PROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF *Psidium guajava* BARK EXTRACT AGAINST CADMIUM TOXICITY

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Abstract

The protective effect of ethanolic extract of *Psidium guajava* (EEPG) bark on cadmium-induced oxidative damage in male albino rats' liver was investigated. The activities of serum enzymes: AST, ALT, ALP, GGT; protein determination in liver, kidney and testes and serum cholesterol level were analysed. In the presence of cadmium, there were significant increase in ALP (87.45 ± 2.89) and GGT (103.35± 8.53) activities and no significant increase in ALT (71.23 ±1.87) and AST (121.59±5.14) when compared with the control. There were reductions in activities of AST (90.57 ± 8.02), GGT (44.29 ± 8.52) and ALP (33 ± 3.81) in presence of extracts when compared to control. Protein estimation results show that cadmium significantly reduced the protein levels of liver (59 ± 6.93), Kidney (55.33 ± 0.19) and testis (35 ± 8.08) compared to control. The protein levels in the kidney (54.5 ± 3.18) and testis (49.5 ± 0.8) were also significantly reduced in presence of extract when compared to the control. The results of the plasma cholesterol levels in rats treated with cadmium show a non-significant increase in the cholesterol level (0.37 ± 0.04) when compared with the control. In the group treated with the extract, the plasma cholesterol level (0.26± 0.023) was significantly reduced relative to control. EEPG appears to act as an antioxidant in scavenging the oxidative damage induced by cadmium.

Keyword: *Psidium guajava*, cadmium, serum enzymes, oxidative damage, antioxidant

INTRODUCTION.

Several authors have reported toxic and carcinogenic effects induced when humans and animals are exposed to certain metals. (Thompson & Bannigan, 2008; Leonard *et al*., 2004; Cuajungco & Faget 2003; Roy& Saha, 2002; Miller *et al*., 1990; Grankvist *et al*., 1981). It is also known that several essential transition metals, such as zinc, iron, copper, cadmium, cobalt and manganese participate in the control of various metabolic and signaling pathways. (Valko *et al.*, 2005).

The best evidence supporting the hypothesis of the oxidative nature of metal-induced genotoxic damage is provided by the wide spectrum of nucleobase products typical for the oxygen attack on DNA in cultured cells and animals exposed to carcinogenic metals (Valko *et al.*, 2005). Detailed studies in the past two decades have shown that metals like iron, copper, cadmium, chromium, mercury, nickel, vanadium possess the ability to produce reactive radicals, resulting in DNA damage, lipid peroxidation, depletetion of protein sulfhydryls and other effects. Reactive radical species include a wide range of oxygen-, carbon-, sulfur- radicals, originating from the superoxide radical, hydrogen peroxide, and lipid peroxides but also in chelates of amino-acids, peptides, and proteins complexed with the toxic metals. The toxic effects of metals involve hepatoxicity, neurotoxicity and nephrotoxicity (Waalkes *et* al., 2004; Roy& Saha, 2002; Valko *et al.*, 2005; Valko *et al*., 2006).

Cadmium is the 48th element and a member of group 12 in the Periodic table of elements. The most common oxidation number of cadmium is +2. Cadmium is a heavy metal; roughly 13,000 tons of cadmium is produced, worldwide, each year for nickel-cadmium batteries, pigments, chemical stabilizers, metal coatings and alloys. The toxicity of cadmium relates to smelting where the main route of exposure is through the lungs. In contaminated areas, there is evidence to suggest increased body burdens of cadmium among a proportion of the exposed population, with some evidence of increased urinary excretion of β-2- microglobulin and some loss of bone density among people with the highest urinary cadmium concentrations (Buchet *et* al., 1990; Staessen *et al*.,1999).

Cadmium is a highly toxic metal. Cadmium itself is unable to generate free radicals directly, however, indirect generation of various radicals involving the superoxide radical, hydroxyl radical and nitric oxide has been reported (Galan *et al.*,2001). Some experiments also confirmed the generation of (non-radical) hydrogen peroxide which itself in turn may be a significant source of radicals via Fenton chemistry (Watanabe *et al.*, 2003).

