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COMPARATIVE EVALUATION OF HEPATOPROTECTIVE EFFECTS OF EXOTIC FRUITS AND COMMON VEGETABLES EXTRACTS ON CCL₄ INDUCED HEPATOTOXICITY: AN *IN VITRO* STUDY

Kavitha Raj V.¹, J. R. Kumar^{*1}, S. Balasubramanian¹, S. Najunda Swamy³, D. Keerthini⁴, N. Shambhavi⁴, B. M. Kanthesh² and K. O. Avinash¹

Division of Biochemistry¹, Division of Molecular Biology², Faculty of Life Science, JSS University, Mysuru- 570015 Karnataka India.

Department of Biotechnology³, Sri Jayachamarajendra College of Engineering, Mysuru - 570006 Karnataka India.

Department of Biochemistry⁴, JSS College of Arts, Commerce and Science, Mysuru-570025 Karnataka India.

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Correspondence to Author:

Dr. J. R. Kumar

Assistant Professor
Division of Biochemistry
Faculty of Life Sciences, JSS
University, JSS Medical Institutions
Campus, Sri Shivarathreshwara
Nagara, Mysuru, Karnataka 570015,
India


Email: kumarjr2013@gmail.com

ABSTRACT: Liver toxicity faces a major challenge in world health issues. Many drugs are being withdrawn from the market by Food and Drug Administration (FDA) for causing drug induced hepatotoxicity. In the present study, ameliorative activity of methanoic extracts of different parts (Peel, Pulp and seed) of exotic fruits and vegetables on carbon tetrachloride (CCl₄)-induced hepatotoxicity were studied in *in vitro* condition. Chicken liver cell suspension treated with CCl₄ was used for lipid peroxidation (LPO), free radical scavenging and sodium dismutase (SOD) assay. Extracts from different parts of exotic fruits and common vegetables showed potent antioxidant effect. In the present study even, methanoic extract of some unused parts (peel & seed) of vegetables and fruits showed hepatoprotective action, but among all these bottle gourd pulp (vegetable) and passion fruit pulp (exotic fruit) showed the noteworthy hepatoprotective action. Thus it is concluded that, the methanoic extracts of exotic fruits and vegetables was observed to have notable protective activity on CCl₄ induced hepatotoxicity.

INTRODUCTION: The liver is the body's largest glandular and principle organ for maintaining the body's intramural environment. The liver supports almost every organ in the body in some facet and is vital for survival.

Some of these major functions include protein, carbohydrate and fat metabolism, detoxification, secretion of bile and storage of vitamin. It performs multiple critical functions to keep the body pure by filtering toxins and many drugs from the blood; transforms and excretes. Thus, to maintain a healthy liver is a crucial factor for the overall health and well being of human¹.

Even though liver has tremendous regeneration capacity, the liver injury morbidity has been increasing vigorously. Among all the causes for

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hepatotoxicity, drug induced liver injury is prominent. It has an estimated annual incidence between 10 and 15 per 10,000 to 100,000 persons exposed to prescription medications² and Drug-induced acute liver failure (ALF) accounts for approximately 20% of ALF in children and a higher percentage of ALF in adults³. Hence hepatotoxicity is nowadays one of the most important pharmacovigilance concerns and the leading cause for drug withdrawal on safety grounds⁴.

Chemical toxins (including acetaminophen, carbon tetrachloride, galactosamine and thioacetamide are often used as the model substances causing experimental hepatocyte injury in both in vivo and in vitro conditions⁵⁻⁸. Most of the hepatotoxic chemicals cause damage to the hepatocytes by inducing lipid peroxidation⁹. Liver cirrhosis induced by CCl₄ is perhaps the best-studied model of liver cirrhosis¹⁰. CCl₄ was the first toxin for which it was shown that the injury produced is largely or entirely mediated by a free-radical mechanism. It is believed that CCl₄ is metabolized by the cytochrome P450 system to give the trichloromethyl radical, a carbon-centred radical. Covalent binding of trichloromethyl free radicals to cell proteins is considered to be the initial step in a chain of events that eventually lead to membrane lipid peroxidation (LPO) and finally to cell apoptosis and necrosis¹¹⁻¹³.

