

## Hepatoprotective effect of *Brassica oleracea* vegetable and its leaves in Paracetamol induced liver damage in albino rats

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**Abstract:** The liver is the key organ involved with almost all the biochemical pathways related to energy production, growth and fight against disease, nutrient supply, and reproduction. Drug-induced toxicity has turned out to be a major trouble worldwide. Paracetamol is a commonly used drug and continued use may cause liver damage. In this present investigation the commonly used vegetable cabbage (*Brassica oleracea*) and its leaf is used for the hepatoprotective action against paracetamol induced liver damage. The animals are divided into seven groups, in that four groups are treated with cabbage and its leaf. The parameters like AST, ALT, gamma GT, bilirubin, total protein and antioxidant levels of Superoxide Dismutase (SOD), catalase, Glutathione Peroxidase levels in normal range due to the treatment with cabbage and its leaf extracts. The histological change shows the good hepatoprotective action of cabbage and its leaf extracts.

**Keywords :** Hepato protective, *Brassica oleracea*, Ethanolic extract, liver damage, markers.

### Introduction

Cabbage is the common leafy green vegetable used as a good food material in worldwide especially India. Plants are the major sources having a variety of application in pharmaceutical to nanotechnology [1-6]. The scientific name of cabbage is *Brassica oleracea* coming under the family of Brassicaceae. It has many biological applications like antimicrobials, against the bacterial (*Pseudomonas aeruginosa*, *Shigella flexneri*, *E.coli* and *Klebsiella pneumoniae*) and fungal (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Cladosporium* species) isolates. It contains many phytochemicals such as secondary metabolites like phenolics, alkaloids, flavonoids and saponins and functional group of phenols, alcohol, amines and carboxylic acid [7].

The liver is a very important organ, with different function. It plays a significant role in metabolism of carbohydrate, lipid and protein etc. and apart from plays a key role in detoxification of a lot of endogenous and exogenous compounds changing them to not as much of toxic substance and is excreted.

The various plants and its parts from different families have been involved in the hepatoprotective activity such as *Withania frutescens* leaves (Ethanolic extract) of Solanaceae [8], *Vitex trifolia* leaves (Aqueous and ethanolic extracts) of Verbenaceae, *Vitis vinifera* Leaves (Ethanolic Extract and *n*-BuOH fraction from ethanolic extract) Vitaceae [9], *Trianthema portulacastrum* Whole plant (Ethanol extract) Aizoaceae [10], *Taraxacum Officinale* Root (Hydro-alcoholic acid extract) Asteraceae [11], *Tridax procumbens* Aerial part (Chloroform insoluble fraction from ethanolic extract) Compositae [12], *Sarcostemma Brevistigma* Stem (Ethyl acetate extract) Asclepiadaceae [13], *Swertia chirata* Whole Plant (Chloroform soluble fraction and Methanol Extract) Gentianaceae [14], *Rubia cordifolia* Root (Aqueousmethanol Extract) Rubiaceae [15], *Phyllanthus amarus* Leaf (Methanolic Extract) Euphorbiaceae [16], *Phyllanthus urinaria* whole plant (Alcohol extract) Euphorbiaceae [17], *Phyllanthus maderaspatensis* (hexane extract) Phyllanthaceae [18], *Pergularia daemia*

Aerial parts (Acetone sub fraction of ethanolic fraction) Asclepiadaceae [19], *Pterocarpus Santalinus* Stem bark (Aqueous Ethanol Extracts) Fabaceae [20], *Mamordica subangulata* (Leaf Aqueous Suspension) Cucurbitaceae [18], *Oenothera biennis* (Semen Fatty oil) Oenotheraceae [21], *Lygodium flexuosum* Leaves (*n*-hexane extract) Lygodiaceae [22], *Hedyotis corymbosa* Whole plant (methanolic Extract) Rubiaceae [23], *Hypericum perforatum* dried aerial parts (50% Alcoholic Extract) Clusiaceae [24], *Glycosmis arborea* Aerial parts (Butanol extract) Rutaceae [25], *Echinacea pallid* (Hydroalcoholic Extract) Asteraceae (Rusu et al, 2005), *Eclipta prostrate* Whole plant (Aqueous powder extract) Asteraceae [26], *Calendula officinalis* (Hydroalcoholic extract) Asteraceae, *Corylus avellana* Folium (Hydroalcoholic extract) Betulaceae [21], *Chrysanthemum balsamita* Herba (Hydroalcoholic extract) Asteraceae [21], *Beta vulgaris* Root (Ethanolic extract) Chenopodiaceae [27].

