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Research Article

ACUTE TOXICITY AND ANTIOXIDANT ACTIVITY OF *PARINARI CURATELLIFOLLIA* ROOT METHANOLIC EXTRACT IN CARBON TETRACHLORIDE-INDUCED TOXICITY IN WISTAR RATS

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ABSTRACT

The present study is aimed at evaluating the toxicological and antioxidant activity of *Parinari curatellifolia* (P.C) methanolic root extract on oxidative stress induced by carbon tetrachloride (CCl_4) in rats. The degree of treatment in this activity has been measured by using biochemical parameters such as serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD) and catalase (CAT) in tissues and serum. The extract produced significant antioxidant effect by decreasing the activity of serum enzymes and TBARS level, and increasing the SOD and CAT activities in the organs. From these results, it was suggested that P.C could ameliorate the tissue injuries perhaps, by its antioxidative effect, hence eliminating the deleterious effect of toxic metabolites from CCl_4.

Key Words: *Acute Toxicity, Antioxidant Activity, Parinari Curatellifollia*

INTRODUCTION

Recently, a great interest has been given to naturally occurring antioxidants which may play important roles in inhibiting both free radicals and oxidative chain-reactions within tissues and membranes (Nsimba *et al.,* 2008). Edible plants contain a wide variety of chemicals such as flavonoids and other phenolic compounds that have potential antioxidant activity through a number of different mechanisms. The proposed mechanisms for their actions include direct radical scavenging, iron chelating, direct inhibition of lipid peroxidation and inhibition of enzymes such as NO-synthase, xanthine oxidase, cyclooxygenase and lopoxygenase, (Xanthopoulou *et al.,* 2009).

Parinari curatellifolia is an evergreen tropical tree of Africa, 10-13m high, although heights of 23-26m have been recorded in certain regions. It is found in various kinds of deciduous woodland most frequently in poorly drained areas and inland at moderate altitudes (National Research Council, 2008). It is traditionally used in healing of wound and skin problems, treatment of malaria, typhoid fever, washing of fracture and gastrointestinal disorders. The leaf extracts and bark may be used as a remedy for symptoms of pneumonia or to treat ailments of the eye or ear (Venter and Venter, 1996). *Parinari curatellifolia* also called ganzakuisa in Hausa language which is widely used in some parts of Northern Nigeria as a recipe in traditional management of diseases, including liver-related illnesses has not been scientifically evaluated. Earlier unpublished *in vitro* research conducted in our laboratory revealed high concentrations of Polyphenols, flavonoids and antioxidant activity in the root methanolic extract of the plant; hence there was high positive correlation between the flavonoids concentration and antioxidant activity exhibited. In spite of the medicinal claim for the root as an antioxidant, there are no reports in the literature regarding this effect. Thus the present study was performed to evaluate the toxicological and antioxidant activity of the methanolic extract against CCl_4-induced hepatic injury in rats.

MATERIALS AND METHODS

Plant Material

The root of the plant was collected from Katsina State, Nigeria. The plant specimen was identified by Mal A.U Gallah of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University and Zaria- Nigeria, where a voucher specimen, 109 was assigned.

Preparation of Plant Extract

The pulverized sample was extracted with petroleum ether and methanol or ethanol respectively using soxhlet extractor. Pulverized sample (35g) was weighed into a thimble and placed in the soxhlet extractor. The extraction was carried out by first, using petroleum ether (300ml) then absolute methanol (300ml).

Animal Management

Male albino rats (7-8weeks old) were purchased from the animal house of National Research Institute for Chemical Technology (NARICT), Basawa-Zaria, Nigeria. They were acclimatized for two weeks prior to commencement of experiment. They were kept at room temperature and were maintained *ad libitum* on growers mash (Vital feed, Jos, Nigeria) and weighed prior to experiment.

Experimental Design

Thirty animals were randomly allotted into six experimental groups of five animals, each was administered extract intraperitoneally on daily basis for ten days, while CCl_4 was administered 72 hourly to the respective groups thus;

Animals of group 1 received vehicle only and served as normal control. Animals of group 2, 4 and 6 received CCl_4 (72 hourly) for 10 days and group 2 served as experimental control. Group 3 and 5 received treatments of extract and vitamin E respectively for 2 weeks (daily). Blood was collected from retro orbital venous plexus after 48 hrs of the last administration. The animals of entire groups were euthanized under chloroform anaesthesia; liver, kidney and heart were immediately excised and processed for biochemical analyses. Scheme of different treatments are given below;

Group 1.Solvent only (1.8mg/kg); Group 2.Solvent (1.8mg/kg) + CCl_4 (0.6ml/kg) an hour interval; Group 3.Methanolic extract (5mg/kg); Group 4.Methanolic extract (5mg/kg) + CCl_4 (0.6ml/kg) an hour interval; Group 5, vitamin E only (10mg/kg); Group 6, vitamin E (10mg/kg) + CCl_4 (0.6ml/kg) an hour interval. The solvent used here is corn oil.