High levels of reactive oxygen species (ROS) damage cells and are involved in several human pathologies, including liver cirrhosis and fibrosis. Among the degradation products of fatty acids are reactive aldehydes, malondialdehyde (MDA), and 4-hydroxynonenal, which bind easily to functional groups of proteins and inhibit important enzyme activities. Disturbed cellular processes are most likely due to increased levels of these thiobarbituric acid reactive species (TBARS)¹⁴, lactate dehydrogenase (LDH) leakage as a result of membrane breakdown and concomitant increase in membrane permeability¹⁵, loss of cell protection, witnessed by GSH depletion and as a result of all these changes - cell death. The second phase of CCl₄ - induced hepatotoxicity involves the activation of Kupffer cells, which is accompanied by the production of proinflammatory mediators¹⁶.

Hence, CCl₄ – induced hepatotoxicity serves as an excellent model to study the molecular, cellular and morphological changes in the liver¹⁷.

Hence extracts with high free radical scavenging activity/antioxidants may show hepatoprotection. Antioxidants also turn free radicals into waste byproducts, and they eventually get eliminated from the body. They also have the ability to repair previous damage to cells. However, consumption of fruits and vegetables is known to lower the risk of several diseases, and such health benefits are mainly imposed due to the presence of phytochemicals, such as polyphenols, carotenoids and Vitamin E and C.

There are approximately 5000 known plant phenolics and model studies have demonstrated that many of them have antioxidant activity¹⁸. Ayurveda, Siddha, Unani etc., are practiced in India and these practices take totally rely on the plant source. As a natural way, consumption of fruits and vegetables has showed therapeutic values in these traditional medicines. Majority of edible plants and its parts such as fruits, seeds, leaves and roots are also observed to have medicinal value substances, such as antioxidants, flavonoids, tannins and other phenolics compounds¹⁹. Therefore in the present study we have tried extracts different parts of exotic fruits and common vegetables on hepatoprotectivity.

MATERIALS AND METHODS:

Chemicals:

Analytical grade Carbon Tetrachloride (CCl₄) was procured from Hi-Media Laboratory Pvt Ltd, Bengaluru. Methanol was purchased from Chethana chemicals, Mysore. All other chemicals used in the present study were of analytical grade.

Liver sample:

Fresh liver sample of healthy adult chicken weighing approximately 1.2-1.5 kg obtained from local slaughter house was brought to laboratory under frozen condition and used immediately.

Liver cell suspension:

Liver cell suspension was prepared by continuous shaking method²⁰.

Assay for Lipid Peroxidation:

Lipid peroxidation was estimated colorimetrically by measuring malondialdehyde (MDA) ^{21, 22}. In brief, 0.5 ml of homogenate treated with 0.5 ml carbon tetrachloride and 100µg/ml of fruit/vegetable extract along with 2 ml of a 1:1:1 ratio of TBA-TCA-HCl (TBA 0.6%, TCA 15%, HCl 0.25 N) reagent added and placed in water bath at 65°C for 15 min, cooled, and centrifuged at 5,000 rpm for 10 min at room temperature. The optical density of the clear supernatant was measured at 535 nm. Controls were prepared similarly without CCL₄ for each extract. The MDA formed was calculated by using the molar extinction coefficient of thiobarbituric acid reactants (TBARS; 1.56×10^5 l/mole cm⁻¹). The product of LPO was expressed as nmol of MDA formed per g of tissue.

Free Radical Scavenging Assay:

Free radical scavenging activity was measured by a decrease in absorbance at 517 nm of a solution of coloured 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) in methanol brought about by the sample. To the 1ml methanolic solution of DPPH, 0.5 ml of homogenate treated with 0.5 ml carbon tetrachloride and 100µg/ml of fruit/vegetable extract was added. The reaction mixture was allowed to incubate for 30 min at room temperature in the dark and the absorbance of the resulting mixture was measured at 517 nm against ascorbic acid as standard ²³. Controls were prepared similarly without CCL₄ for each extract. The scavenging activity was calculated as follows:

% Radical Scavenging

Activity = $[\text{Abs control} - \text{Abs sample} / \text{Abs control} \times 100]$