The main objective of the study is to evaluate the hepatoprotective activity of the ethanolic extract of the *Brassica oleracea* and its leaf in validated experimental animal models. The Study of hepatoprotective activity extracts of *Brassica oleracea* using Paracetamol Induced Hepatotoxicity. The changes in the activity of AST, ALT, gamma GT, Bilirubin and Total protein and changes in the Antioxidant levels of Superoxide Dismutase (SOD), catalase, Glutathione Peroxidase were evaluated. The histological changes in Normal and Experimental Rats were analysed.

## Materials and Methods

### Chemicals

Analytical grade paracetamol, Silymarin and other chemicals were purchased from Himedia laboratories private limited, Mumbai. *B. oleracea* plant was collected from Arcot, South India.

### Preparation of plant extracts

The leaves and vegetable of *Brassica oleracea* were collected and shade dried. The shade leaves were subjected to pulverization to get coarse powder which was then used for extraction with ethanol. 100g of dry powder was loosely packed in the thimble of Soxhlet apparatus and extracted with ethanol at 55° c for 24 hours. The extract was air dried at 25-30°c and weighed. In the same way, the cabbage extract was prepared. For oral administration, extracts were dissolved in distilled water.

### Experimental Design for Hepatoprotective Activity of *Brassica oleracea*

Adult male Wistar albino rats maintained at the college weighing 150g-170g were used for the hepatoprotective studies. Ethical clearance was obtained from the Institutional Animal Ethical Committee, CPCSEA, India (Reg No.282/ac/09/CPCSEA).

Animals were divided into 7 groups, each comprising 6 rats as:

**Group I (Normal):** Orally received distilled water for 7 days.

**Group II (Induced):** Orally received Paracetamol (2g/kg body weight) dissolved in distilled water for 7 days.

**Group III (Standard):** Orally received Paracetamol (2g/kg body weight) along with Silymarin (20mg/kg body weight) dissolved in distilled water for 7 days.

**Group IV (Treatment):** Orally received Paracetamol (2g/kg body weight) along with *Brassica oleracea* leaf extracts (300mg/kg body weight) dissolved in distilled water for 7 days.

**Group V (Treatment):** Orally received Paracetamol (2g/kg/body weight) along with *Brassica oleracea* leaf extracts (500mg/kg body weight) dissolved in distilled water for 7 days.

**Group VI (Treatment):** Orally received Paracetamol (2g/kg body weight) along with *Brassica oleracea* vegetable extracts (300mg/kg body weight) dissolved in distilled water for 7 days.

**Group VII (Treatment):** Orally will received Paracetamol (2g/kg/body weight) along with *Brassica oleracea* vegetable extracts (500mg/kg body weight) dissolved in distilled water for 7 days.

### Collection of blood

On the 8<sup>th</sup> day, all the animals were sacrificed by mild ether anaesthesia. Blood samples were collected in glass tube from retro-orbital puncture to obtain haemolysis for 30 min at 37° C. The clear serum obtained after centrifugation was used for the estimation of Alanine amino transferase (ALT), Aspartate amino transferase (AST),  $\gamma$  -Glutamyl transferase ( $\gamma$  GT), Serum bilirubin and Serum protein

### Liver homogenate preparation

Liver homogenates were obtained by using a tissue homogenator, Ultra turrax T-25 Polytron at 4°C. The homogenates (1:10 w/v) were prepared by using a 100 mMol KCl buffer (pH 7.0) containing 0.3 mM EDTA. All homogenates were centrifuged at 6000 RPM for 45 min at 4°C and the supernatant was used for biochemical analysis. The tissue homogenate was the following antioxidant levels were analyzed Superoxide dismutase (SOD), Catalase and Glutathione Peroxidase.

