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Anti icteral effect of the leaf extract of *Psidium guajava* in diethylnitrosamine induced albino rats

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Abstract

Anti icteral effect of ethanolic leaf extract of *Psidium guajava* was examined in diethylnitrosamine induced female albino rats. The pathophysiology was evaluated using the biochemical parameters such as TBARS, protein, AST, ALT, bilirubin, GSH, Vit-E, Vit-c, SOD, catalase, GPx. The elevated hepatic anti oxidants enzymes suggests oxidative stress in jaundice induced animals. The oral administration of the ethanolic herbal extract, restored all the values to near normal. The result of the present study clearly indicates that the ethanolic leaf extract of *P.guajava* has a potent hepatoprotective activity against diethylnitrosamine induced jaundice in female albino rats.

Keywords : Anti icteral effect, diethylnitrosamine, *Psidium guajava.*

INTRODUCTION

Jaundice or icterus is defined as yellow pigmentation of skin and sclera. It is caused by an increase in the amount of bilirubin in the blood (Hyperbilirubinemia). Bilirubin is an yellowish pigment that is produced from the breakdown of heme, primarily from hemoglobin and red blood cells (RBCs). Bilirubin is transported by the blood to the liver, where the liver processes it and allowing it to be excreted in bile. Bile is a thick, greenish brown fluid that is secreted into the upper small intestine (duodenum) to get rid of waste products such as bilirubin and excess cholesterol and to aid in the digestion of fats. Jaundice may arise from increased breakdown of red blood cells, inherited changes in bilirubin metabolism, liver diseases or damage, and whenever there is interference with bile excretion.

Typically, the concentration of bilirubin in the plasma must exceed 1.5mg/dL, and three times the usual value of approximately 0.5mg/dL is needed for the coloration to be easily visible. One of the first tissues to change colour as bilirubin level rises in jaundice is the conjunctiva of the eye, a condition sometimes referred to as scleral icterus. Many drugs can cause jaundice and / or cholestasis. The notable example of a drug that causes this type of cholestasis and jaundice is estrogen.

Jaundice is classified into three categories, viz., pre hepatic, hepatic and post hepatic jaundice. Pre hepatic jaundice is caused by increased rate of hemolysis. The cause of hepatic jaundice include acute hepatitis, hepato toxicity and alcoholic liver disease, whereby cell necrosis reduces the liver's ability to metabolise and excrete bilirubin leading to hyper bilirubinaemia. Post hepatic jaundice, also called obstructive jaundice, is caused by an interruption to the drainage of bile in the biliary system.

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Traditional systems of medicine continued to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatment, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments..

P.guajava L., a valuable farm fruit plant, has many medicinal uses. A survey of the literature showed that *P. guajava* has many pharmacological activities such as antioxidant (Hui-Yin Chen and Gow – Chin Yen, 2007), hepatoprotective (Roy *et al.,* 2006), antimicrobial (Grover and Bala, 1993; Limsong *et al.,* 2004; Abdelrahim *et al.,* 2002), anticough (Jaiarj *et al.,* 1999) antidiabetic (Cheng and yang, 1983; Ojewole, 2005; Oh *et al.,* 2005), antibacterial (Lozoya *et al.,* 1989; Tona *et al.,* 2000), antimutagenic (Matsuo *et al.,* 1994), antispasmodic (Lozoya *et al.,* 2002), antiproliferative (Manosroi *et al.,* 2006), anti inflammatory (Qadan *et al.,* 2005) and antinociceptive activities, supporting its traditional uses. It is also used for the treatment of infantile rotoviral enteritis and diarrhea (Pina and Moraes, 2000). The present paper deals with the protective effect of *P. guajava* leaves against diethyl nitrosamine induced jaundice in albino rats.

MATERIALS AND METHODS

Experimental Animals

Albino Wistar female rats weighing 150-200g were divided into 4 groups of six each, and housed in polypropylene cages. They were maintained at standard husbandry conditions, provided with standard feed and water *ad libitum.*

Preparation of ethanolic extracts of *Psidium* guajava leaf extract

P. guajava leaves were shade dried at room temperature. The dried leaves were ground into fine powder using

pulverizer. The powder was sieved and kept in deep freezer until the time of use. 100g of dry fine powder was suspended in 400 ml of 95% ethanol for 72h. The extract was filtered using a muslin cloth and concentrated at $40^{o} \pm 5^{o}C$.

Experimental design

A total number of 24 rats were randomized into 4 groups of 6 animals each.

Group I : Rats served as control and were treated with saline (i.p.,).

Group II: Rats were administered with diethyl nitrosamine (100 mg /kg b.w., i.p.,).

Group III : Rats were administered with diethyl nitrosamine (100 mg / kg b.w., i.p.,) followed by *Psidium guajava* leaf extract (200 mg/kg b.w., p.o.,).

Group IV: Rats were treated with *Psidium guajava* (200 mg/kg b.w., p.o.,)

Biochemial analysis

Serum, plasma and tissue homogenate preparations were used for the estimations of TBARS, Bilirubin, Catalase, SOD, GPx, AST and ALT.