Superoxide Dismutase (SOD) assay:

The assay of SOD is based on the inhibition of the formation of NBT- diformazan which is measured at 560nm. Cuvets containing 1.17×10^{-6} M riboflavin, 0.01 M methionine, 2×10^{-5} M sodium cyanide, 5.6×10^{-5} M NBT, and 0.05 M potassium phosphate at pH 7.8 were illuminated and the absorbance was read at 560nm ²⁴. Controls were prepared similarly without CCL₄ for each extract. The % inhibition was calculated by

% inhibition = $[\text{Abs control} - \text{Abs sample} / \text{Abs control} \times 100]$

Fruit and vegetable extract preparation:

Fresh exotic fruit (**Table 1a**) and vegetables (**Table 1b**) were purchased from hopcoms and were washed under running tap water and their peel, pulp and the seeds were separated. They were shade dried separately for over a month. The dried fruit parts were ground using a mechanical grinder. Each of the samples was approximately weighed and subjected to extraction by cold maceration with 80% methanol at room temperature. The macerate was allowed to soak in 80% methanol for 72 hours ²⁵. The samples were filtered after 72 hours using regular filter paper. The methanolic extracts thus obtained was collected in the vials and stored at 4° C.

TABLE 1a: EXOTIC FRUITS USED IN EXPERIMENT

Exotic fruits	Scientific Name
Date Palm	<i>Phoenix Dactylifera</i>
Grape Vine	<i>Vitis Vinifera</i>
Passion Fruit	<i>Passiflora Edulis</i>
California Wild Grape	<i>Vitis Californica</i>
Kiwi Fruit	<i>Actinidia Deliciosa</i>
Wood Apple	<i>Limonia Acidissima</i>
Indian Jujube	<i>Ziziphus Mauritiana</i>
Avocado	<i>Persea Americana</i>
Litchi	<i>Litchi Chinensis</i>
Java Plum/Black Palm	<i>Syzygium Cumini</i>
Rose Apple	<i>Syzygium Jambos</i>
Soursop	<i>Annona Muricata</i>

TABLE 1 b: COMMON VEGETABLES USED IN EXPERIMENT

Common Vegetables	Scientific Name
Eggplant	<i>Solanum melongena</i>
Bitter Gourd	<i>Momordica charantia</i>
Ivy Gourd	<i>Coccinia grandis</i>
Tomato	<i>Solanum lycopersicum</i>
Green chilli	<i>Capsicum frutescens</i>
Ladies finger	<i>Abelmoschus esculentus</i>
Cucumber	<i>Cucumis sativus</i>
Bottle Gourd	<i>Lagenaria siceraria</i>
Bell peppers	<i>Capsicum annum</i>
Snake Gourd	<i>Trichosanthes cucumerina</i>

RESULTS AND DISCUSSION: Liver is the very important part of our body responsible for the maximum metabolic and secretory activities and therefore appears to be a sensitive target site for substances modulating biotransformation. Liver is also associated in detoxification from the

exogenous and endogenous challenges like xenobiotics, drugs, viral infections and chronic alcoholism. CCl_4 has been extensively used to study liver injury induced by ROS in the animal models, which is closely analogous to hepatotoxicity in humans. The toxic effects of CCl_4 are the consequences of production of free radicals. Unless neutralized by radical scavengers, these peroxy radicals in turn abstract hydrogen atoms from other lipid molecules, thereby propagating the process of LPO²⁶.

The present study was planned to evaluate toxic effects of carbon tetrachloride by a biochemical parameter indicative of oxidative stress and its amelioration by different fruits and vegetables *in vitro*.

All the plant samples with different parts of fruit and vegetable extracts showed considerable values. The peel extract of litchi fruit and california grapes was found to inhibit lipid peroxidation very effectively compared to the peel extracts of other fruits. Black plum and passion fruit showed less effectiveness (**Fig.1a**). Among vegetables peel extracts of Brinjal and Cucumber was found to inhibit peroxidation very effectively compared to the peel extracts of others but ladies finger had least effect (**Fig.1b**). Apart from peel extract, the pulp extracts also showed LPO inhibition.