### Histological studies

The histological studies were conducted as per standard procedure and prepared tissues were stained with hematoxylin and eosin and for granulated  $\beta$ -cells, tissue sections were stained with modified aldehyde fuchsin staining.

### Statistical Analysis

The difference of biochemical parameters were measured using the statistical method of, Analysis of Variance (ANOVA). Analysis of Variance refers to the examination of differences among the samples.

## Results and Discussion

### Hepatoprotective studies of ethanolic extract from *Brassica oleracea* leaves and vegetables

The higher doses of paracetamol to animal may cause liver damage. The paracetamol-induced liver disorders were treated with ethanolic extracts from *Brassica oleracea* and its leaf (300 mg/kg and 500 mg/kg of body weight) for 7 days. Silymarin is noticeable as one of the standard herbal formulation, which used for Hepatoprotective activity. Estimating the activities of serum marker enzymes, like aspartate amino transferase, alanine aminotransferase, and  $\gamma$ -glutamyl transferase can make evaluation of liver function.

### Changes in the activity of AST, ALT, and $\gamma$ -GT

Table 1 represent the changes in the activity of serum AST, ALT and  $\gamma$ -GT. Here, paracetamol-induced liver damage was characterized by increased activity of these enzymes and the same returns to normal level after 7 days of treatment of ethanolic extracts from *Brassica oleracea* and its leaf.

Aminotransferase contribute a group of enzymes that catalyze the interconversion of amino acids and  $\alpha$ -keto acids by the transfer of amino groups. These are liver specific enzymes and are considered to be very sensitive and reliable indices for essential hepatotoxic as well as hepatoprotective or curative effect of various compounds [28].

The both AST and ALT level increased due to toxic compounds affecting the integrity of liver cells [12]. The ethanolic extracts of *Brassica oleracea* and its leaf seems to preserve the structural integrity of the hepatocellular membrane as evident from the significant reduction in paracetamol – induced rise in serum AST and ALT in rats. The decreased serum enzymes level in paracetamol – induced liver damage by *Brassica oleracea L. var. Capitata* and its leaf and may be due to the prevention of leakage of the intracellular enzymes by their membrane stabilizing activity, which was supported by the limited extent of histological changes.  $\gamma$  - Glutamyl transferase is a microsomal enzyme, which is widely distributed in tissues throughout the body and including liver. The activity of serum  $\gamma$ -GT is generally elevated as a result of liver disease involving cholestasis and acute hepatic damage.

**Table 1- Changes in the serum levels of AST, ALT AND  $\gamma$ -GT.**

Parameters	AST (IU/L)	ALT(IU/L)	$\gamma$ -GT(IU/L)
Group I	37.75 $\pm$ 3.20	33.12 $\pm$ 0.64	6.15 $\pm$ 0.14
Group II	165.17 $\pm$ 1.23	115.2 $\pm$ 1.2	9.98 $\pm$ 0.65
Group III	38.24 $\pm$ 2.2	34.48 $\pm$ 1.48	6.42 $\pm$ 0.31
Group IV	43.23 $\pm$ 1.34	38.6 $\pm$ 1.62	7.48 $\pm$ 0.35
Group V	46.09 $\pm$ 1.60	48.23 $\pm$ 1.42	7.59 $\pm$ 0.73
Group VI	40.23 $\pm$ 1.54	39.6 $\pm$ 0.62	6.58 $\pm$ 0.39
Group VII	39.09 $\pm$ 1.30	37.23 $\pm$ 0.42	6.59 $\pm$ 0.23
Level of Significance	<0.05	<0.05	<0.05

$p < 0.05$  value are considered statistically significant (BMRT)

The results show that increased activities of serum marker enzymes are decreased on the administration of ethanolic extracts of *Brassica oleracea* and its leaf indicating that the natural tissues injury mechanism is kept intact and oxidative degeneration of tissues is prevented by the compounds present in the *Brassica oleracea* and its leaf. Thus *Brassica oleracea* and its leaf protects the cellular membrane from damage, thereby preventing the leakage of hepatic enzyme.