An interesting mechanism explaining the indirect role of cadmium in free radical generation was presented some years ago (Price *et al.*,1983). In this mechanism it was proposed that cadmium can replace iron and copper in various cytoplasmic and membrane proteins (e.g. ferritin, apoferritin), thus increasing the amount of unbound free or chelated copper and iron ions which then participate in oxidative stress via Fenton reactions (Casalino *et al.*, 1997). Similar findings were very recently presented by Watjen and Beyersmann (2004). Cadmium is a potent human carcinogen and occupational exposure to it has been associated with cancers of the lung, the prostate, pancreas and kidney (Waisberg, *et al.*, 2003). Because of its characteristics as a lung carcinogen, cadmium has been classified as a category #1 human carcinogen by the International Agency for Research on Cancer and the National Toxicology Program of the USA. It has also been suggested that cadmium might also be implicated in the pathogenesis of human pancreatic cancer and renal carcinoma (Waisberg, *et al.*, 2003).

Flavonoids are one of the most numerous and widespread group of naturally occurring antioxidants and as potent inhibitors of lipid peroxidation in biological membrane. They are widely distributed in plant origin such as vegetables, fruits, nuts, seeds, leaves, flowers and barks of plants (Middleton et al., 2000). They usually contain one or more aromatic hydroxyl groups in its moiety, which is responsible for the antioxidant activity of flavonoids (Van Akre et al., 2000). One of such flavonoid is morin (2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one), the active ingredient of *Psidium guajava.*

However, no study has been carried out on the effect of ethanolic extract of *Psidium guajava* on cadmium-induced toxicity. Hence, this present study was designed to evaluate the protective role of ethanolic extract of *Psidium guajava* on cadmium-induced toxicity in rat liver.

Materials and method

2.1. Plant material

Psidium guajava bark was obtained peeled into pieces and dried under room temperature for 5 weeks. The weight of the dried bark was 508.30g.

2.2. Method of Plant Extraction

Extraction was carried out according to the method as described by Qian and Nihorimbere (2004). 200g of the dried of the dried guava bark was ground and extracted using ethanol for 48 hours. The extract was filtered through Whatman paper using a chilled Buchner funnel connected to sorption machine and concentrated to obtain solid extract. 16.09g of the solid extract was obtained. Extract was prepared as an emulsion in distilled water. The final concentration of the extract being 10g/100ml

2.3. Animals

40 (male) albino rats of Wistar breed weighing between 120–250 g were housed in cages and maintained under well ventilated conditions. The animals were allowed free access to standard pellet diet and water ad libitum.

2.4. Preparation of drugs and administration

Cadmium salt (3 $CdSO_4.8H_20$) was the toxicant used for this work. The toxicant (4mg/kg body weight) was administered intraperitoneally for a day. Ascorbic acid was dissolved in distilled water; 100mg/kg body weight was administered orally for seven days. 100mg/kg body weight of the extract was administered orally for seven days.

2.5. Experimental time line

The animals were randomly divided into eight groups of five rats in each group. Group I served as control (saline), Group II animals were orally administered with extract, Group III animals received ascorbic acid, Group IV animals were treated with the toxicant, Group V animals were treated with toxicant and extract, Group VI animals received toxicant and ascorbic acid, Group VII animals were treated with extract and ascorbic acid and Group VIII animals were treated with toxicant, extract and ascorbic acid.

At the end of the experimental period, animals in different groups were sacrificed by cervical decapitation. Blood samples were collected in two different tubes, i.e. one is heparinised, for plasma and another without heparin for serum collection. Serum and plasma separated by centrifugation was used for various biochemical estimations.

2.6. POST MITOCHONDRIAL FRACTION PREPARATION

The tissue excised was rinsed in 1.15% KCl and dried on filter paper, weighed and recorded. The tissue was then homogenized in 5 part of homogenizing buffer and centrifuge was carried out at 17,000g for 20 mins. The supernatant was then stored between 0-4^{0}C.

