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# Hepatoprotective and antioxidant activity of *Coldeniaprocumbens* Linn on paracetamol induced liver damage in albino wistar rats

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## ABSTRACT

In the present study protective effect of coldeniaprocumbens hydroalcoholic extract on acetaminophen induced hepatotoxicity on albino wistar rats was evaluated. Acetaminophen at the dose of 750mg/kg produce hepatotoxicity as manifested by the significant rise of Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) and ALP activities were significantly ( $p < 0.05$ ) elevated in the acetaminophen alone treated animals and decrease in total proteins levels. Pretreatment with coldeniaprocumbens hydroalcoholic extract (100 mg and 200mg) significantly ( $P < 0.05$ ) decrease the SGPT, SGOT, ALP and increase in total proteins. The antioxidant studies reveals that the levels of hepatic superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase and glutathione-S-transferase (GST), reduced glutathione (GSH) were increased in APAP treated animals with significantly reduced in malondialdehyde (MDA) content, which is reversed in pretreatment with hydroalcoholic extract of coldeniaprocumbens. In conclusion the protective effect of coldeniaprocumbens against acetaminophen-induced acute toxicity is mediated either by preventing the decreasing of hepatic antioxidant status or due to its direct radical scavenging capacity.

**Keywords:** Coldeniaprocumbens, Acetaminophen, Hepatotoxicity, Antioxidant, Hydroalcoholic

## INTRODUCTION

Acetaminophen (acetyl-para-aminophenol or APAP) is a commonly used for safe and effective as analgesic and antipyretic at therapeutic doses [1] (Ayman et al). However it causes hepato and nephro toxicity in humans and as well as experimental animals by centrilobular hepatic necrosis [2] (Yun-Hee Shon et al.,).

CYP2E1 converts Acetaminophen (APAP) into reactive intermediate, *N*-acetyl-*p*-benzoquinoneimine which is responsible for liver necrosis. NAPQI depletes glutathione and covalently binds to proteins [3] (Susanna S. T. Lee), loss of glutathione with an increased formation of reactive oxygen and nitrogen species in hepatocytes undergoing necrotic changes [4] (Hartmut Jaeschke et al.,). Increased oxidative stress, linked with alterations in calcium homeostasis and beginning of signal transduction responses, causing mitochondrial permeability transition mitochondrial membrane potential, and loss of the ability ATP production, leads to the necrosis [5] (Margo Moore et al.,).

*Coldenia Procumbens* is an annual herb (CSIR) and common weed in India [7] (Krishnarao Mangeshrao Nadkarni). It is found widely in south India on waste lands, common in dry rice grounds. It is used as folk medicine for the

treatment of rheumatic swelling[22](Aleemuddin.MA et al), supparation of boils(RashtraVardhana), wound healing(White Law Anisile) Wedelolactone was identified as an active constituent [10](beena et al) .

## MATERIALS AND METHODS

### 2.1 Plant material

Fresh aerial parts of *ColdeniaProcumbens*Linn (Boraginaceae) were collected from Thanjavur(India) and it was identified and authenticated by Dr.G.V.S.Murthy, Scientist 'F' & Head of Botanical Survey of India, Coimbatore.

### 2.2 Preparation of hydro alcoholic extract

The leaves were dried under environmental temperatures and protected from direct sunlight, and then milled with electrical grinder to obtain a powder particle. Milled material (1.5 kg) was extracted in 60% ethanol solution by maceration process for 7days. Afterwards, the extract was filtered and evaporated to dryness in a rotary evaporator with reduced pressure. The yield of the extract was quantified (12.98%) and the material obtained was protected from light and stored[11](L. Guaraldo et al).