Statistical Analysis

All quantitative measurements were expressed as mean SD for control and experimental animals. The data were analyzed using one way analysis of variance (ANOVA) on SPSS/PC (Statistical package for Social Sciences, Personal computer) and the group means were compared by Duncan's Multiple Range Test (DMRT). The results are considered statistically significant if the p values were less than 0.05.

RESULTS AND DISCUSSION

Bilirubin is a by product of the liver, when the liver is not functioning properly, bilirubin may begin to build up in the body. Hyperbilirubinaemia leads to jaundice which is a sign of an underlying disease process associated with yellow discolouration of the skin, mucous membranes and the whites of eyes, light coloured stools, dark coloured urnie and pruritis associated with nausea, vomiting, abdominal pain, fever, weakness, loss of appetite, headache, confusion, swelling of legs and of abdomen. In the present study, the level of bilirubin was increased in the serum of diethyl nitrosamine treated rats (Table 1). Treatment with the herbal extract, significantly brought the values to near normal.

Table 1: Serum Bilirubin level in control and experimental animals

Groups	Bilirubin (mg/dL)
Group I	0.59 ± 0.06
Group II	1.72 ± 0.12 *
Group III	0.48 ± 0.04 * *
Group IV	0.60 ± 0.05 * *

Values are given as mean ± S.D (n=6)

* Statistically significant when compared with group I

** Statistically significant when compared with group II

In the present study, the activities of liver profile enzymes were significantly increased (Table 2). These enzymes are localised in the cell cytoplasm and cell mitochondria as well as found in bile. Elevated serum enzymes like AST, ALT are indicative of cellular damage and loss of functional integrity of cell membrane in liver. Damage of liver cells causes leakage of cellular enzymes into serum (Das *et al.,* 2007). The increased concentration of bilirubin and significant rise in the activity of liver profile enzymes could be taken as an index of liver damage. After treatment with the herbal extract, the altered parameters were brought back to near normal.

Table 2 : Levels of Liver Marker enzymes in control and experimental animals

Groups	Aspartate Amino transferase (U/L)	Alanine Amino transferase (U/L)
Group I	118.13 ± 6.41	64.46 ± 5.60
Group II	174.32 ± 8.09 *	91.63 ± 3.56 *
Group III	133.5 ± 1.75 * *	70.37 ± 2.78 * *
Group IV	118.12 ± 6.41 * *	64.92 ± 2.24 * *

Values are given as mean ± S.D (n=6)

* Statistically significant when compared with group I

** Statistically significant when compared with group II

Lipid peroxidation is used as an indicator of oxidative stress in cells and tissues. Diethyl nitrosamine administration resulted in a significant enhancement of lipid peroxidation which was assessed by the increased production of TBARS (Table 3). Treatment with the herbal extract prevented the increased levels of TBARS.

Table 3 : Changes in the levels of TBARS in Control and experimental animals

Groups	TBARS in plasma (mg/dL)	TBARS in liver (mg/100g)
Group I	0.18 ± 0.03	0.75 ± 0.06
Group II	0.41 ± 0.04 *	3.28 ± 0.29 *
Group III	0.22 ± 0.02 * *	1.41 ± 0.12 * *
Group IV	0.17 ± 0.10 * *	0.73 ± 0.07 * *

Values are given as mean ± S.D (n=6)

*Statistically significant when compared with group I

**Statistically significant when compared with group II

SOD is an important antioxidant enzyme having scavenging effect against superoxide anion and catalase is responsible for detoxification of H_2O_2 produced by the action of superoxide dismutase there by inhibits

formation of superoxide radicals, (Wendel, 1980) due to increased oxidative stress. The activity of SOD, GPx, and catalase were decreased in the present study might be causing oxiradical – mediated injury and thus may contribute to liver damage in diethylnitrosamine treated rats (Table 4). After treatment with the herbal extract the activities of the antioxidant enzymes were restored indicating the regeneration of liver cells.

Table 4: Changes in the levels of Enzymatic antioxidants in control and experimental animals

Groups	CAT (U/L)	SOD (U/L)	GPx (U/L)
Group I	148.14 ± 12.71	7.1 ± 0.56	13.16 ± 1.93
Group II	74.27 ± 5.61 *	4.65 0.27 *	7.49 ± 0.61 *
Group III	127.22 ± 11.52 **	6.18 0.64 **	10.31 ± 0.95 **
Group IV	147.39 ± 12.43 **	7.19 0.53 **	12.93 ± 1.2 **

Values are given as mean ± S.D (n=6)

* Statistically significant when compared with group I

** Statistically significant when compared with group II

Thus, the present study demonstrates that leaf extract of *Psidium guajava* has hepatoprotective potential in curing jaundice by reducing the oxidative damage induced by diethyl nitrosamine. Further studies are required to evaluate the exact mechanisms of the active principles present in *Psidium guajava* leaf extract.

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