2.7. ACTIVITIES OF SERUM MARKER ENZYMES

The activities of serum enzymes: Alanine aminotransferase (ALT) (E.C.2.6.1.2), Aspartate aminotransferases (AST) (E.C.2.6.1.1), Alkaline phosphatase (ALP) (E.C.3.1.3.1), and Gama-gluthamyltransferases (GGT) (E.C.2.3.2.2) were assayed. The activities of alanine aminotransferase and aspartate aminotransferases were measured as described by Reitman and Frankel (1957). Alkaline phosphatase activity was assayed by optimized standard method according to the recommendation of the Deutshe Geselleschaft for clinical chemistry (1972). γ-Gluthamyltransferases activity was determined as described by Szasz (1976) and Szasz and persyn (1974).

2.8. PROTEIN ESTIMATION

The protein content of the microsome was determined by the Biuret method using Bovine Serum Albumin (BSA) as standard as described by Gornall et al., (1949) with some modifications. Potassium iodide was added to biuret reagent to prevent the precipitation of Cu^{2+} ion as cuprous oxide. Cu^{2+} ion in an alkaline pH form complexes with maximum absorbance at 540nm.

2.9. STATISTICAL ANALYSIS

Values are given as means±S.D. for six rats in each group. Data were analyzed by one-way analysis of variance followed by Duncan's Multiple Range Test (DMRT) using SPSS version 13 (SPSS, Chicago, IL) (Duncan, 1957). The limit of statistical significance was set at Pb0.05 and the values sharing a common superscript did not differ significantly.

3.0 RESULTS

3.1 EFFECTS OF ETHANOLIC EXTRACT OF *PSIDIUM GUAJAVA* BARK (EEPGB), ASCORBIC ACID AND CADMIUM ON THE ACTIVITIES OF SOME SERUM ENZYMES.

Table 1 shows the levels of serum hepatic marker enzymes in control and experimental rats. Intraperitoneal administration of cadmium salt caused abnormal liver function in rats. In cadmium treated rats, the activities of serum hepatospecific enzymes such as serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma glutamyl transferase were significantly ($P<0.05$) increased, when compared with control rats. But, oral administration of EEPG (100 mg/kg body weight) to normal rats did not show any significant ($P<0.05$) effect on hepatic markers.

However, these biochemical impairments were ameliorated by the administration of EEPG. There was a significant decrease in the toxic effect of the toxicant when EEPG and Ascorbic acid were co-administrated.

TABLE 1: EFFECTS OF ETHANOLIC EXTRACT OF *PSIDIUM GUAJAVA* BARK, ASCORBIC ACID AND CADMIUM ON THE ACTIVITIES OF SOME SERUM ENZYMES.

GROUP	**ALT**	**AST**	**GGT**	**ALP**

Control	62.05±0.48	100.30±2.83	59.06±7.05	44.64±7.62
Bark extract	63.46±8.32	90.57±8.02[b]	44.29±8.52[ab]	33.00±3.81[ab]
Ascorbic acid	58.19±2.28[b]	82.80±0.58[ab]	29.53±0.23[ab]	41.25±0.95[ab]
Cadmium toxicant	71.23±10.87	121.59±514[a]	103.35±8.53[a]	87.45±2.89[a]
Bark extract + Cadmium toxicant	67.24±8.40	112.23±2.44	88.27±16.88[a]	52.80±13.33[b]
Ascorbic acid + Cadmium toxicant	47.01±1.22[ab]	109.12±2.30	49.21±4.36[b]	41.25±2.86[b]
Bark extract + Ascorbic acid	53.78±1.72[b]	83.64±2.66[b]	59.06±16.82[b]	46.70±6.00[b]
Bark extract+Ascorbic acid+Cadmium toxicant	42.28±6.03[ab]	112.32±3.95	78.58±11.33[ab]	54.45±4.76[b]

Table 3.1

Tabulated results are means of five determinations ±SEM. Values carrying different notations are significantly different ($p<0.05$)

a. Significantly different compared to control ($p<0.05$)
b. Significantly different compared to cadmium ($p<0.05$)

3.2 EFFECTS OF ETHANOLIC EXTRACT OF *PSIDIUM GUAJAVA* BARK (EEPGB), ASCORBIC ACID AND CADMIUM ON PROTEIN ESTIMATION IN SELECTED RAT TISSUE.

