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HEPATOPROTECTIVE ACTIVITY OF *BRASSICA OLERACEA* IN PARACETAMOL INTOXICATED ALBINO RATS

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ABSTRACT
Hepatoprotective activity of ethanolic extract of *Brassica oleracea* L. var. *Italica* flower was studied against paracetamol induced acute hepatotoxicity in albino rats. We studied the effect of *B. oleracea* on serum glutathione, protein, serum marker enzymes, serum bilirubin, and thiobarbutiric acid reactive substances against paracetamol induced damage in rats has been studied to find out the possible mechanism of hepatoprotection. Pre and post-treatment with extract showed a dose-dependent reduction of paracetamol induced rats were elevated levels of enzyme activity with parallel increase in total protein and bilirubin, indicating the extract could preserve the normal functional status of the liver. The weight of the organs such as liver, heart, lung, spleen and kidney in paracetamol induced experimental animals administered with *B. oleracea* showed an increase over paracetamol control group.

KEYWORDS: Hepatoprotective activity, *Brassica oleracea*, hepatotoxicity, enzyme activity, Biochemical studies.

INTRODUCTION

Liver diseases are a serious health problem. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional system of medicine in India. However, we do not have satisfactory remedy for serious liver disease; most of the herbal drugs speed up the natural healing process of liver. So the search for effective hepatoprotective drug continues (Ayyadurai and Ramamurthy, 2009).

This organ plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. It produces bile, an alkaline compound which aids in digestion, via the emulsification of lipids. The liver's highly specialized tissues regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Ramamurthy and Raveendran, 2010).

The liver is a vital organ present in vertebrates and some other animals. The liver is a reddish brown organ with four lobes of unequal size and shape. A human liver normally weighs between 1.4–1.6 kg (3.1–3.5 lb) and is a soft, pinkish-brown, triangular organ. It is both the largest internal organ (the skin being the largest organ overall) and the largest gland in the human body (Cotran et al., 2005). Liver is the vital organ of metabolism and excretion. About 20,000 deaths are found every year due to liver disorders. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2,50,000 new cases each year. In India, about 40 polyherbal commercial formulations reputed to have hepatoprotective action are being used. It has been reported that 160 phytoconstituents from 101 plants have hepatoprotective activity (Handa et al., 1986). Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthines. Plant extracts of many crude drugs are also used for the treatment of liver disorders. Extracts of different plants of about 25 plants have been reported to cure liver disorders (Sharma et al., 2002).

In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cell (Chaterjee, 2000). There are however, members of drugs employed in traditional system of medicine for liver affections (Chattopadhyay, 2003). Many formulations containing herbal extracts are sold in the Indian market for liver disorders. But management of liver disorders by a simple and precise herbal drug is still an intriguing problem. Several Indian medicinal plants

have been extensively used in the Indian traditional system of medicine for the management of liver disorder. Some of these plants have already been reported to posses strong antioxidant activity (Achuthan et al., 2003).

Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione (GSH) depletion. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses (Ramamurthy and Raveendran, 2010). Paracetamol toxicity is due to the formation of toxic metabolites when a part of its metabolized by cytochrome P-450. Introduction of cytochrome (Dahlin et al., 1984) or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity (Gupta et al., 2006).

Broccoli (*Brassica oleraceae* L. var. *italica*) belongs to the Brassicaceae family and is closely related to the cabbage, cauliflower and brussels sprouts. Broccoli is an edible green plant in the cabbage family whose large flowering head is eaten as a vegetable. The word broccoli comes from the Italian plural of broccolo, which means "the flowering crest of a cabbage", and is the diminutive form of brocco, meaning "small nail" or "sprout" (Marino *et al*., 2016). Broccoli is often boiled or steamed but may be eaten raw. Broccoli is classified in the Italica cultivar group of the species *Brassica oleracea*. Broccoli has large flower heads, usually green in color, arranged in a tree-like structure branching out from a thick, edible stalk. The mass of flower heads is surrounded by leaves. Broccoli resembles cauliflower, which is a different cultivar group of the same species (Mahn *et al*., 2014; Li and Blande, 2015). Therefore, the present investigation has been designed to study the possible mechanism of ethanolic extract of *Brassica oleracea* on the biochemical parameter against paracetamol induced hepatic damage in albino rats.

MATERIALS AND METHODS

For the study, the flower of *Brassica oleracea* L. *var italica* plenck belongs to family Brassicaceae was collected from Super market, Thanjavur, Tamilnadu, South India. The flower was identified with the help of flora of presidency, Tamil Nadu and Karnatic flora (Gample, 1967) and standard references (Kirtikar and Basu, 1993).