#### Changes in the level of serum total bilirubin and total proteins

Table 2 represent the changes in the level of serum total bilirubin and total protein. Here, paracetamol-induced liver damage was characterized by increased level of total bilirubin and decreased level of total protein and they return to normal level after 7 days of treatment of ethanolic extracts of *B. oleracea* and its leaf. Administration of ethanolic extracts from *B. oleracea* and its leaf decreased the level of bilirubin to near normal values, suggesting that it offered protection.

**Table 2 - Changes in the serum levels of bilirubin and total protein.**

Parameters	Serum bilirubin (mg/dl)	Serum Protein (g/dl)
Group I	0.41 $\pm$ 0.03	6.68 $\pm$ 0.06
Group II	1.86 $\pm$ 0.10	3.42 $\pm$ 0.04
Group III	0.43 $\pm$ 0.04	6.36 $\pm$ 0.16
Group IV	0.58 $\pm$ 0.06	5.45 $\pm$ 0.08
Group V	0.45 $\pm$ 0.04	6.24 $\pm$ 0.07
Group VI	0.49 $\pm$ 1.06	6.45 $\pm$ 1.08
Group VII	0.47 $\pm$ 1.34	6.39 $\pm$ 1.37
Level of Significance	<0.05	<0.05

$p < 0.05$  value are considered statistically significant (BMRT)

Liver is the major organ which involved in the process of protein synthesis and its metabolism. Many of the diseases related with protein are based on the liver functions only. The lowered level of total protein in the serum of paracetamol-treated rats suggests the severity of hepatopathy. The ability of near normal levels in total protein content of serum of *Brassica oleracea* and its leaf-treated rats further elucidate its hepatoprotective effect.

#### Changes in the antioxidant levels of superoxide dismutase, catalase and glutathione peroxidase

In the removal of toxic intermediates antioxidants are playing a vital role in scavenging ROS. The cabbage having various components which act as a electron donars and it involved for the process with free radicals and terminating of radical chain reaction. Table 3 present the changes in the levels of antioxidants such as superoxide dismutase, catalase and glutathione peroxidase. In the present study, decrease in enzyme activity of superoxide dismutase (SOD) is a responsive key in liver damage and is the most sensitive enzymatic key in liver damage. Curtis and Mortiz (1972), SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. The renal SOD level

registered a considerable decrease in paracetamol treated rats. The values returned to normal in *Brassica oleracea* and its leaf treated rats, and were comparable to those obtained with the values of standard treatment of silymarin. The reduction in the activity of CAT may result in a number of harmful effects due to the incorporation of superoxide radical and hydrogen peroxide. The catalase level recorded a considerable decrease in paracetamol treated rats. The values returned to normal in *Brassica oleracea* and its leaf treated rats, and were comparable to those obtained with the values of standard treatment of silymarin.

Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. The role of glutathione is removing of free radical species like hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Also it is substrate for glutathione peroxidase (GPx) [30]. The hepatocellular glutathione peroxidase level registered a significant decrease in paracetamol treated rats. The values returned to normal in *Brassica oleracea.L.Var.Capitata* and its leaf treated rats, and were comparable to those obtained with the values of standard treatment of silymarin.