Isolation of Serum and Homogenate Preparation

Serum was isolated after keeping the blood for 1 hr at room temperature followed by centrifugation at 1000 g for 15 min and stored in the deep freezer until analyzed. Tissue samples of liver and kidney were homogenized with ice-cold potassium phosphate buffer (pH 7.4) for the determination of TBARS and CAT activity and SOD activity determination.

Acute Toxicity Study

The lethal dose (LD_{50}) of the plant extract was determined by method of Lorke (1983). The animals were handled in accordance with international principles guiding the use and handling of experimental animals (United State National Institute for Health, 1985). In the first phase rats were divided into 3groups of 3 rats each and were treated with the extract at doses of 10, 100 and 1000mg/kg body weight intraperitoneal. They were observed for 24 hours for signs of toxicity. In the second phase 4 rats were divided into 4 groups of 1 rat ineach group and were also treated with the extract at doses of 600, 1000, 1600and 2900mg/kg body weight (i.p). The median lethal dose (LD_{50}) was calculated using the second phase.

Thiobarbituric Acid Reactive Substances (TBARS)

Lipid peroxidation was determined as thiobarbituric acid reactive substances as described by Torres *et al.*, (2004). Lipid peroxidation generates peroxide intermediates which upon cleavage release malondialdehyde, a product which react with thiobarbituric acid. The product of the reaction is a coloured complex which absorbs light at 535nm. About 2 ml of 14% trichloroacetic acid was measured into a test tube, 2ml thiobarbituric acid (0. 67 %) was added and 100µl of the tissue homogenate was added. The mixture was incubated at 80°Cfor 30 minutes in a water bath and allowed to cool immediately under ice

before centrifugation at 3000xg for 10 minutes. Thiobarbituric acid reactive substances (TBARS) content was read spectrophotometrically at 535nm.

Determination of Liver Marker Enzymes in Serum

Serum was used for the determination of aspartare aminotransaminase (AST) (Reitman and Frankel, 1957), alanine aminotransaminase (ALT) (Reitman and Frankel, 1957), by Randox assay kit (U.S.A) according to the manufacturer's instructions.

Determination of Antioxidant Enzyme Activity

Catalase (CAT)

Catalase (CAT) activity was measured using the method of Abei (1974). Exactly 10µl of serum was added to test tube containing 2.80ml of 50mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1ml of fresh 30mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240nm for 5 minutes on a spectrophotometer. A molar extinction coefficient of 0.041 $mM^{-1}cm^{-1}$was used to calculate the catalase activity.

Superoxide Dismutase (SOD)

Superoxide dismutase activity was carried out according to the method described by Martin *et al.*, (1987). Exactly 920µl of assay buffer was added into clean test tube containing 40µl of added to the sample, mixed and incubated for 2 mins at 25^0C. 40µl of hematoxylin solution was added, mixed quickly and the absorbance was measured at 560nm.

Statistical Analysis

The results obtained were statistically analyzed using Analysis of Variance (ANOVA) to get the grouped mean and student's t-test was used to separate the significant difference between the grouped means at 95% confidence level (Duncan *et al.*, 1977).

RESULTS

Acute Toxicity Studies

In the animals that were administered 10mg/kg- 1000mg/kg, no visible adverse reaction was observed up to 72nd hour post-administration. In the second phase, sign of toxicity was first noticed after 10-12 hours of extract administration. There was decreased locomotor activity and decrease in sensitivity to touch. Also there was decreased feed intake, and prostration after 18 hours of extract administration. However, all animals in the second phase (1,600-5,000mg/kg) died.In view of the above; the LD50 was calculated to be 1,265mg/kg.

