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Evaluation of Antioxidant Activity of *Averrhoa bilimbi* Linn. Fruit Juice in Paracetamol Intoxicated Wistar Albino Rats

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Abstract

Free radicals are reactive molecules involved in many physiological processes and human diseases. As a result of which, much attention has been directed towards the studies regarding free radical scavenging activity or antioxidant activity of plants and plant extracts. The present study was undertaken to assess the antioxidant potential of fresh fruit juice of *Averrhoa bilimbi* in the paracetamol intoxicated Wistar albino rats. The rats were divided into five groups of six animals each comprising of Healthy Control, Disease Control (paracetamol challenged), Standard Drug treated (paracetamol and silymarin), Test extract treated (paracetamol and *A. bilimbi*) with Lower Dose (LD) (250 mg/kg b.wt) and Higher Dose (HD) 500 mg/kg b.wt). Blood and tissue samples were collected and biochemical investigations were carried out. The antioxidant parameters including Superoxide dismutase (SOD), Glutathione, Glutathione peroxidise (GPx) levels were evaluated. The study showed that *A. bilimbi* extract had increased the antioxidant activity significantly both in blood and tissues of animals and the efficacy of the extract was dose dependent. The phytochemical studies showed the presence of flavanoids, phenols, and glycosides in the extract. The antioxidant property of *A. bilimbi* may be the contribution of these phytoconstituents.

Keywords Free radical scavenging; Antioxidant activity; *Averrhoa bilimbi*; Liver markers; Phytoconstituents

Introduction

The primary health care of 70-80% of the world's population is based on the use of medicinal plants derived from traditional system of medicine and local health practices [1,2]. Plants are used as pharmaceutical, nuturaceutical, cosmetics and food supplements. The modern pharmacopoeia contains at least 25 % drugs that are directly derived from plants and many others are synthetic analogue built on prototype compound isolated from plants [3-8].

India is the largest producer of medicinal herbs and is appropriately called Botanical Garden of the World. The present study has been taken up for evaluating antioxidant potential of Averrhoa bilimbi fruit juice in the paracetamol intoxicated wistar albino rats. The plant Averrhoa bilimbi commonly known as bilimbi, is essentially a tropical tree, less resistant to cold. The bilimbi tree is long lived, reaches 5 to 10 meter in height. The leaves are alternate, and cluster at branch extremities [9]. Flowers are found on the trunk and branches. Fruits of bilimbi are too sour to eat raw. The uncooked bilimbi is prepared relish and served with rice in natives of Kerala, India.

Materials and Methods

Plant Material

The fruits of *A. bilimbi* were collected from Western ghat region of Kerala, India (Thrissur and Palakkad) and the authentication of the plant was done in the Pharmacy Division, National Research Institute for Panchakarma, Thrissur.

Preparation of Extract

The fresh fruits were taken (100 gm) and crushed using mixer grinder, the juice was filtered through Whatman filter paper No.7. The clear filtrate was obtained and was stored in the refrigerator for experimental purpose.

Phytochemical Studies

The phytochemical analysis of the test extract was carried out as per the standard protocols including Salkowski test, Dragendorff's test, Keller Killani test and Ellagic acid test protocols [10-13].

Animal Experiment

The animal studies were carried out in the National Research Institute for Panchakarma, Cheruthuruthy as per CPCSEA guidelines and with the approval of Institutional Animal Ethical Committee (IAEC).

Six to seven months old Wistar albino rats weighing 150-200 gm were used for the experiment. The animals were fed with standard laboratory pellet chow (Amrit, Bangalore) and given water *ad libitum*. All rats were clinically healthy based on the random sampling and screening of biochemical parameters [14]. The animals were randomly divided into five groups of six animals each for the present experiment [14-15].

Group I: Healthy Control Group (HC)

Animals received standard food and water throughout the experiment period i.e. 10 days.

Group II: Disease Control Group (DC)

Animals received standard food and water throughout the experiment period. Oral administration of Single dose of Paracetamol 2.5 gm/kg.b.wt on 8th day.

Group III: Positive Control Group (PC)

Animals received standard food and water throughout the experiment period. Oral administration of Silymarin 100 mg/kg.b.wt. daily. Silymarin is a well known standard drug used for hepatoprotective function. Single dose of Paracetamol 2.5 gm/kg. b.wt on 8th day. This group is also called Standard Control group.

Group IV: Experimental Group: Test Drug- Lower Dose (LD)

Animals received standard food and water throughout the experiment period. Oral administration of *A. bilimbi* extracts 250 mg/kg.b.wt. daily. Single dose of Paracetamol 2.5 gm/kg.b.wt on 8th day.