Table 2 illustrates the changes protein level in three different organs: liver, kidney and testes. Results obtained show a significant reduction in the protein level in all the organs of the rats treated with cadmium when compared to the control ($p<0.05$). Oral administration of ascorbic acid produced a significant decrease in the protein levels of both liver and kidney when compared to the control. When compared to the cadmium toxicant, there was significant increase in the protein levels of kidney and testes.

In the presence of the extract and cadmium toxicant, there was significant reduction in the level of protein and testes when compared to control. However, a significant increase in protein level of kidney was observed when compared to control. When compared to cadmium administered group, only the

kidney showed a significant increase in the level of protein while the increase in liver and testes were not significant at $p<0.05$.

TABLE 2: EFFECTS OF ETHANOLIC EXTRACT OF *PSIDIUM GUAJAVA* BARK, ASCORBIC ACID AND CADMIUM ON PROTEIN ESTIMATION IN SELECTED RAT TISSUE.

GROUP	**Liver**	**kidney**	**Testes**
Control	72.25±1.15	63.50±0.87	53.00±0.01
Bark extract	68.00±6.93	54.50±3.18[ab]	49.50±0.08[b]
Ascorbic acid	60.33±0.19[a]	58.00±1.15	53.10±2.89[b]
Cadmium toxicant	59.00±6.93[a]	55.33±0.19[a]	35.00±8.08[a]
Bark extract + Cadmium toxicant	63.00±1.63	76.67±4.81[ab]	39.00±5.20[a]
Ascorbic acid + Cadmium toxicant	65.00±2.89[a]	56.50±2.02	25.50±2.60[a]
Bark extract + Ascorbic acid	72.00±0.01[b]	74.50±2.60[ab]	50.00±2.87[b]
Bark extract+Ascorbic acid+Cadmium toxicant	60.00±1.83[a]	69.00±0.01[ab]	32.50±1.44[a]

Tabulated results are means of five determinations ±SEM. Values carrying different notations are significantly different ($p<0.05$)

a. Significantly different compared to control ($p<0.05$)
b. Significantly different compared to cadmium ($p<0.05$)

3.3 EFFECTS OF ETHANOLIC EXTRACT OF *PSIDIUM GUAJAVA* BARK, ASCORBIC ACID AND CADMIUM ON PLASMA CHOLESTEROL LEVEL RAT.

Table 3 depicts the effects of EEPG, ascorbic acid and cadmium on plasma cholesterol level rat. The results show that cadmium increases the plasma cholesterol non-significantly ($p<0.05$) when compared

to control. In the presence of the extract, there was a significant ($p<0.05$) reduction in the plasma cholesterol when compared to control and cadmium

Administration of ascorbic acid also reduced the rats' plasma cholesterol significantly when compared to control and cadmium. There was non-significant reduction in the plasma cholesterol when compared to control and cadmium in the groups administered extract and cadmium. But in the presence of ascorbic acid and cadmium, there was a significant reduction in the plasma cholesterol relative to control and cadmium.

Also, the co-administration of ascorbic acid, extract and cadmium lower the plasma cholesterol significantly when compared to control and cadmium.

TABLE 3: EFFECTS OF ETHANOLIC EXTRACT OF *PSIDIUM GUAJAVA* BARK, ASCORBIC ACID AND CADMIUM ON PLASMA CHOLESTEROL LEVEL RAT.

Treatment	Mg/ml
Control	0.28±0.001
Bark extract	0.26±0.023[b]
Ascorbic acid	0.21±0.005[ab]
Cadmium toxicant	0.37±0.040[a]
Bark extract + Cadmium toxicant	0.33±0.098
Ascorbic acid + Cadmium toxicant	0.23±0.046[ab]
Bark extract + Ascorbic acid	0.20±0.005[ab]
Bark extract+Ascorbic acid+Cadmium toxicant	0.22±0.001[ab]

Tabulated results are means of five determinations ±SEM. Values carrying different notations are significantly different ($p<0.05$)

a. Significantly different compared to control ($p<0.05$)

b. Significantly different compared to cadmium ($p<0.05$)