Fresh flower was dried thoroughly under shade and cut into small pieces and added with deionized water about 2 cm over, boiled for 15min and filtered through three layers of fine cleaned gauze. Residues were boiled again in deionized water for 15min and then filtered. The filtrate of both extractions were combined and centrifuged at 2000 rpm for 10min. The supernatant was filtered using the filter paper no. 1. The clear filtrate was lyophilized to dry by a freeze dryer and kept in the light protected bottle at 4^0C. The extract was suspended in 5% gum acacia and used for studying hepatoprotective activity.

Paracetamol toxicity: The capitula paracetamol and saline were given with the help of feeding cannels. Four groups (Group I, Group II, Group III and Group IV) of rats, three rats in each group were taken. The *Brassica oleracea* extract at a fixed dose (250 mg/kg) that was daily fed for fifteen days to one group (Group III) of rats and paracetamol (200 mg/kg) was administered on five day after 5^{th} day's administration of the extract. The paracetamol treated group (Group II) received normal saline in place of plant extract. After 48h of paracetamol feeding rats were sacrificed by cervical dislocation for estimation of blood biochemical parameters and serum marker enzymes were analyzed following standard methods.

Biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) by the methods of Reitman and Frankel (1957), alkaline phosphatase (Kind and King, 1954), total bilirubin (Mallay and Evelyn, 1937) and protein (Lowry et al., 1951) were analyzed. Reduced glutathione (GSH) was estimated using DTNB (Sedlak and Lindasy, 1968). The blood glutathione was estimated by the method of Beutler *et al*. (1963). The concentration of Thiobarbutiric acid reactive substances (TBARS) was measured in liver using the method of Ohkawa *et al*. (1979).

RESULTS

Natural therapy for various human ailments purified with plant products has gained much attention now a day's *Brassica oleracea* L. var *italica* is frequently used for the food. The use of herbal preparations in the treatment of diseases is very common in the rural communities of world. The treatment with the extract did not decrease water and food consumption. The body weight of the rats treated with hydroalcoholic extract once a day during 15 days (sub-acute treatment) did not show any significant change when compared with the control group, although had a tendency to decrease body weight (100 mg/kg). This decrease can be associated with the decrease of liver weight at the dose of 250 mg/kg in comparison with the control group without any concomitant alteration in the activity of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. Estimation of the serum activity of total bilirubin, protein, reduced glutathione, TBARS, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase is one of the most widely used means of measuring hepatocellular injury (Table 1). The macroscopic analysis of the target organs of the treated rats (liver, lung, heart, spleen & kidney) did not show significant changes in colour and texture when compared with the control group (Table 2).

Table 1: Effect of *Brassica oleracea* extracts on some biochemical and serum marker enzyme parameters in Paracetamol intoxicated albino rats.

Parameters	Control	Paracetamol treated (200 mg/kg)	*Brassica oleracea* (250mg/kg)	Silymarin (25 mg/kg)
Bilirubin (mg/dl)	0.90 ± 0.22	2.67 ± 0.18	1.25 ± 0.15	1.01 ± 0.12
Protein (g/dl)	7.82 ± 0.16	5.15 ± 0.12	7.19 ± 0.19	7.52 ± 0.18
TBARS (n moles/ml)	2.85 ± 0.18	5.70 ± 0.16	3.00 ± 0.16	2.90 ± 0.15
GSH (µ mole/g of tissue)	7.68 ± 0.12	3.50 ± 0.11	7.02 ± 0.19	7.25 ± 0.17
SGOT (IU/L)	128 ± 1.22	189 ± 2.48	142 ± 2.36	134 ± 2.29
SGPT (IU/L)	45 ± 1.25	120 ± 2.12	63 ± 2.18	51 ± 2.34
ALP (IU/L)	138 ± 2.16	287 ± 4.62	165 ± 5.84	143 ± 4.28

Results are mean of three observations ± S.E.M.

Table 2: Effect of Brassica Oleracea extracts on body and organs weight in Paracetamol intoxicated rats.

Dose (mg/kg)	Control	Paracetamol treated (200 mg/kg)	*Brassica oleracea* (250mg/kg)	Silymarin (25 mg/kg)
Body (g)	195 ± 3.17	170 ± 1.90	181 ± 3.8	186 ± 3.77
Liver (g)	1.90 ± 0.200	1.56 ± 0.140	1.59 ± 0.177	1.68 ± 0.225
Heart (g)	0.164 ± 0.018	0.144 ± 0.017	0.155 ± 0.028	0.163 ± 0.037
Lungs (g)	0.213 ± 0.037	0.191 ± 0.016	0.202 ± 0.064	0.235 ± 0.040
Spleen (g)	0.186 ± 0.092	0.136 ± 0.031	0.156 ± 0.013	0.175 ± 0.051
Kidney (g)	0.85 ± 0.021	0.56 ± 0.017	0.72 ± 0.027	0.82 ± 0.025

Mean values of 3 animals ± S.D.