Group V: Experimental Group: Test Drug- Higher Dose (HD)

Animals received standard food and water throughout the experiment period. Oral administration of *A. bilimbi* extracts 500 mg/kg.b.wt. daily. Single dose of Paracetamol 2.5 gm/kg. b.wt on 8th day.

At the end of the experiment, blood samples will be collected by retro orbital route after overnight fasting of the animals. These blood samples were allowed to clot by keeping the test tube in slanting position for 15 minutes. Then, they were centrifuged for 5000 rpm for 7 minutes, and clear supernatant portion, i.e. serum was collected for the biochemical investigation purpose. The antioxidant parameters including Superoxide dismutase (SOD), glutathione peroxidase and glutathione levels were evaluated [16,17]. Then the animals were sacrificed under anaesthesia using diethyl ether and the tissue samples of liver, kidney and heart were collected for evaluation of antioxidant levels in tissues.

Antioxidant Assays

Superoxide Dismutase

The role of superoxide dismutase (SOD) was to accelerate the dismutation of the toxic superoxide radica (O2), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. The method employed xanthine and xanthine oxidase to generate superoxide radicals, which reacted with 2-(4-idophenyl)-superxoixde dismutase 3-(4-nitrohpenol)-5-phenyltetrazolium chloride to form a red formazan dye. The superoxide dismutase activity was then measured by the degree of inhibition of the reaction.

Glutathione and Glutathione Peroxidase

Glutathione peroxidise (GPx) catalyses the oxidation of Glutathione by Cumene Hydroperoxide. In the presence of Glutathione reductase and NADPH the oxidized Glutathione (GSSG) was immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured.

Statistical Analysis

The data were expressed as mean ± SEM and statistically analyzed by one way ANOVA.

Results

The phytochemical analysis of the fruit extract showed the presence of carbohydrates, flavonoids, phenols, glycosides and amino acids (Table 1).

Table 1: Phyto-constituents of fruit juice of *A. bilimbi*

Phytochemical Analysis	Results
Carbohydrate	+++
Lignans	+++
Flavonoids	+++
Tannins	+
Steroids	-
Terpenoids	-
Alkaloids	+
Glycosides	++
Saponins	-
Aminoacids	+++

+++ Very strongly present ++ Strongly present + Present - Absent

The present study showed the antioxidant efficacy of Averrhoa bilimbi fruit extract in the paracetamol intoxicated experimental rats. The experiment was carried out at two different concentrations of extract, 250 and 500 mg/kg.bwt. as Lower dose (LD), and Higher dose (HD) respectively.

At the end of the 10 days experiment, the Disease control group showed the depleted levels of antioxidant enzymes in blood and tissues when compared with Healthy control group. The decreased levels of antioxidant enzymes exhibited the illness caused by the intoxication of paracetamol. *A. bilimbi* extract administered groups showed the elevated levels of antioxidant enzymes superoxide dismutase, glutathione peroxidase and glutathione (Table 2). The efficacy of the extract was also found to be significant and dose dependent. The antioxidant enzymes were also measured in the tissue samples of liver, kidney and heart tissues of the experimental animal groups (Table 3). This also showed that the antioxidant status have been significantly ($p<0.05$ and $<p0.01$) improved in the *A. bilimbi* fruit extract administered groups when compared with the disease control group (Figure1,2). The overall experiment demonstrated that *Averrhoa bilimbi* fruit extract has potential antioxidant activity.

Table 2 Effect of *A. bilimbi* fruit extracts on blood antioxidant enzyme levels in Rats subjected to paracetamol induced toxicity

Parameters	**SOD (Units/mg protein)**	**GPx (units/µg protein)**	**GSH (µg/mg protein)**
Healthy Control (Group I)	8.81 ± 0.96	69.16 ± 0.41	1.52 ±0.02
Disease Control (Group II)	4.66 ± 0.74*	34.39 ± 0.67	0.73 ± 0.11**
Standard Drug Group (Slilymarin treated- Positive Control Group) (Group III)	7.93 ± 0.22*	58.311 ± 0.69**	1.68 ± 0.18**
A. bilimbi (Lower Dose) (Group IV)	6.36 ± 0.31*	54.82 ± 0.69*	1.31 ± 0.35*
A. bilimbi (Higher Dose) (Group V)	6.84 ± 0.76*	59.83 ± 0.91*	1.63 ± 0.49**

Values are mean ± SEM, n=6 animals in each group. *p<0.05, **p<0.01 when compared to disease control.