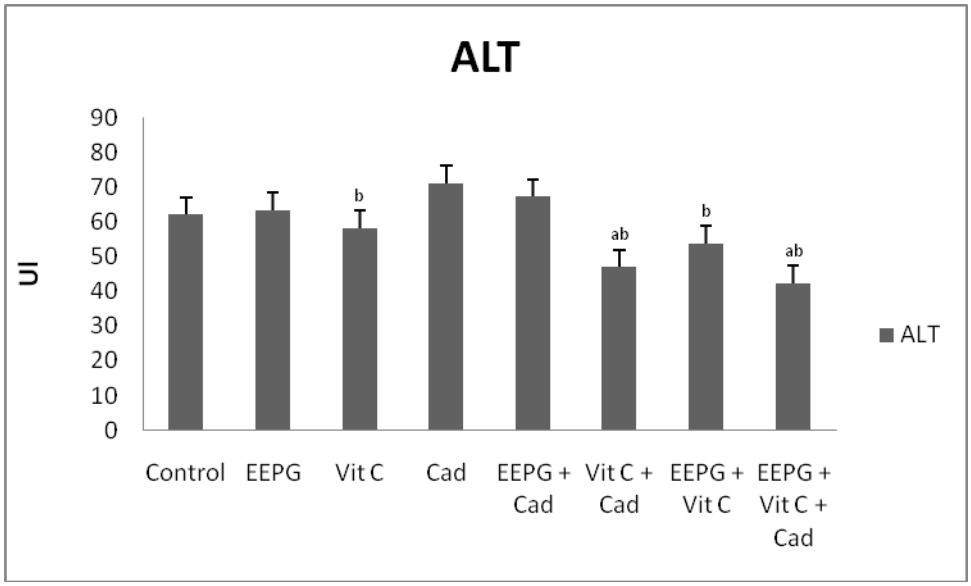

Figure 2: Effects of EEPG, Ascorbic acid (Vit C) and Cadmium on the activities of Alanine Amino Transferace (ALT) in rats. Each bar represents mean ± SD of five rats. [a] -Significantly different compared to control ($p<0.05$). [b]- Significantly different compared to cadmium ($p<0.05$)

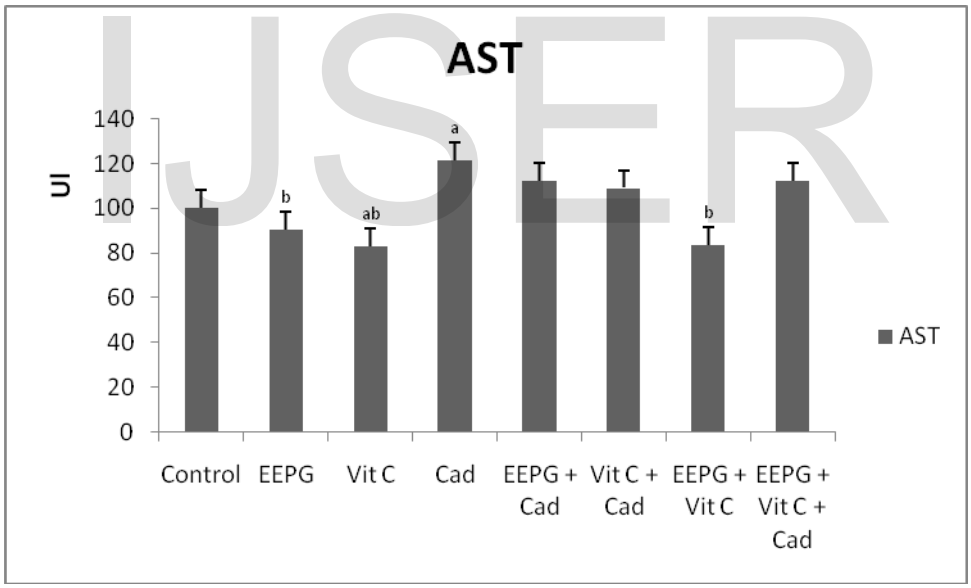

Figure 3: Effects of EEPG, Ascorbic acid (Vit C) and Cadmium on the activities of Aspartate aminotransferases (AST) in rats. Each bar represents mean ± SD of five rats. [a] -Significantly different compared to control ($p<0.05$). [b]- Significantly different compared to cadmium ($p<0.05$)