Hepatoprotective Activity

Alanine aminotransferase (ALT) activity of group 2 animals (corn oil + CCl_4) increased rapidly owing to CCl_4 intoxication while treatment with the extract or vitamin E in the respective group significantly ($p< 0.05$) reduced ALT activity close to normal. The group treated with extract without CCl_4 remained within the normal range with the control. Like ALT, aspartate aminotransferase (AST) activity in CCl_4 intoxicated animals was elevated across the group, but extract treatment wasable to reduce the activity close to normal. There was no statistical difference between the effect exhibited by extract and vitamin E. however, administration of extract to normal group had no significant difference on the enzyme activity.

Anti-Lipidperoxidation Andantioxidant Activity

Oxidative stress marker thiobarbituric acid reactive substance (TBARS) levels as well as SOD and catalase activities incontrol, CCl_4 intoxication, P.C treated rats are shown in figure 2, 3 and 4. TBARS levels were assessed as indicator oflipid peroxidation, CCl_4 treatment significantly ($P<0.05$)increased the level of TBARS in the liver, kidneys and heart tissues ascompared to control.However, treatment with P.C extract significantly ($P<0.05$) decreased the level of TBARS as compared to the CCl_4 treated rats.

Data presented showed that CCl_4 administration causedsignificant decrease ($P<0.05$) in SOD activity as comparedto control. Treatment of rats with P.C extract significantly increased ($P<0.05$) the the activity as compared to CCl_4treated group. Concerning the effect of CCl4 on thecatalase (CAT) activity, a significant decrease ($P<0.05$) inthe CAT activity was recorded as compared to the controlrats (figure 4).

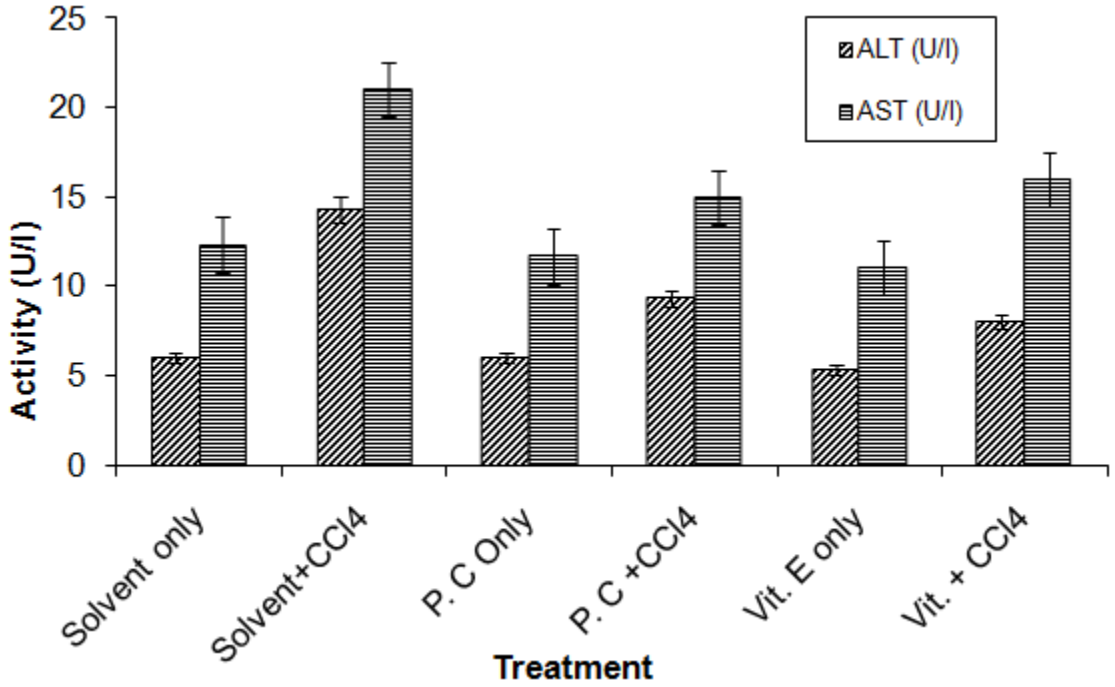

Figure 1: Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activities in rats treated with *Parinari curatellifolia* (P.C) methanolic root extract owing to CCl_4 intoxication

