quantity was made to soxhlet repeatedly; about 14-15 through drug and the constituents which were active in the flask was collect.

Successive Solvent Extraction

After the selection, collection and drying of rhizomes of *Colocasia esculanta Linn* extraction was done. In pharmacy the solvent in extraction purposes called menstrum and after extracting desired constituent the residue left after is known as marc. The effective extraction of plant materials depends largely on solubility, and functional group consideration. The powder rhizomes were subjected to cold maceration and successive Soxhlet extraction using various solvent and their polarity increase in petroleum ether, chloroform acetone and methanol. Before extracting each time with next solvent the powdered was dried in air oven below 50 degree centrigate. The extract was concentrated by distilling off the solvent and evaporating to dryness on water bath.

Extractive Value

It determines the method in which the amount of active constituent in a given amount of medical plant material when extracted with solvents. It employed for plant materials in which no chemical or biological assay method exists. The extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of phytoconstituents depends on nature of drug and solvent used. The use of single solvent can be the means of providing preliminary information about the quality of a particular drug sample [2-9].

Table 1. Extractive Values of Leaves of *Colocasia Esculanta Linn* In Different Solvents

Solvent Used	Extractive Value (%w/w)
Distilled water	12.46
Petroleum ether (60-80)	12.46
Chloroform	6.88
Ethyl acetate	9.84
Methanol	14.42

Preliminary Phytochemical Screening [10-11]

Preliminary tests were carried out for phytoconstituents presence or absence in Glycosides, Alkaloids, Flavonoids, Saponins, Sterols, Terpenes and Tannins in all the extracts by using the above four solvents individually. A description of methods adopted for performing the tests are summarized below.

Test for Alkaloids

The portion of extract was made acidic with dilute sulphuric acid. This portion which was divided was two parts and was tested with the following precipitating reagents

a) ***Mayer's Reagent:*** 1.36 gram of mercuric chloride dissolved in 60 ml of water, and was added Potassium iodide solution of 5 gram in 20 ml distilled water. They were mixed properly and volume was made upto 100 ml with distilled water. Precipitate of Buff colored considered as positive
b) ***Dragendorff's Reagent****:* 1 gram of bismuth subnitrate acetic acid of 20mlwas added to 20 gram of potassium iodide water in 100ml.Precipated of orange or brown colored appear as positive test.

Test for Carbohydrates

a) ***Molisch's test:*** It was performed for the conformation of carbohydrates. 1 ml of 10% acidic solution of a-napthol was added with extract and then mixed. Then concentration

of 1ml of sulphuric acid was carefully poured in the sides of the test tubes. In the juncture of two layers was considered as positive test.

Test for Glycosides

a) **Kedde test (for aglycone):** The evaporated extract was dried with one drops 90% alcohol and two drops of 2% dinitrobenzoic acid in 90% alcohol was added and the above mixtures was made with 20% Sodium hydroxide to alkaline to get purple colour. Appearance of purple colour showed the presence of free aglycone moiety.

b) **Keller-Killani Test (for sugar):** To the dried extract 0.4 ml acetic glacial acid contains trace Feric choloride was added. To the mixture, 0.5ml of concentrated H2SO4 added. Presence of sugar moiety indicates by green blue colour in the upper acetic acid.

Test for Flavonoids

a) The magnesium (dust) and concentrated HCL was treated with extract. The presence of flavonoids indicated by pink tomato colour.

b) **Shinoda's Test:** 5-10 drops of dilute hydrochloric acid were added to 0.5ml of extract. Magnesium in small piece was added to it. The appearance of pink, reddish pink or brown colouration was considered positive test.

The appearance of yellow, orange, red indicates by brick colour precipitate with lead acetate with dilute sulphuric acid extract of was 10ml hydrolyzed. This was extracted with ether and divided into two portions. 1 ml dilute ammonia solution was added to one portion, a greenish yellow colour conform presence of flavonoids.

Liebermann-Burchard Reaction: It was also performed for the conformation of Sterols and Terpenes. 1 ml of acetic anhydride was added to 1 ml of previously cooled sulphuric acid in cold condition.

Then extract of 1ml was dissolved in chloroform and added to this mixture. Appearance of bluish green was considered positive for Terpenes.

Test for Reducing Sugars

a) ***Benedict's Test:*** Benedict's reagent of 5ml in few drops in water bath few drops of extract was added and boiled for few minutes. Presence of reducing sugar indicates by appearance of green, yellow or orange-red.

b) ***Fehling's test for Reducing Sugars:*** To the mixture of equal volume of Fehling A and Fehling B added with 2ml of extract and boiled for 5 minutes in water bath. The presence of reducing sugar indicates by red precipitate.