These observations support the hypothesis that the mechanism of hepatotoxicity in paracetamol treated animals is related to the depletion of antioxidant defence system. Treatment with *Brassica oleracea* and its leaf (300 mg/kg and 500 mg/kg body wt, p.o) prevents the depletion of hepatocellular antioxidants. Administration of *Brassica oleracea* and its leaf to rats increased the levels of SOD, CAT and GPx because of its free radical scavenging activity as compared to normal. The decrease in SOD activity after paracetamol administration might be due to the loss of copper and zinc, which are essential for enzyme activity [31]. Paracetamol has been demonstrated to induce the loss of copper and zinc in the liver. The decreased SOD activity is insufficient to scavenge the superoxide anion produced during the normal metabolic process. The superoxide

Thus ethanolic extracts of *Brassica oleracea.L.Var.Capitata* and its leaf used in the present study seems to prevent and cure hepatotoxicity by maintaining the level of bilirubin, AST, ALT and  $\gamma$ -GT which normally increases during hepatotoxicity. The extracts also maintain the antioxidant levels by its free radical scavenging activity.

**Table 3 - Changes in the Antioxidant Levels of SOD, CAT and GPX.**

Parameters	SOD ( $\mu\text{mol}/\text{min}/\text{mg protein}$ )	CAT ( $\mu\text{mol}/\text{min}/\text{mg protein}$ )	GPx ( $\mu\text{mol}/\text{min}/\text{mg protein}$ )
Group I	4.91 $\pm$ 0.31	58.99 $\pm$ 4.80	5.98 $\pm$ 2.46
Group II	1.94 $\pm$ 0.21	41.89 $\pm$ 3.43	3.98 $\pm$ 0.35
Group III	4.63 $\pm$ 0.39	55.67 $\pm$ 3.13	5.34 $\pm$ 2.11
Group IV	4.26 $\pm$ 0.18	53.68 $\pm$ 0.51	5.12 $\pm$ 1.92
Group V	4.02 $\pm$ 0.28	51.23 $\pm$ 3.14	4.94 $\pm$ 1.70
Group VI	4.86 $\pm$ 0.10	59.38 $\pm$ 0.15	5.42 $\pm$ 1.02
Group VII	4.72 $\pm$ 0.08	57.43 $\pm$ 1.14	5.39 $\pm$ 0.23
Level of Significance	<0.04	<0.04	<0.04

$p < 0.04$  value are considered statistically significant (BMRT)

### Histopathological studies in liver

Histological studies showed the normal appearance in control rats (1. a) and that the administration of Paracetamol to rats cause liver damage (Fig. 1 (b)). The pattern showed almost normal appearance in standard treatment of Silymarin (Fig. 2), Ethanolic extracts of *B. oleracea* leaf - treated rats (300 mg/kg b.wt) (Fig. 2 (a) and (b) 500mg/kg b.wt) and *B. oleracea* vegetable-treated rats (300 mg/kg b.wt), (500mg/kg b.wt),(Fig.2 (c and d)

In histopathological studies, the control rats showed normal appearance of liver without any histological alterations. Paracetamol-induced rats showed pathological changes in liver including a number of congestions, like occasional necrosis. The liver was having near normal appearance with mild changes in congestions, degeneration and necrosis, in rats treated with silymarin. Same pattern was obtained in rats treated with ethanolic extracts from *B. oleracea* and its leaf (300 mg/kg and 500 mg/kg of b.wt). The results of efficient tests and histopathological studies suggest that paracetamol toxicity leads to serious histological changes in the liver cells. The properties such as membrane-protective and antioxidant character of ethanolic

extracts from *Brassica oleracea* and its leaf might be helpful in alleviating the pathological changes caused by paracetamol in liver.