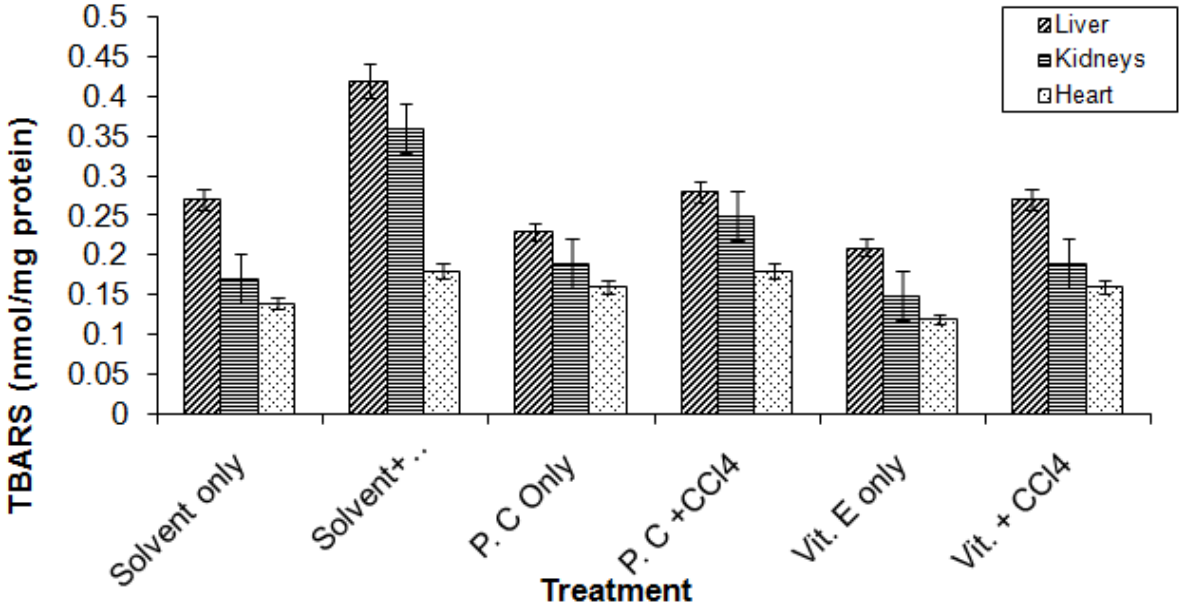

Figure 2: Thiobarbituric Acid Reactive Substances (TBARS) levels in rats treated with *Parinari curatellifolia* (P.C) methanolic root extract owing to CCl_4 intoxication

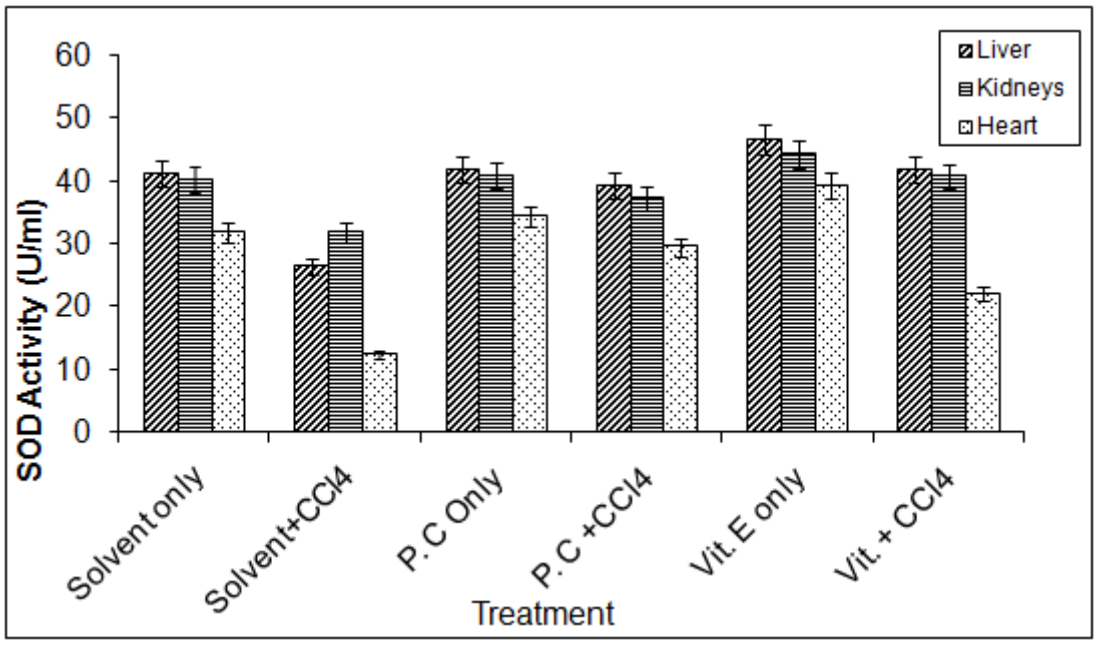

Figure 3: Superoxide Dismutase (SOD) activity in rats treated with *Parinari curatellifolia* (P.C) methanolic root extract owing to CCl_4 intoxication