DISCUSSION

For centuries medicinal plants have been used for the treatment of various diseases. Over the centuries, the use of medicinal herbs has become an important part of daily life despite the progress in modern medical and pharmaceuticals research. Approximately 6000 plants species are known to have medicinal properties in India. The Rigveda mentions the use of medicinal plants. Our traditional systems of medicines, viz., Ayurveda, Yunani, Siddha and Homeopathy etc. use herbs for treatment. It is estimated that 40% of the world populations depends directly on plant based medicine for their health care.

The results of biochemical parameters revealed the elevation of enzyme level in Paracetamol treated group, indicating that Paracetamol induces damage to the liver (Table 1). Liver tissue rich in both transaminases increased in patients with acute hepatic diseases, SGPT, which is slightly elevated by cardiac necrosis, is a more specific indicator of liver disease (Murugaian et al., 2008 and Sukumaran et al., 2008). A significant reduction ($P < 0.001$) was observed in SGPT, SGOT, ALP, total bilirubin and protein levels in the groups treated with silymarin and alcoholic extract of *S. brevistigma*. The enzyme levels were almost restored to the normal (Ayyadurai and Ramamurthy, 2009).

The present study was observed that *B. oleracea* has a significant hepatoprotective effect in paracetamol administrated rats that hepatocellular degenerative and necrotic changes are slight without advanced fibrosis and cirrhotic process in *B. oleracea* treated group. However, Ramamurthy and Raveendran (2010) found that *Nigella sativa* L can prevent liver fibrosis and cirrhosis, suggesting that *Nigella sativa* L protects liver against fibrosis possibly through immunomodulator and antioxidant activities. Paracetamol is a common antipyretic agent, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses. Protection against paracetamol-induced toxicity has been used as a test for potential hepatoprotective activity by several investigations (Kumar et al., 2004).

Liver is the most important and main part of the animal body. It is highly affected primarily by toxic agents and that is why the above-mentioned parameters have been found to be of great importance in the assessment of liver damage. The abnormal high level of serum ALT, AST, ALP and bilirubin observed in our study (Table 1) are the consequence of paracetamol induced liver dysfunction and denotes the damage to the hepatic cells. Treatment with AET of *Brassica oleracea* reduced the enhanced level of serum ALT, AST, ALP and bilirubin, which seem to offer the protection and maintain the functional integrity of hepatic cells.

Liver is plays an important role in the protein synthesis. It is considerably affected when there is a disturbance in protein metabolism. The site-specific oxidative damage of some of the susceptible amino acids of proteins is now recorded as the major cause of metabolic dysfunctions during pathogenesis (Uday Bandyopandhyay et al., 1999). Decrease in serum bilirubin after treatment with the extract in liver damage indicated the effectiveness of the extract in normal functional status of the liver. In the present study the lowered level of total proteins and bilirubin recorded in blood sample of paracetomal treated rats reveals the severity of hepatopathy. In the *Brassica oleracea* treated group, the protein and bilirubin level of

animal was almost normal. This result is support by stimulations of protein synthesis have been advanced as a contributory hepatoprotective mechanism, which accelerates the regeneration process and the protection of liver cell (Ayyadurai and Ramamurthy, 2009).

Reduced glutathione is tripeptide consisting of glutamate, cysteine, and glycine. It acts as antioxidant both intracellular and extracellular. Glutathione reductase is a major enzyme in GSH regeneration which produces hydroxyl redical by reaction with H_2O_2 (Murugaian et al., 2008). In the present study the GSH level was decreased in paracetamol induced animals, while treatment with *Brassica oleracea* extract clearly enhanced the GSH levels. The restoration of GSH indicates hepatoprotective effect of herbal extract (Uday Bandyopandhyay et al., 1999).

ALT and AST are the specific markers to assess hepatocellular damage leading to liver cell necrosis. In present study ALT and AST activities were assessed as it is the more specific index of liver cell damage. High level of SGOT indicates liver damage such as due to cellular damage. SGPT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore SGPT is more specific to the liver and a better parameter for detecting liver damage (Ramamurthy and Raveendran, 2010). In the present study paracetamol injection significantly increased serum ALT and AST indicating induction of hepatic damage. Ethanol extracts of *Brassica oleracea* at the dose of 250 mg/kg decreased the levels of both SGOT and SGPT. In the present investigation, it was observed that serum SGOT, SGPT and ALT levels were significantly reduced in animals receiving *Brassica oleracea* and paracetomal than those given paracetomal alone indicating that the degree of hepatic cell damage was lesser magnitude in treated groups. In conclusion, the results of present study demonstrate that extracts of *Brassica oleracea* has potent hepatoprotective activity against paracetamol induced liver damage in rats. Hence our present investigation reveals that the *Brassica oleracea* species possess the hepatoproductivity activity.

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