Among all the pulps of vegetables, Tomato and Bottle gourd pulps were found to inhibit LPO more effectively compared to other vegetable pulp extracts (**Fig.1c**) and in fruit extracts, passion fruit and soursop were found to inhibit lipid peroxidation more effectively (**Fig.1d**). The fruit and vegetable seeds were found to exhibit some of the beneficial effects in controlling hepatotoxicity by inhibiting lipid peroxidation. Avocado and rose apple among fruits (**Fig.1e**); Capsicum and bottle gourd among vegetables (**Fig.1f**) showed efficient inhibition of lipid peroxidation compared with the others.

The free radical scavenging capacity of the fruit and vegetable extracts was determined using a stable free radical DPPH. In the present investigation, snakegourd seed, bottle gourd pulp and brinjal peel showed high free radical

scavenging activity among all the other vegetables; in fruits, litchi peel, passion fruit pulp and rose apple seed showed free radical activity against ascorbic acid as positive standard.

It is well documented that the many life forms have enzymatic and non-enzymatic antioxidant systems, which possess the ability to protect the body from damage caused by free radicals as self defensive mechanism and Superoxide dismutase is one among them. Superoxide dismutase is an antioxidant enzyme which metabolizes toxic oxidative intermediates. SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide. So, the accumulation of peroxide or products of its decomposition may also be aided by a decrease in SOD activity²⁷. In the present study passion fruit pulp among fruits and bottle gourd pulp among vegetables showed high SOD activity.