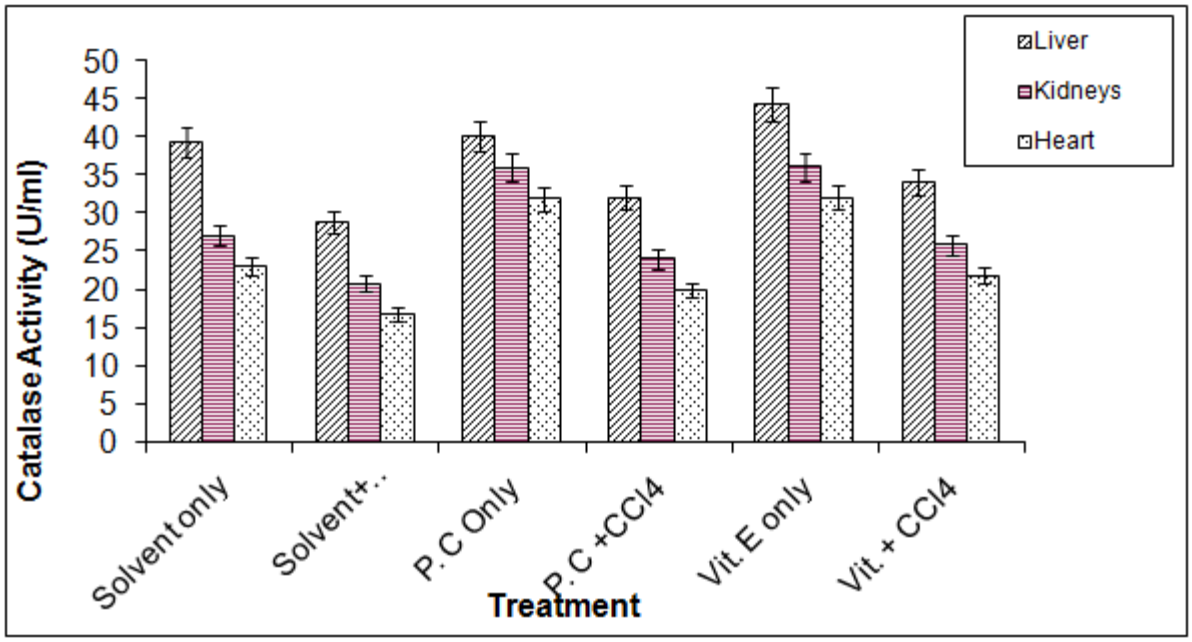

Figure 4: Catalase activity in rats treated with *Parinari curatellifolia* (P.C) methanolic root extract owing to CCl_4 intoxication.

Meanwhile, CAT activity increased significantly ($P<0.05$) after treatment either with extract orvitamin E at the the respective doses. Administration of extract or vitamin E toCCl_4 untreated rats, had no significant effect on the studied oxidative stress markers in the organs as compared to control.

DISCUSSION

For the therapeutic strategies of liver injury and disease, it is important to find antioxidant compounds that are able to block liver injuries through free radicals generated due to toxic chemicals. Liver damage induced by carbon tetrachloride (CCl_4) involves biotransformation of free radical derivatives, increased lipid peroxidation and excessive cell death in liver tissue (Recknagel *et al.,* 1989). Toxic effects of CCl4 on liver have been extensively studied (Junnila *et al.,* 2000; Amin and Mahmoud, 2009; Cui *et al.,* 2009 and Kim *et al.,* 2010). Serum AST and ALT are the most sensitive biomarkers used in the diagnosis of liver diseases (Pari and Kumar, 2002). During hepatocellular damage, varieties of enzymes normally located in the cytosol are released into the blood flow. Their quantification in plasma is useful biomarkers of the extent and type of hepatocellular damage (Pari and Murugan, 2004). Serum ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, serum ALT is more specific to the liver, and is thus a better parameter for detecting liver injury (Williamson *et al.,* 1996). In conjunction with the reports of Cho *et al.,* (2009); Hegde and Joshi, (2009); Kim *et al.,* (2010), data from the present study showed that CCl_4 caused hepatic damage with a significant increase in serum levels of AST and ALT. Treatment with P.C methanolic root extract significantly decreased the activities of these enzymes in CCl_4-treated rats indicating maintenance of functional integrity of hepatic cell membrane. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (The brews and Joice, 1987).