Table 3 Effect of *A. bilimbi* fruit extract on Superoxide dismutase level in different tissues

Groups	**SOD level in tissue samples (U/mg protein)**		
	Liver	Kidney	Heart
Healthy Control (Group I)	9.11 ± 0.63	6.15 ± 0.36	22.6 ± 4.30
Disease Control (Group II)	4.11 ± 0.21*	3.86 ± 0.12*	10.78 ± 1.25*
Standard Drug Group (Slilymarin treated- Positive Control Group) (Group III)	7.65 ± 0.15*	5.80 ± 0.74**	19.81 ± 3.70**
A. bilimbi (Lower Dose) (Group IV)	6.88 ± 0.19*	4.48 ± 0.15*	20.25 ± 3.42*
A. bilimbi (Higher Dose) (Group V)	7.23 ± 0.19*	5.10 ± 0.22*	19.75 ± 2.19*

Values are mean ± SEM, n=6 animals in each group. *p<0.05, **p<0.01 when compared to disease control.

Figure 1 Effect of *A. bilimbi* on Glutathione peroxidise level in liver, kidney and heart tissues

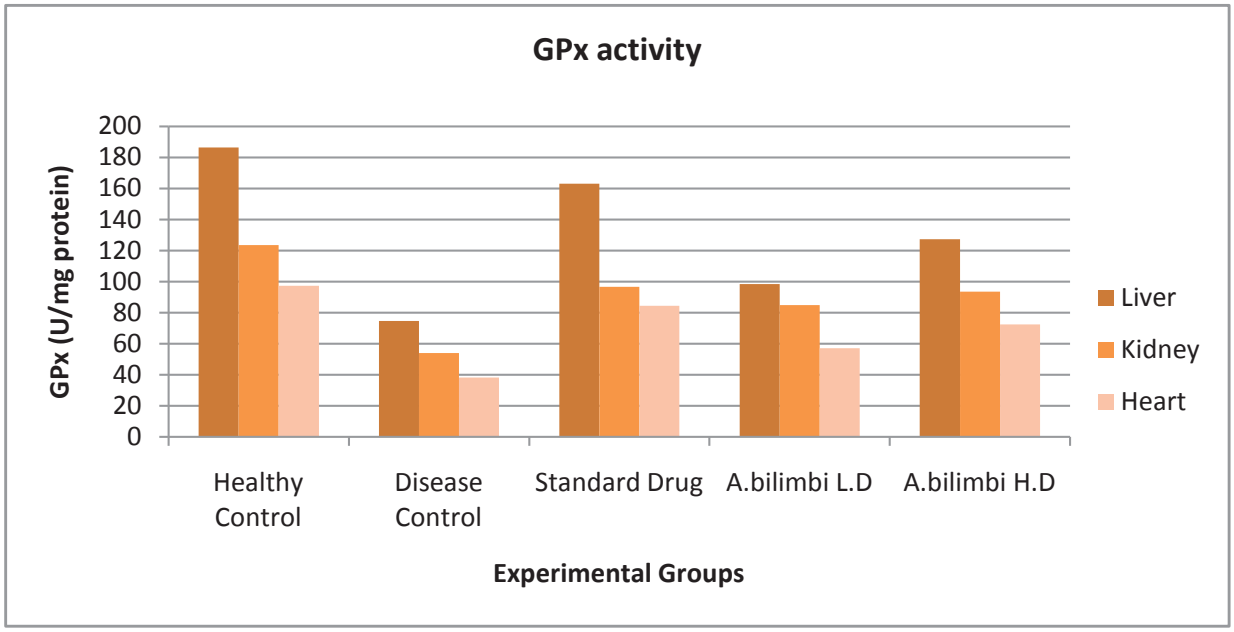
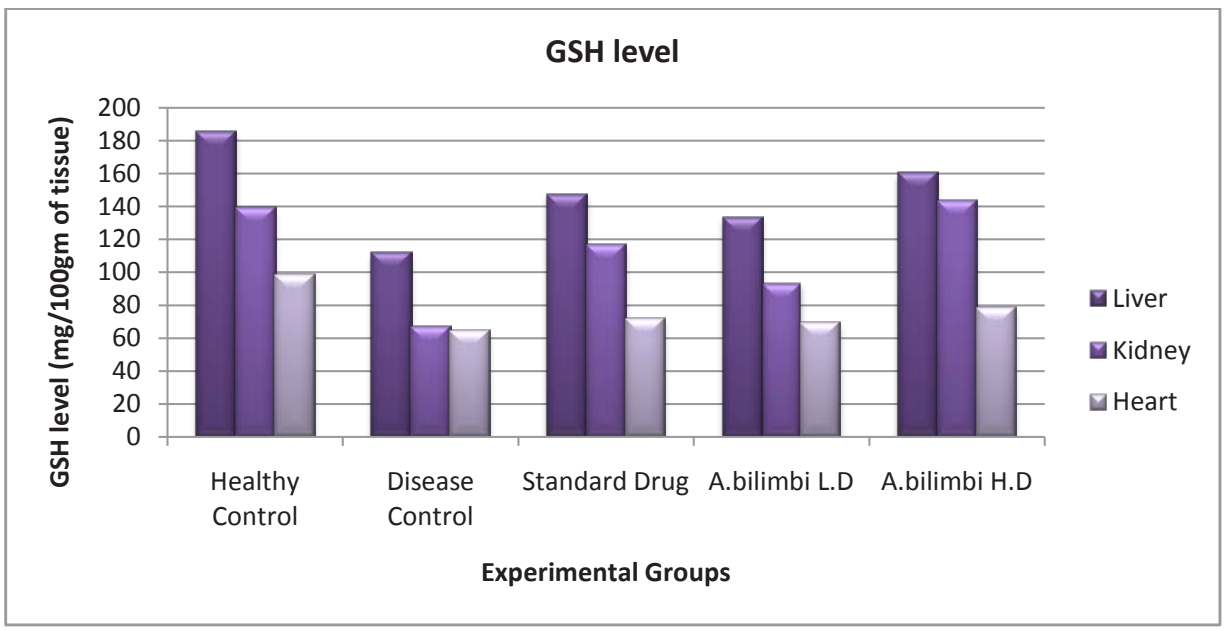