### 2.3 Reagents

Ethanol was purchased from Hayman Ltd., (Witham,Essex) CM8 3YE, England. Bovine serum albumin (BSA), 1-chloro-2,4-dinitrobenzene (CDNB), 2,4-dinitrophenylhydrazine (DNPH), 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), ethylene diamine tetra acetic acid (EDTA), reduced nicotinamide adenine dinucleotide (NADH), nitro blue tetrazolium (NBT), phenazinemethosulfate (PMT), potassium dihydrogen phosphate, reduced glutathione (GSH), sodium azide, sodium pyrophosphate, trichloro acetic acid (TCA), thiobarbituric acid (TBA), 5-thio- 2- nitrobenzoic acid (TNB), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) , sodium carbonate , aluminium nitrite and ascorbic acid were purchased from Merck chemicals , Mumbai, India. All chemicals and reagents used were of analytical grade.

### 2.4 Total Phenolic Content

Total soluble phenolic compounds in the CPE were determined with Folin–Ciocalteu reagent according to the method of 'IlhamiGülçin using standard gallic acid as a phenolic compound. Briefly, 1 ml of the CPE solution (contains 100 mg extract) in a volumetric flask diluted with distilled water (46 ml). One milliliter of Folin–Ciocalteu reagent was added to the mixture and the content of the flask was mixed thoroughly. After 3 min 3ml of Na<sub>2</sub>CO<sub>3</sub> (2%) was added and then allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 765 nm in a spectrophotometer (TechComp UV2310). The total concentration of phenolic compounds in the CPE determined as microgram of gallic acid equivalent by using an equation that was obtained from standard gallic acid graph.

### 2.5 Total Flavonoid Content

Flavonoid concentration was determined as follows: CPE (0.1 ml) was diluted with 80% aqueous ethanol (0.9 ml). An aliquot of 0.5 ml was added to test tubes containing 0.1 ml of 10% aluminum nitrate, 0.1 ml of 1 M aqueous potassium acetate and 4.3 ml of 80% ethanol. After 40 min at room temperature, the absorbance was determined spectrophotometrically at 415 nm. Total flavonoid concentration was calculated using quercetin as standard.

### 2.6 Experimental Animals

Twenty four albino wistar rats either sex weighing between 180 to 220 g were used in this study. They were housed for 72 h before use for acclimatization at a controlled condition of temperature with a 12-hlight:12-h dark cycle before the experiment. Rats were divided into four groups of 6 animals each. Animals were handled according to the rules and regulations ofInstitutional Animal Ethics committee (IAEC).

### 2.7 Experimental Protocol

Group I served as a vehicle control and received normal saline orally.

Group II received acetaminophen (750 mg/kg suspended in 1% methylcellulose) orally 1 h post-treatment of the last dose (on 16 and 17).

Group III was treated with plant extract 100 mg/kg for 15 days and follows received acetaminophen (750 mg/kg suspended in 1% methylcellulose) orally 1 h post-treatment of the last dose (on 16 and 17).

Group IV was treated with plant extract 200 mg/kg for 15 days and follows received acetaminophen (750 mg/kg suspended in 1% methylcellulose) orally 1 h post-treatment of the last dose (on 16 and 17).

Acetaminophen was administered 24 h later to both the third and fourth groups. The rats were sacrificed; 24 h after the acetaminophen administration, by decapitation blood was collected from retro orbital. Serum was separated, and used for different biochemical assays. The liver was carefully dissected, cleaned of extraneous tissue and cut into approximately 50–100 mg portions on ice and stored separately at 70°C.

## 2.8 Biological assays:

Serum aspartate and alanine aminotransferase activities (Reitman and Frankel, 1957), alkaline phosphatase (ALP) (Kind and King, 1954) and Serum Bilurubin (Mally and Evelyn, 1937) were determined. Liver tissues were homogenized in four volumes of ice-cold Tris–HCl buffer (20 mM, pH 7.4) containing KCl (0.15 M) using a Potter–Elvehjem homogenizer with a Teflon pestle. The homogenate mixture was centrifuged at 3200g for 20 min at 4°C and the supernatant fractions were separated into two volumes. A small volume of supernatant (1.0 ml) was kept at 4°C for estimating reduced glutathione (GSH) levels (Griffith, 1980). The activities of hepatic glutathione reductase (GSH-R) (Stahl *et al.*), total glutathione peroxidase (GSH-Px) (Rotruck *et al.*, 1973), were determined. Lipid peroxidation, Superoxide dismutase [27](Kakkar *et al.*) and catalase activity[23](Aebi *et al.*) in liver was ascertained by the formation of malondialdehyde (MDA) and measured by the thiobarbituric acid-reactive method according to [24]Ohkawa *et al.* (1989). The protein concentration was measured by the mentioned method of Lowry *et al.* (1951).