Test for Saponins

a) ***Foam Test:*** A dry extract in small amount was boiled with water than it cooled. Than vigorously shaken for a minute. The formation of persistent honeycomb like forth was taken as positive results for saponin.

Test for Tannins

a) The extract in small portion was treated with 5% ferric chloride solution green to blue

color appearance test positive for tannins

b) A creamy precipitate with lead acetate was considered test positive of tannins.

Table2; Result of Preliminary Phytochemical Screening of Extracts for Various Phytoconstituents

Extracts	Alkaloids	Carbohydrates	Flavonoids	Glycosides	Red sugar	Saponins	Sterols	Terpenes	Tannins
Petroleum ether	--	--	--	++	--	--	++	++	--
Chloroform	+	--	--	+	++	-	+	--	+
Ethyl acetate	--	+	+++	+	--	+	--	--	--
Methanol	+++	+	+++	++	++	+	+	+++	--
Water	+++	+++	+++	++	++	--	--	--	++

+++ Prominently present; ++moderately present, +slightly present, --Absent

Acute Toxicity and Gross Behavioral Studies

Acute toxicity was carried for the methanolic extract using the Acute Toxic Method as described in OECD (Organization of Economic Cooperation & Development) Guide Line No: 423. Animals were given increasing doses of 30,100,600&1000 mg/kg p.o of the methanolic extract suspended in 2 % tween-80 solution. The animals were observed continuously for 2 hour and finally at the end of 24 hour and 72 hour to note any toxic sign Experimental Design:

Group–I: Negative Control (Normal Saline)
Group–II: Positive control (ccl_4 1 ml/kg/day) 9 days
Group–III: Standard (Silymarin (100 mg/kg /day) +CCl4 (1 ml/kg /day) 9 days
Group–IV: Test (1) (Methanolic extract of *Colocosia esculenta Linn* 500 mg/kg/day) +CCl4 (1 ml/kg /day) 9 days.
Group–V: Test (2) (Methanolic extract of *Colocasia esculenta Linn* 1000 mg/kg/day) +CCl4 (1ml/kg /day)

Assessment of Liver Function

The extract of hepatoprotective effect was evaluated by parameter of lever function assay such as Serum Glutamic Oxaloacetate, (SGOT) Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphates (ALP) and Total Serum Bilirubin (SB) according to the standard methods.

Histopathological Studies

Formalin were fixed subjected to graded dehydration in ascending strength of alcohol 70%,80%,90% and 100% and subsequent wash with xylene. The tissue were embedded in liquid paraffin to facilitate preparation of histopathological blocks which were essential for imparting strength to tissue so that it can withstand the abrasive force of the blade while being sectioned. The 5 um sections which were obtained by trimming with manual rotary microtome are subjected to staining by Harris haematoxylin and counterstained by Eosin. The sections were viewed under trinocular microscope of different magnifications which were photographed by Motif software inbuilt in system.

Statistical Analysis

Result has been expressed as mean±SEM. One way ANOVA has been employed for comparing majority of parameters. Post hoc tests were used for identification of groups

having significant differences for one-way ANOVA.

Turkey's Multiple Range Test was used for comparisons. Whereas for two-way ANOVA. Bonferroni's test was used for the post hoc analysis. The significant groups were indentified on the figure by designed alphabet

Table 3. Serum Biochemical Analysis Result of Hepatoprotective Activity

Group	Treatment regimen	SGP(IU/L)	SGOT(IU/L)	ALP(IU/L)	SB (IU/L)
I	Normal Saline	62.58±4.12	155.45±6.32	264.35±8.22	0.42±0.04
II	CCl_4	245.58±13.54[a]	495.68±15.58[a]	544.82±19.43[a]	1.43±0.32[a]
III	Silymarin +CCl_4	74.42±5.45***	182.43±6.75***	285.76±10.41***	0.48±0.04***
IV	MCH (500mg/kg +ccl_4	110.45±7.56***	236.43±8.68***	326.96±12.47***	0.64±0.05***
V	MCH (1000mg/kg+CCl_4	93.65±7.42***	208.54±8.62***	302.82±10.32	0.52±0.5***

Values are expressed as mean±SEM (n=6) and analyzed by using ANOVA followed by Bonferroni's multiple comparison test. Percentage change of protective effect compared with the CCl_4 treated control was expressed within brackets. P: [a]<0.001 vs. vehicle control, [ns]>0.05, *<0.05, **<0.01, ***<0.001vs. CCl_4 treated control.