Figure 2 Effect of *A. bilimbi* on Glutathione level in liver, kidney and heart tissues



Discussion and Conclusion

The present study has well demonstrated the antioxidant activity of *A. bilimbi* fruit extract in the experimental animal system. The antioxidant system is comprised of different types of functional components classified as first line, second line, third line and fourth line defences. The first line defence preventive antioxidants are which act by quenching of O_2-, decomposition of H_2O_2 and sequestration of metal ions. The antioxidants belonging to this category are mainly enzymes, like superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase and non-enzymatic molecules like minerals and some proteins. Super oxide dismutase mainly acts by quenching of super oxide radical, produced in different aerobic metabolism. Glutathione peroxidase is a selenium containing enzyme which catalyses the reduction of H_2O_2 and lipid hydroperoxides, generated during lipid peroxidation, to water and oxygen [18,19].

The paracetamol is a well known antipyretic and analgesic agent, which is safe in therapeutic doses but can produce fatal hepatic necrosis when ingested in very large doses. The involvement of free radicals in the pathogenesis of liver injury has been investigated for many years in well defined experimental systems and concluded that ROS and lipid peroxidation may play a role in pathogenesis of hepatic fibrosis with loss of normal liver architecture [20-23]. The liver contains high SOD, catalase, glutathione peroxidise activity. These are major enzymes which catalyze the elimination of reactive oxygen species (ROS) derived from the redox process of xenobiotic in liver tissue.

In the present study, the *A. bilimbi* extract treated animal groups showed the elevated levels of the antioxidant enzymes superoxide dismutase, glutathione peroxidase and glutathione in blood and tissues (liver, kidney and heart), when compared with the Disease control group. The efficacy of the extract was found to be significant and dose dependent.

The phytochemical analysis showed the presence of flavanoids, lignans, amino acids and glycosides. The antioxidant activity of flavonoids, phenols and lignan compounds from various plants has been well documented for their individual and synergistic effect [24,25]. The results obtained from the present study indicated that *A. bilimbi*, has potential antioxidant activity and it may be the due to the synergistic effect of the major phyto-constituents like flavonoids, lignans and phenols that are highly present in the extract. Flavonoids are the polyphenols, with C6-C3-C6 skeleton that consists of two aromatic rings joined by a three-carbon link. Flavonoids generally include anthocyanins, flavanols, flavones, flavanones and flavonols. These flavanoids have significant antioxidant activity through their metabolic reaction in the system. Similarly lignans are a group of compounds found in plants. Plant lignans are polyphenolic substances derived from phenylalanine via dimerization of substituted cinnamic alcohols known as nomolignols. When a part of diet, some plant lignans are metabolized by intestinal bacteria to mammalian lignans enterodiol and enterolactone. Lignans can be metabolized to mammalian lignans such as pinoresinol, lariciresinol, matairesinol etc. Lignans are one of the major classes of phytoestrogens, which are estrogen –like chemicals and also act as antioxidants. So, antioxidant property of the *A. bilimbi* may be due to the presence of such phytochemicals and their synergistic effect. The further research is very much required in the aspects of isolation and characterization of potential compounds from the extract of *A. bilimbi* and validating them for the development of new drugs.

Conflict of Interest: Nil

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