## 2.9 Statistical Analysis.

The results are expressed as Mean±S.E.M. and all statistical comparisons were made by means of One way ANOVA  $P < 0.05$  were considered significant

## RESULTS

### 3.1 Total phenolic and flavonoid compounds

The level of total phenolic and flavonoid content of hydroalcoholic extract of *Coldeniaprocumbens* had been showed in the table 1.

Table:1

Solvent	Total phenolics mg GAE/100g fresh mass	Total flavonoids mg QC/100g fresh mass
Hydroalcoholic extract	77.21	53.12

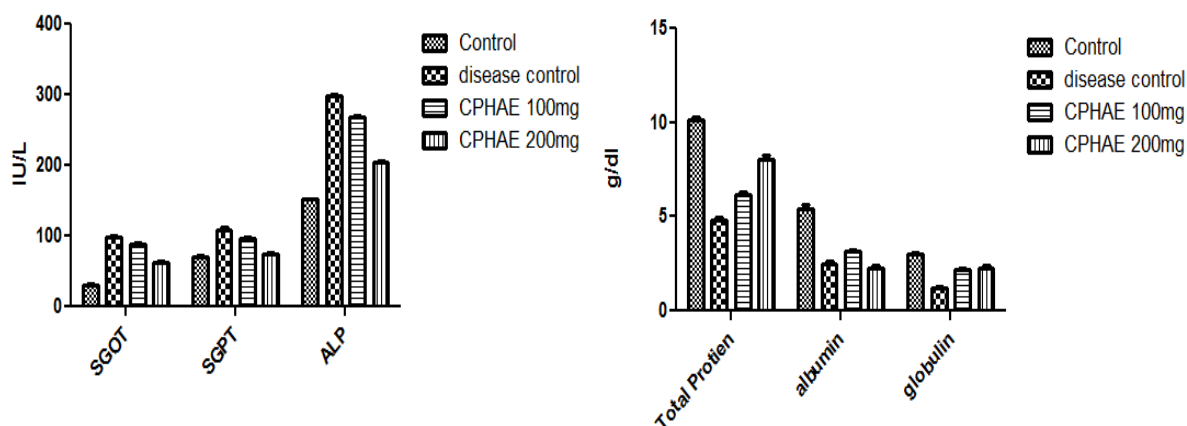
### 3.2 Biochemical approach

Overdose of APAP leads to the hepatic damage as evidence by significantly ( $P < 0.05$ ) alteration of serum levels of glutamate oxalo acetate transaminase (AST), glutamate pyruvate transaminase (ALT), Alkaline phosphate, Total protein, albumin, globulin and bilirubin in comparison with control group. Pretreatment of rats with 100mg and 200mg of *Coldeniaprocumbens* hydroalcoholic extract markedly lowered the elevated serum biochemical markers (Fig- 1).

Table:2

PARAMETERS	GROUP I	GROUP II	GROUP III	GROUP IV
SGOT(IU / L)	31.5±0.894	98.16± 2.227*	88.66± 0.9888*#	63± 1.770*#
SGPT(IU / L)	70.16±1.249	108.5±3.631*	96±2.066*#	74.83±1.815*#
ALP(IU/L)	151.6±1.333	298.3±2.499*	269.1±1.078*#	203.5±2.141*#
Total Protein(g/dl)	10.15±0.1607	4.83±0.1308*	6.2±0.09661*#	8.05±0.2391*#
Bilirubin(g/dl)	0.6±0.06325	1.26±0.1145*	1.23±0.09189*	0.95±0.08466*
Albumin(g/dl)	5.43±0.1706	2.51±0.1376*	3.13±0.08433*#	3.93±0.1116*#
Globulin(g/dl)	2.98±0.1249	1.18±0.08333*	2.18±0.09098*#	2.26±0.1116*