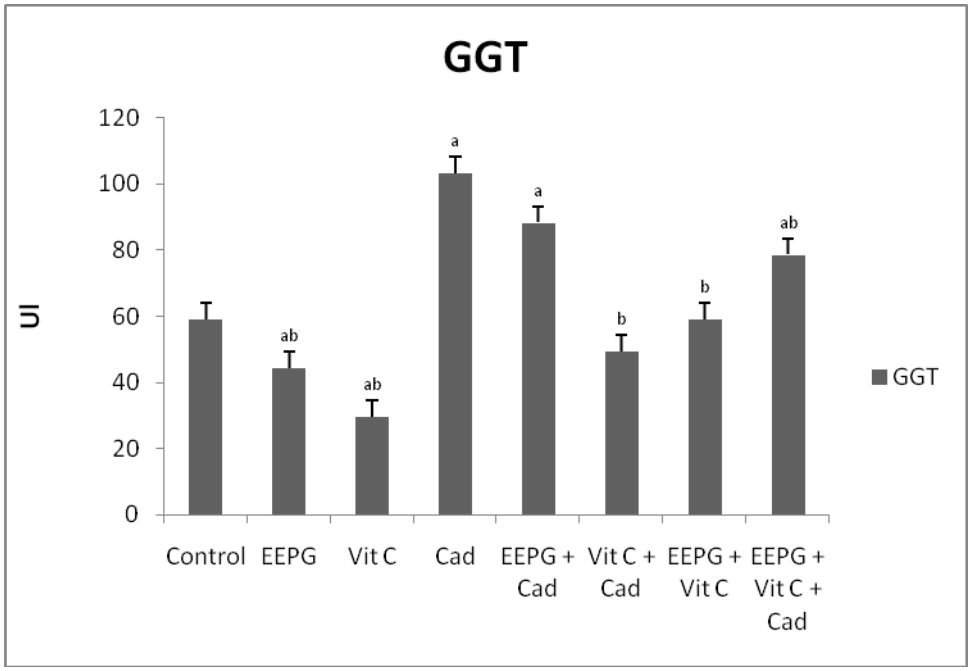

Figure 4: Effects of EEPG, Ascorbic acid (Vit C) and Cadmium on the activities of Gama-gluthamyltransferases (GGT) in rats. Each bar represents mean ± SD of five rats. [a] -Significantly different compared to control ($p<0.05$). [b]- Significantly different compared to cadmium ($p<0.05$).

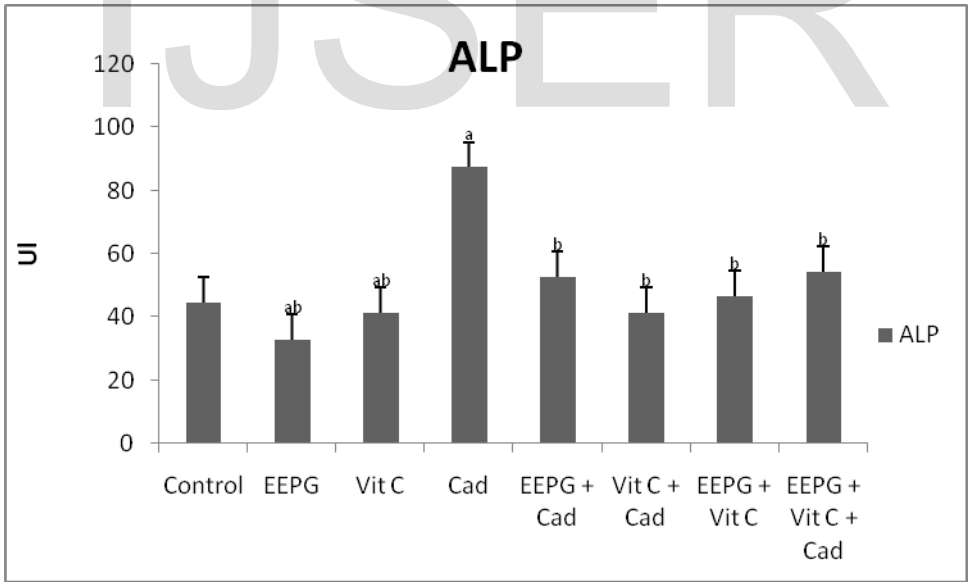

Figure 5: Effects of EEPG, Ascorbic acid (Vit C) and Cadmium on the activities of Alkaline phosphatase (ALP) in rats. Each bar represents mean ± SD of five rats. [a] -Significantly different compared to control ($p<0.05$). [b]- Significantly different compared to cadmium ($p<0.05$).