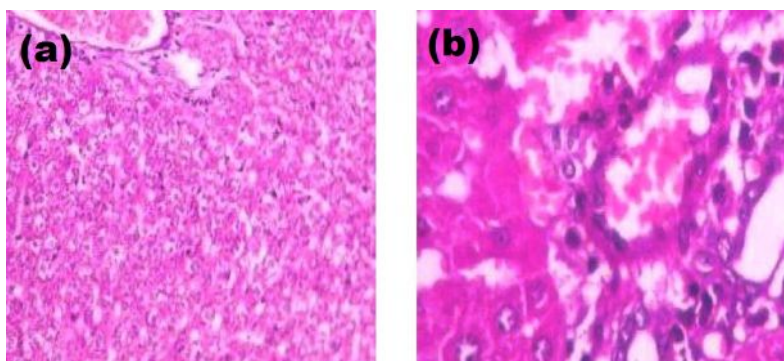


Figure : 1 (a) Normal appearance in liver cells in control rats (b) Paracetamol induced rat cells

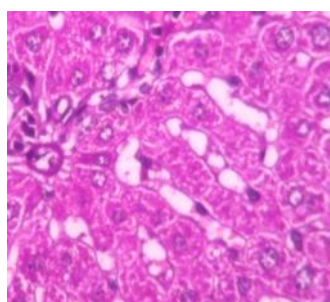


Figure: 2 Kidney cells of normal appearance in standard treatment of Silymarin

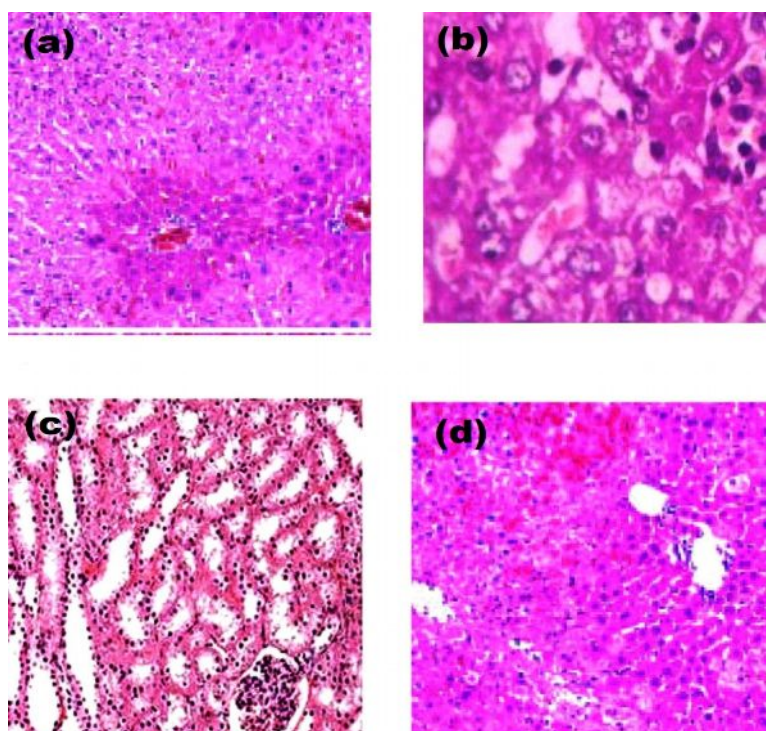


Figure 3: Ethanolic extracts of *B. oleracea* leaf - treated rat cells (a) 300mg/kg b.wt (b) 500mg/kg b.wt and *B. oleracea* vegetable-treated rat cells (c) 300mg/kg b.wt (d) 500mg/kg b.wt

## Conclusion

*Brassica oleracea* has previously been pharmacologically appraised for its potential therapeutic importance. The present study was undertaken to examine the efficacy of the ethanolic extract from *Brassica oleracea* and its leaf against paracetamol-induced hepatotoxicity in rats, with the help of different hepatic markers. Based on these results, It can be concluded that the ethanolic extracts from *Brassica oleracea* and its



leaf compared with (300 mg/kg and 500 mg/kg body weight), 500mg/kg possesses high protective activity against paracetamol-induced liver damage in rats. According to the results obtained in this study, it may be inferred that, in general, *Brassica oleracea* and its leaf reverse the hepatic damage induced by paracetamol. In future the various components of the samples responsible for this activity can be isolated and the purified components can be tested to predict the efficacy of the plant drug. More investigations have to be done to propose the mechanism of action.

### Conflict of interest

We declare that we have no conflict of interest.

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