Figure 1. Biochemical approaches of rat's serum treated with Paracetamol follows treated with *Coldeniaprocumbens* extract at doses of 100 and 200 mg/kg for 15 days as compared to the control group

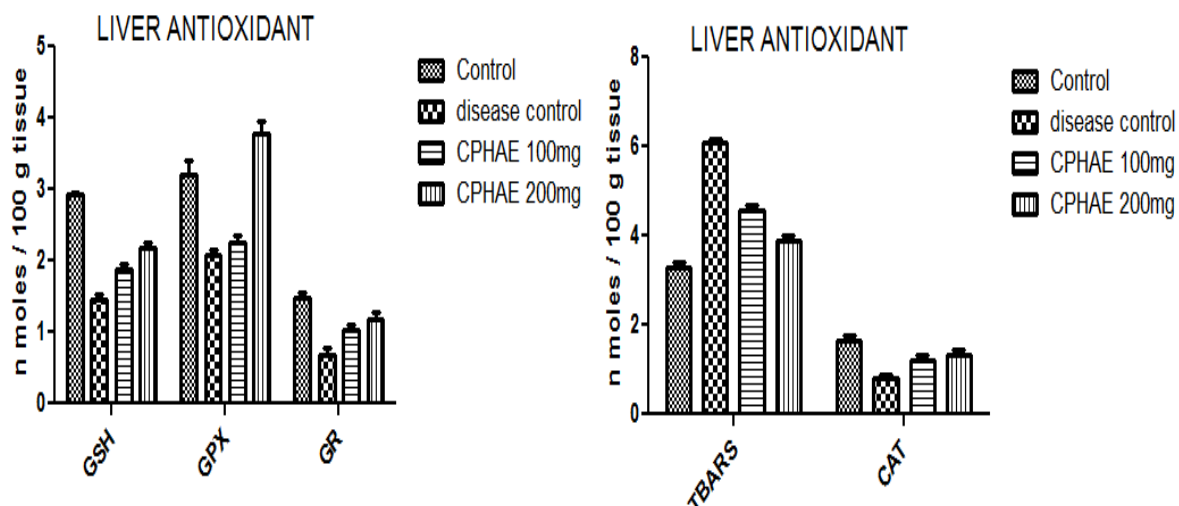


Values are expressed as mean  $\pm$  S.E.M. (n=6), \*P<0.01, When compared with the control group, #P<0.01, When compared with the disease control group, (One way ANOVA followed by Bonferroni's Multiple Comparison Test)

### 3.3 Oxidative approach

The effects of CPHAE on rat liver glutathione, glutathione peroxidase, glutathione reductase, superoxide dismutase, lipid peroxidation and catalase levels were determined (Fig-2). Liver enzymatic and nonenzymatic antioxidant levels were significantly altered in paracetamol treated rats compared with normal group ( $p<0.05$ ). GSH, GPx, GR, SOD and CAT levels were significantly increased ( $p<0.05$ ) in CPHAE pretreated group. TBARS level (expressed in term of malondialdehyde (MDA) formation) are significantly increased in paracetamol treated group compared with the normal group ( $p<0.05$ ). Pretreatment with CPHAE (100mg and 200mg/kg) significantly prevented the increase in MDA levels ( $p<0.05$ ) and brought back near to normal level.

Figure 2. Antioxidant activity of rat's liver treated with Paracetamol follows treated with *Coldeniaprocumbens* extract at doses of 100 and 200 mg/kg for 15 days as compared to the control group



Values are expressed as mean  $\pm$  S.E.M. (n=6), \*P<0.01, When compared with the control group, #P<0.01, When compared with the disease control group, (One way ANOVA followed by Bonferroni's Multiple Comparison Test).