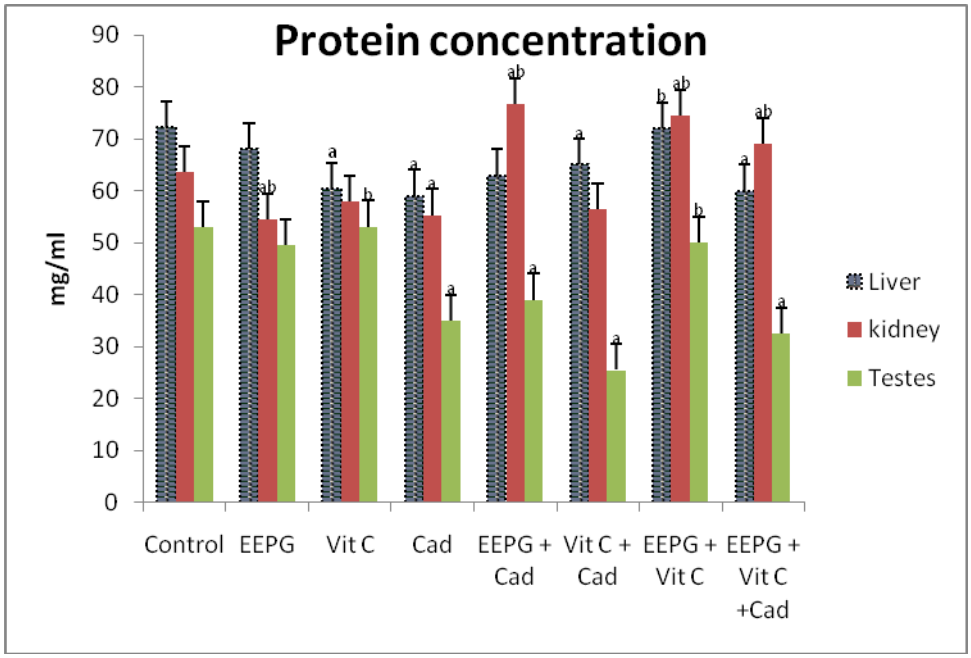

Figure 6: Effects of EEPG, Ascorbic acid (Vit C) and Cadmium on the protein concentration in liver, kidney and testes of rats. Each bar represents mean ± SD of five rats. [a] -Significantly different compared to control ($p<0.05$). [b]- Significantly different compared to cadmium ($p<0.05$).