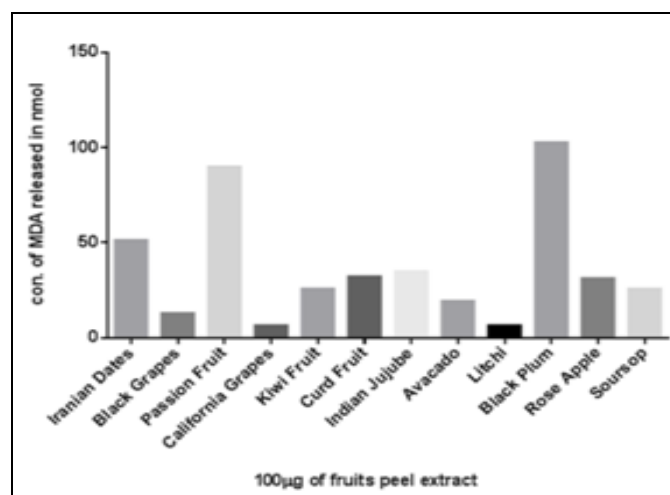


FIG.1a: LPO ASSAY OF FRUIT PEEL

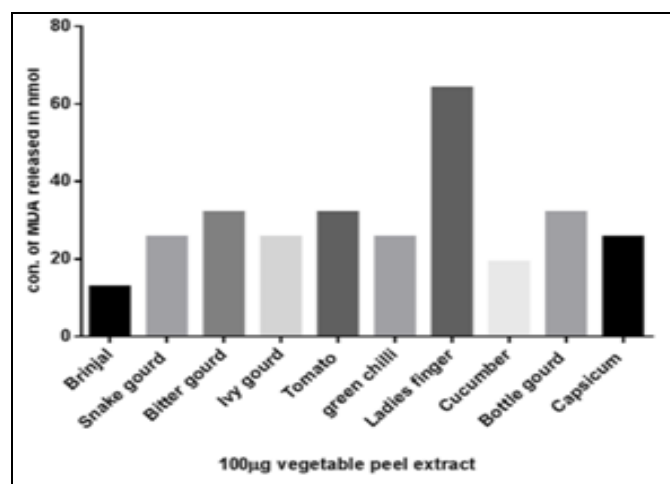


FIG.1b: LPO ASSAY OF VEGETABLE PEEL EXTRACT

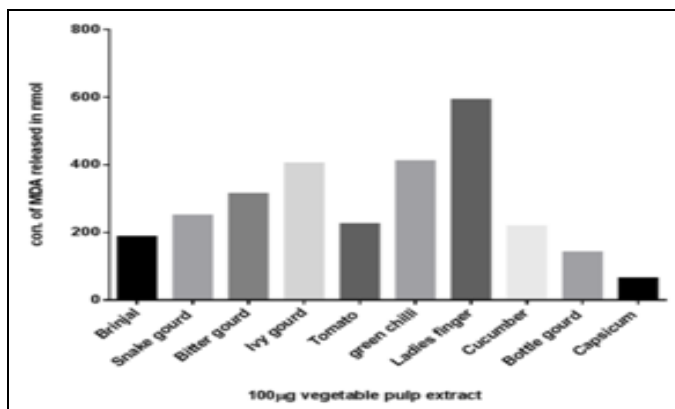


FIG.1c: LPO ASSAY OF VEGETABLE PULP EXTRACT

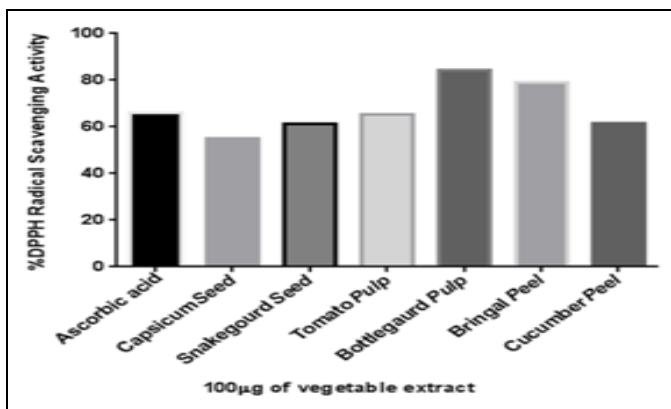


FIG.2a: DPPH ASSAY OF VEGETABLES

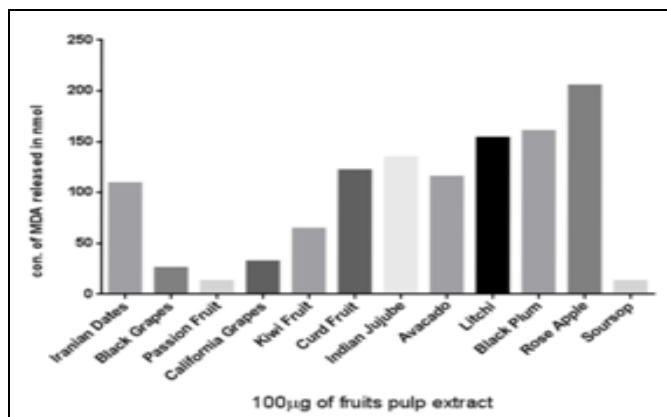


FIG.1d: LPO ASSAY OF FRUIT SEED EXTRACT

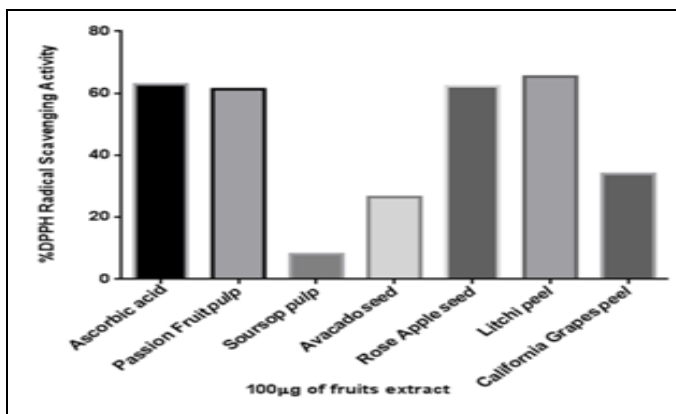


FIG.2b: DPPH ASSAY OF FRUITS

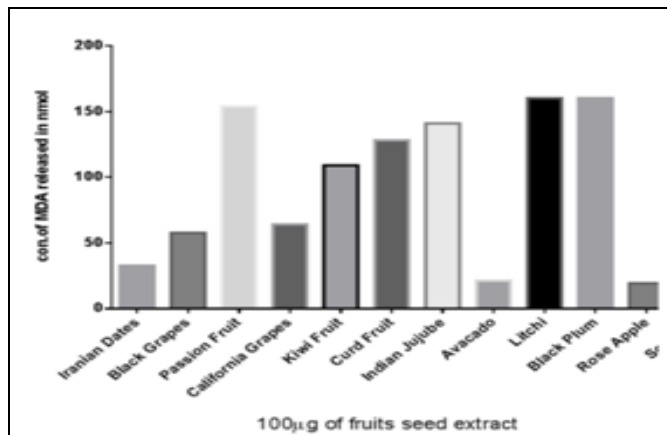


FIG.1e: ASSAY OF FRUIT SEED EXTRACT

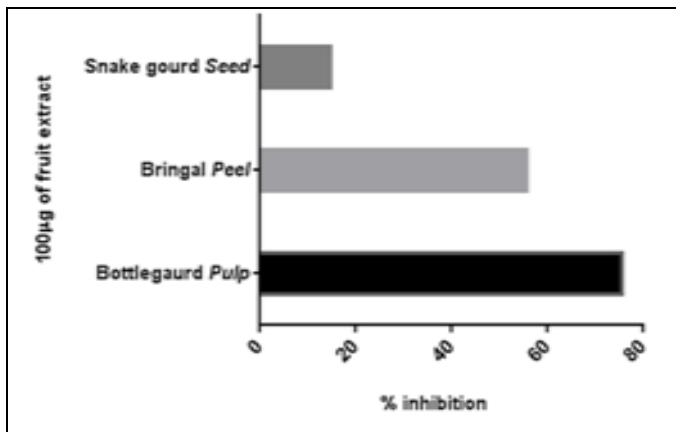


FIG.3a: SOD ASSAY OF VEGETABLES

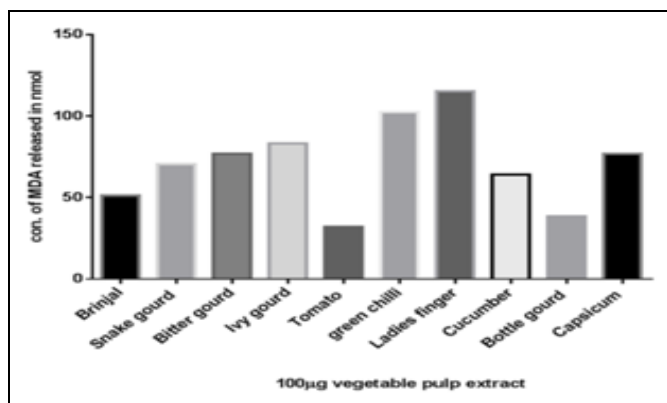