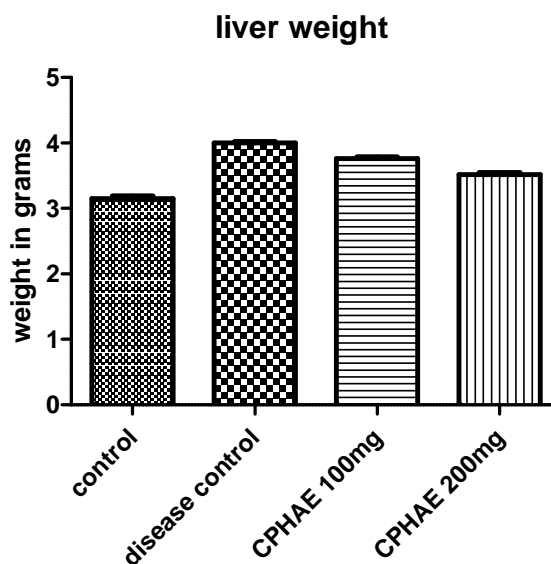
PARAMETERS	Group I Control	Group II Disease Control	Group III CPHAE 100mg/kg	Group IV CPHAE 200mg/kg
GSH(n moles / 100 g tissue)	2.91±0.04773	1.45±0.07188*	1.86±0.08819 <sup>#</sup>	2.16±0.09888 <sup>#</sup>
GPX(n moles / 100 g tissue)	3.2±0.1949	2.06±0.07601*	2.25±0.1147*	3.76±0.1856 <sup>#</sup>
GR(n moles / 100 g tissue)	1.48±0.07491	0.66±0.1116*	1.01±0.07491 <sup>#</sup>	1.16±0.1229 <sup>#</sup>
TBARS(n moles / 100 g tissue)	3.26±0.1453	6.1±0.08165*	4.55±0.1384 <sup>#</sup>	3.9±0.1033 <sup>#</sup>
SOD (units / mg)	1.78±0.09458	0.66±0.06146*	0.93±0.07149*	1.31±0.1078 <sup>#</sup>
CAT (n moles / 100 g tissue)	1.65±0.1285	0.8±0.07303*	1.2±0.1125	1.31±0.1195 <sup>#</sup>

### 3.4 Liver weight:

Treatment with APAP leads to the significantly ( $p<0.05$ ) increase in the weight of the liver and it was reversed by the pretreatment with hydroalcoholic extract (Table 2).

GROUP	LIVER WEIGHT(gms)
Group I Control	3.150±0.03751
Group II Disease Control	4.002±0.02242 *
Group III CPHAE 100mg/kg	3.762±0.02301 <sup>#</sup>
Group IV CPHAE 200mg/kg	3.520±0.02620 <sup>#</sup>

Values are expressed as mean±S.E.M. (n=6), \* $P<0.01$ , When compared with the control group, <sup>#</sup> $P<0.01$ , When compared with the disease control group, (One way ANOVA followed by Bonferroni's Multiple Comparison Test).



### DISCUSSION

Acetaminophen is widely used analgesic and antipyretic, in high doses causes hepatotoxicity as well as nephro toxicity. The primary metabolism of acetaminophen in liver leads to the generation of free radical NAPQI by cytochrome P450 enzyme which is responsible for the toxicity (M.Cekmen).

In the assessment of hepatic damage by acetaminophen was determined by estimating the enzyme levels such as AST, ALT and ALP. Hepatocellular necrosis or membrane damage releases the enzymes into circulation and hence it can be measured in the blood serum (Ahmed Imran Siddiqi *et al.*,2007). High level of AST/SGPT indicates liver damage, such as viral hepatitis, cardiac infarction and muscle injury. AST catalyses the conversion of alanine to pyruvate and glutamate, and released in a similar manner. Therefore, ALT/SGOT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. On other hand, serum levels of ALP, bilirubin and Total Protein was accompanied by the function of hepatic cell (Adak *et al.*,2010). Increase in serum level of ALP is due to increased synthesis (Deepak kumari *et al.*,2010), in the presence of increasing biliary pressure (Raj Kapoor B *et al.*,2008). The parenchymal cells are responsible for synthesis of fibrinogen, albumin and other coagulation factors

and most of a and b globulins. Bilirubin is an endogenous anion derived from haemoglobin degradation from the RBC (Thapa B.R *et al.*, 2007).