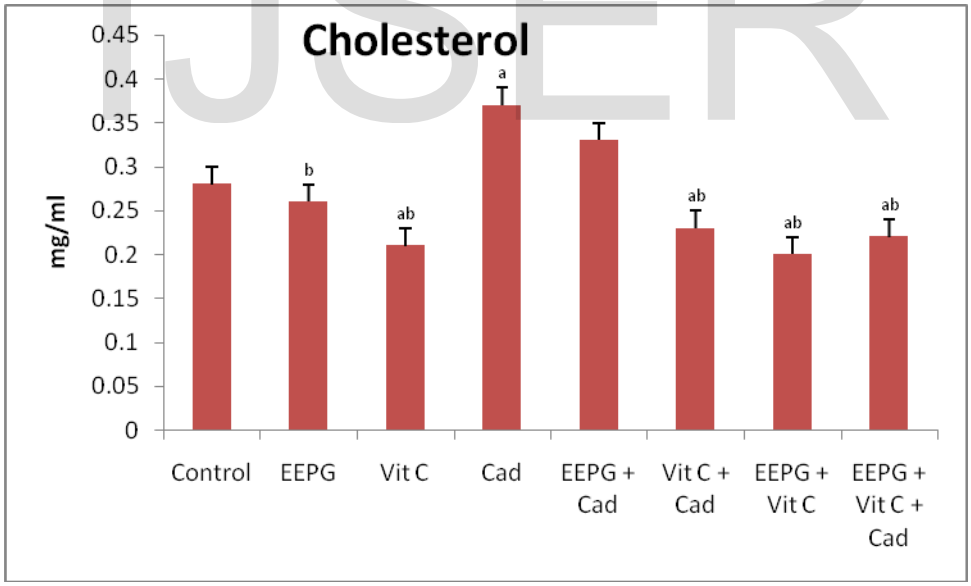

Figure 7: Effects of EEPG, Ascorbic acid (Vit C) and Cadmium on the plasma cholesterol level in rats. Each bar represents mean ± SD of five rats. [a] -Significantly different compared to control ($p<0.05$). [b]- Significantly different compared to cadmium ($p<0.05$).

Discussion

Polyphenolic compounds constitute one of the most commonly occurring and ubiquitous groups of plant metabolites and represent an integral part of human diet (Rice-Evans et al., 1996). Recent interest in phenolic compounds in general and flavonoids in particular, has increased greatly owing to their antioxidant capacity and their possible beneficial implications in human health (Schroeter et al., 2002). These include the treatment and prevention of cancer, cardiovascular disease and other pathological disorders (Rice-Evans 2001).

Several soluble enzymes of blood serum have been considered as indicators of the hepatic dysfunction and damage. The increase in the activities of these enzymes in plasma is indicative for liver damage and thus causes alteration in liver function (Pari and Amudha 2011). All these morphological changes observed in cadmium administered rats were attenuated by treatment with EEPG.

In this work, the group of rats administered acute dose of cadmium (4mg/kg body weight) showed significant increase in the level of serum ALP and GGT (figures 4 and 5) and no significant increase in the level of ALT and AST when compared to control (figure 2 and 3). However, there were reductions in cadmium toxicity activities in presence of extracts when compared to control. It was also observed that the co-administration of ascorbic acid (Vitamin C) and the extract were able to attenuate the effect of Cadmium toxicity. These findings corroborate the works of some authors on these enzymes (Li *et al*., 2010, Amara *et al*., 2008).

Protein estimation results show that cadmium significantly reduced the protein levels of liver, Kidney and testis compared to control (figure 6). The protein levels in the kidney and testis were also significantly reduced in presence of extract when compared to the control. Groups given acute dose of cadmium (4mg/kg body weight) show significant reduction in protein level of kidney, liver and testes. Suzuki (1980) reported that injections of 0.5mgCd/kg daily for 30, 38 or 54days on groups 3-5 male rats caused increase urinary protein in the 5^{th} week, when the kidney cadmium concentration was

100mg/kg. Tanaka (1982) also demonstrated the effect of cadmium on endocrine gland and testes. In the three organs observed, administration of the extract and co-administration of the extract and ascorbic acid were able to ameliorate the effect

The results of the plasma cholesterol levels in rats treated with cadmium show a significant increase in the cholesterol level when compared with the control (figure 7). In the group treated with the extract, the plasma cholesterol level was significantly reduced relative to control. EEPG appears to act as an antioxidant in scavenging the oxidative damage induced by cadmium.

Awad et al. (1998) found that cell damage exhibited good correlation with the enzyme leakage. Hence, cellular damage caused by toxic substances is frequently accompanied by increasing cell membrane permeability. In our study, the increased activities of serum aspartate aminotransferase, alanine aminotransferase, serum alkaline phosphatase and serum gamma glutamyl transferase in serum obviously indicate that liver is susceptible to cadmium induced toxicity. This increase could be attributed to the hepatic damage resulting in increased release of functional enzymes from biomembranes or its increased synthesis and has been widely used as an index of liver dysfunction. (Pari and Prasath, 2008).

Morin is the active ingredient isolated from *Psidium guajava* (common guava) (WU *et al.*,1993). Previous studies have shown that Morin-hydrate, a wood pigment, is an antioxidant that can protect rat liver cells against oxyradical damage (Kok et al., 2000).

Morin may stabilize the hepatic cellular membrane and protect the hepatocytes against toxic effects of cadmium, which may decrease the leakage of the enzymes into blood stream. This can be attributed to the antioxidant property of Morin the active ingredient of the ethanolic extract of *Psidium guajava*.

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