FIG.1f: ASSAY OF VEGETABLE SEED EXTRACT

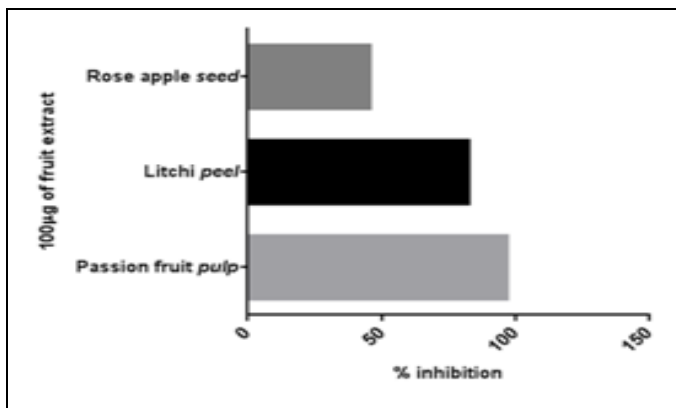


FIG.3b: SOD ASSAY OF FRUITS

CONCLUSION: This paper reports the use of whole plant (peel, pulp, seed) extracts of commonly used vegetables and exotic fruits on hepatoprotection. It showed all the extracts with potent action on hepatotoxicity. This it is concluded from the above results that even the unused parts of the exotic fruits and common vegetables methanoic extracts showed a significant anti-hepatotoxic effect on CCl₄ induced liver toxicity in Invitro condition.

CONFLICT OF INTEREST: The authors have no conflict of interest.

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