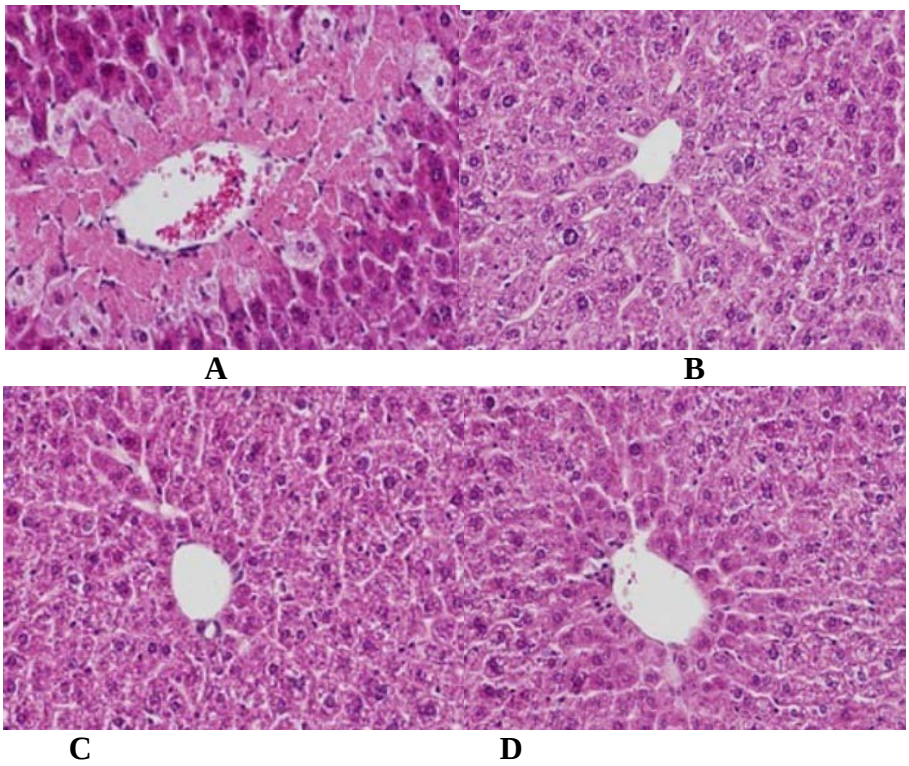

Fig1. Histopathological Evaluation of Hepatoprotective

Group 1: Negative control (Normal saline).
Group 2: Positive control ($CCl_{4)}$.
Group 3: Standard (Silymarin+CCl_4).
Group 4: Test (1) (Methanolic extract of *Colocasia esculanta Linn* 500 mg/kg +CCl_4).
Group 5: Test (2) (Methanolic extract of *Colocasua esculanta Linn* 1000 mg/kg +CCl_4).

DISCUSSION

In this present study was found that tuber extract of *Colocasia esculenta* can modulate hepatotoxicity of CCl4 that has been induced and then reported to be due to the formation of the highly reactive dichloromethane (CCl_3) free radical, which alters function of endoplasmic reticulum .Further Phytoconstituents like flavonoids, alkaloids, saponins and terpeniods posses hepatoprotective activity. In this study, the CCl4 induced liver damage was characterized by increased level of SGPT, SGOT, ALT and SB. Pre-treatment of animal with silymarin 100mg/kg. could reduce the level of SGPT, SGOT, ALP and SB. Similar results were obtained by pre-treatment of animal with MCE (500 and 1000 mg/kg) compared with the CCl4 treated group. However the effectiveness of the extract at the dose levels tested was less compared to the standard hepatoprotective drug used in the study, Silymarin. It has very low toxicity and possesses a good safety profile. At high doses, a laxative effect was observed due to increased bile secretion and bile flow. Adverse effects related to the GI tract such as dyspepsia, bloating, nausea, and diarrhea were reported in 2-10% of patients in a clinical trial. Serious adverse effects, which are rare, include gastroenteritis associated with collapse and allergy. Thus, combination therapy may up to some extent reduce these adverse effects of Silymarin. This study was carried by earlier researchers that the tuber of the plant contained higher flavonoid and phenolic content and scavenging activities. The qualitative photochemical investigations carried out on the methanolic extract of *Colocasia esculenta* also showed positive results for flavonoids including by ferric chloride test, alkaline reagent test, and Shinoda test. The results indicate that the methanolic extract of *Colocasia esculenta* has significant hepatoprotective activity. The histopathological evaluation of kidney preparations in treatment group also revealed a decreased induced tubular congestion, tubular cast, epithelial desquamation, glomerular congestion, blood vessel congestion & inflammatory cells.

CONCLUSION

The methanolic extract of tuber of *Colocasia esculenta linn* was found to possess Hepatoprotective. The activity was however found to be less compared to the standard drugs used in the study. The studies were done using the crude lyophilized extract.

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CONFLICT OF INTEREST: [Nil]

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