Results of our study showed that acetaminophen causes a significant elevated levels of AST, ALT, ALP, Bilirubin, Globulin and decrease in Total Protein and albumin ( $P < 0.05$ ). Pretreatment with *Coldeniaprocumbens*, the levels of these enzymes were restored in a dose-dependent manner. The reversal may be because of the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal levels with the healing of hepatic parenchyma and the regeneration of hepatocytes (Wegner T *et al.*, 1999). Effective control of ALP, total protein and bilirubin levels point towards an early improvement in the secretory mechanism of the hepatic cells. The hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by hepatotoxin. The plant extract decreased elevated enzyme levels in tested groups, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells.

Glutathione and glutathione-related enzymes play a key role in protecting the cells against the effects of reactive oxygen species (ROS). Reduced glutathione can act as a reductant, reducing hydrogen peroxide and lipid hydroperoxides directly to  $H_2O$ , which catalyzed by GSH-Px. Depletion of intracellular GSH, under conditions of continuous intracellular oxidative stress, leads to oxidation and damage of proteins, lipids and DNA by the reactive oxygen species (Kaplowitz, 2000). *Coldeniaprocumbens* extract was found to prevent GSH depletion by scavenging reactive oxygen species, therefore, it inhibits the oxidative damage of cellular macromolecules. Also, *Coldeniaprocumbens* can increase GSH levels by increasing cysteine uptake, which is a rate-limiting step for GSH biosynthesis (Mohamed Bastawy Ahmed *et al.*, 2001). Thus, depletion of intracellular GSH and decrease in the activity of GSH-Px during APAP treatment in this study is indicative of the increased lipid peroxidation level (Jack A. Hinson *et al.*, 2010). Peroxidation of membrane lipids has been implicated as a possible mechanism of oxidative stress-induced lethal injury (Jaeschke, 2000). The increased intracellular GSH level and GSH-Px activity in hepatic and renal tissues in response to Pretreatment of *Coldeniaprocumbens* in our study, is indicative of increased free radical scavenging and enhanced detoxification of hydrogen peroxide and lipid hydroperoxides. Inhibition of lipid peroxidation by *Coldeniaprocumbens* may, at least partially, suppress the injury cascade induced by APAP in rat liver and kidney.

Lipid peroxidation (LPO) is an autocatalytic process, which is a common consequence of cell damage or death. It involves a broad spectrum of alterations, and the consequent degeneration of cell membranes may contribute towards the development of other disorders of lipoprotein metabolism, both in the liver and in peripheral tissues (Deepak Kumar Mittal *et al.*, 2010). Thiobarbituric Acid is a reactive substance which formed as a by-product of lipid peroxidation. Because ROS having extremely short half-lives, they are difficult to measure directly. SOD and CAT are a class of enzymes that catalase the ROS. Acetaminophen toxicity leads to the oxidative stress, causes the depletion of the CAT, SOD and increase in the TBARS levels in the liver as well as kidney. Pretreatment with *Coldeniaprocumbens* showed significant ( $P < 0.05$ ) restoration of altered levels in liver.

### CONCLUSION

In conclusion, the protective effect of *Coldeniaprocumbens* against acetaminophen-induced hepatotoxicity in rats appears to be related to the inhibition of lipid peroxidative processes and to prevention of GSH depletion. Although the hepatoprotective effect of *Coldeniaprocumbens* cannot be inferred from these preliminary results, many hypotheses may be proposed. Among these, *Coldeniaprocumbens* may inhibit acetaminophen metabolism, thereby reducing the production of the active metabolite, N-acetyl-p-benzoquinone imine.

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