In recent years, attention has been focused on the role of biotransformation of chemicals to highly reactive metabolites that initiate cellular toxicity. Many compounds, including clinically useful drugs, can cause cellular damage through metabolic activation of the chemicals to highly reactive compounds such as free radicals. CCl_4 has probably been studied more extensively both biochemically and pathologically than any other hepatotoxin (Cui *et al.,* 2009 and Kim *et al.,* 2010). The mechanism of CCl_4 injury involves oxidative damage by metabolism of CCl_4 to CCl_3• in hepatocytes; this causes cell death with accumulation of lipid peroxidation and intracellular calcium ions and triggers secondary damage from the inflammatory process (Medina and Moreno-Otero, 2005).

CCl_4 and its metabolites are capable of initiating a chain of lipid peroxidation reactions by abstracting hydrogen from polyunsaturated fatty acids (PUFA). Peroxidation of lipids, particularly those containingPUFA, can dramatically change the properties of biological membranes, resulting in severe cell damage and play a significant role in pathogenesis of some diseases (Aleynik *et al.,* 1997). Enhanced lipid peroxidation (LPO) is a measure of membrane damage as well as alteration in structure and function of cellular membranes (Halliwell *et al.,* 1995). The increased TBARS level suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to preventformation of excessive free radicals (Szymonik *et al.,* 2003; Liu *et al.,* 2009 and Kim *et al.,* 2010). However, treatment with extract decreased the LPO levels, which may be due to the free radical scavenging activity of the extract. SOD is a major endogenous antioxidant which counterbalances free radical mediated damage. It is well known that SOD is involved in the protection of normal cell structure and function by maintaining the redox homeostasis, quenching of free radicals and by participating in detoxification reactions (Pushpakiran *et al.,* 2004). The present study confirmed the finding of Srivastava *et al.,* (1983) who suggested that enhancement of lipid peroxidation is a consequence of depletion of SOD to certain critical levels. Insufficiency in enzymatic antioxidant SOD, following CCl_4 intoxication could be the consequence of increased utilization for trapping free radicals. In consonance with our study, Hong *et al.,* (2009), Liu *et al.,* (2009) and Kim *et al.,* (2010) have reported depletion in SOD level in the liver of CCl_4 intoxicated rats. Treatment with the extract in the present study restored SOD activity.In accord with our results, Gate *et al.,* (1998) have reported that dietary supplementation of the marine extract of the *Crassostreagigas*clams increased SOD level in the liver of rats.

Catalase (CAT) is one of the important enzymes in the supportive team of defense against reactive oxygen species (ROS). Catalase is a haemoprotein containing four haeme groups, that catalyses the

decomposition of H_2O_2 to water and O_2 and thus, protects the cell from oxidative damage by H_2O_2 and OH (Gupta *et al.*, 2004). Viewed in conjunction with the report of Szymonik *et al.*, (2003), the inhibition of CAT activity following CCl_4 intoxication in the present study may be due to the enhancement of the peroxidation end product TBARS, which is known to inhibit protein synthesis and the activities of certain enzymes. Administration of P.C methanolic extract and vitamin E enhanced the activity of CAT in CCl_4 – induced liver damage. The enhancement in CAT activity may be due to prevent the accumulation of excessive free radicals and protect liver from CCl_4–intoxication. In conjunction with the report of Balamurugan *et al.*,(2009) who reported that the livercells innate ability to arouse and maintain defense against oxidant by secreting more antioxidants isoverpowered by the CCl_4. P.C extract may overpowers CCl_4 onslaught by suppressing the formation of ROS and protecting the antioxidant machinery.

In conclusion, the results of the present study indicate that intraperitoneal administration of P.C methanolic extract attenuate disrupted hepatic and serum ROS metabolism associated with hepatic injury progression in rats intoxicated with CCl_4 through their antioxidant action which is related to their contents. However, further research must be carried out to elucidate the mechanisms of the toxicity and antioxidant effect *Parinari curatellifolia.*

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