ANTITUMOUR PROMOTING POTENTIAL OF *COCCINIA INDICA* AGAINST BENZIDINE INDUCED HEPATOCELLULAR CARCINOMA IN MICE

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Abstract: In the present study, anti-tumour activity of ethanolic extract of leaves of *Coccinia indica* against benzidine induced hepatocellular carcinoma in mice was investigated. Hepatocellular carcinoma (HCC) was induced by treating the mice orally with benzidine (200mg/kg of body weight) for 30 days. HCC was manifested by significantly decreased (p<0.05) levels of serum protein, albumin and liver proteins. Serum globulin and bilirubin levels were significantly increased (p<0.05) on tumour development. Activities of liver marker enzymes (SGOT, SGPT, ALP and γ-GT) were also significantly increased. On treatment with the ethanolic extract of *Coccinia indica* (250mg kg of body weight) the alterations were reversed. Levels of serum protein, albumin, globulin, liver protein and bilirubin were restored in near normal levels in mice treated with the ethanolic extract of *Coccinia indica* (CLEt). Activities of liver marker enzymes were also restored at near normalcy in treatment group. Elevated level of tumour marker Alpha – Feto protein (AFP) was observed in mice treated with benzidine alone, whereas AFP level was markedly reduced on treatment with the ethanolic extract of *Coccinia indica*. In conclusion, these findings indicate that *Coccinia indica* has a protective effect against the hepatocellular carcinoma induced by benzidine.

Key words: *Coccinia indica*, Antitumour, Carcinoma

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and is the third highest cause of cancer related mortality. Prevention of recurrence of HCC remains as one of the most challenging tasks in current hepatology [1]. HCC is the most common type of liver cancer representing 83% of all causes. The five year relative survival rate is about 7% and causes 6,00,000 deaths annually worldwide. It is the first cause of death amongst cirrhotic patients. Hepatitis viral infection, food additives, alcohol, fungal toxins (Aflatoxins), toxic industrial chemicals, air and water pollutants are the major risk factors of liver cancer [2]. Several chemotherapeutic, cytotoxic and immunomodulating agents are available in western medicine to treat cancer. Besides being enormously expensive, these drugs are associated with serious side effects and morbidity. Still, the continuous search for an ideal treatment that has minimal side effects and cost effective is going on. Today, in Western medicine, only a limited number of plant products are being used to treat cancer. However, some of the widely used anticancer drugs such as taxol and vinca alkaloids are obtained from medicinal plants [3]. Recently, 26 Indian medicinal plants were shown to have *in vitro* anticancer activity [4]. Herbal medicines have been used since the dawn of the civilization to maintain health and to treat diseases. The World Health Organization (WHO) estimates that about three quarters of the world’s population currently use
herbs and other forms of traditional medicines to treat diseases. Even though we commence the new century with its exciting prospects of genetherapy, herbal medicine remains as one of the common forms of therapy available to much of world’s population [5].

*Coccinia indica* Wight and Arn (syn) is a tropical plant in the family of *Cucurbitaceae*, commonly known as Little gourd and locally known as ‘Kovai’, grows abundantly and wildly all over India. In Southeast Asia, *Coccinia indica* is cultivated for its edible young shoots and fruits. Indigenous people use various parts of the plant to get relief from diabetes mellitus. The plant has also been extensively used in Ayurvedic and Unani practice in the Indian subcontinent [6]. It has been shown to possess Hypoglycemic [7], Hypolipidemic [8], Antioxidant [6] and Hepatoprotective [9] activities. Benzidine is a toxic industrial pollutant used in the manufacture of dyes in the leather, textile and paper industries. Hepatocellular carcinoma is the principle and best documented toxic effect of benzidine in animals. The objective of the present study is to demonstrate the anti-tumour promoting potential of *Coccinia indica* against benzidine induced hepatocellular carcinoma.

**MATERIALS AND METHODS**

**Chemicals:** Benzidine was purchased from Sigma chemical company, USA. All the other chemicals used were of analytical grade and purchased from Merck India chemicals Ltd., Mumbai.

**Preparation of ethanolic extract of *Coccinia indica***: Fresh leaves of *Coccinia indica* were collected from villages in and around Kumbakonam, Tamilnadu, and authenticated by the Department of Botany, Bishop Heber College, Trichy. The leaves were washed, shade dried and coarsely powdered in a pulverizer. The powdered leaf material was subjected to Soxthlet extraction using 95% ethanol for six hours. The solvent was removed in vacuum to give an appropriate yield of 13 %. The residue was suspended in normal saline and fed orally at a concentration of 250 mg/kg of body weight [10].

**Animals:** Male albino mice (20-25 g) were procured from Madras veterinary college, Chennai. Animals were maintained under standard experimental conditions (Temperature 25 °C ± 2 °C, relative humidity 60 ± 5% and 12 hours light/dark cycle). They were fed with commercial diet (ISO 9001 certified laboratory feed).

**Experimental protocol:** Animals were divided into 4 groups each containing six animals. Group I: normal group receiving saline. Group II: orally treated with benzidine (200mg/kg of body weight) for 30 days. Group III: orally treated with *Coccinia indica* (250mg/kg of body weight) for 30 days. Group IV: orally treated with benzidine (200mg/kg of b.w) and ethanolic extract of *Coccinia indica* (250mg/kg of b.w) for 30 days. After 30 days animals were fasted over night and sacrificed by cervical decapitation. Blood was collected by jugular vein puncture. Liver was quickly excised off, washed in phosphate buffered saline and stored at 4 °C.

**Biochemical Parameters:** Serum and liver proteins were estimated by the method of Lowry et al.[11]. Serum albumin and globulin were estimated by the method of Doumas [12]. Serum bilirubin was estimated by the method of Malloy and Evelyn [13].

**Assay of enzymes:** Activities of serum transaminases were determined by the method of Reitman and Frankel [14]. Serum alkaline phosphatase was assayed by the method of King and King [15]. Gamma glutamyl transpeptidase activity was determined by the method of Rosalki and Rau [16].

**Estimation of Serum AFP level:** Quantitative estimation of tumour marker protein AFP level in serum was done by the method of Sell and Becker [17] using solid phase Enzyme Linked Immuno Sorbent Assay (ELISA) kit.

**Statistical analysis:** One way analysis of variance (ANOVA) has been applied for statistical analysis and a value of p < 0.05 has been considered as statistically significant level. The entire analyses were computed with the help of SPSS software package.

**RESULTS**

Table 1 shows the levels of serum total protein, albumin, globulin, liver protein and serum bilirubin in control and experimental groups. Serum protein (25%), albumin (30%) and liver protein (33%) were significantly decreased (p<0.05), while serum globulin fraction was increased in benzidine alone treated group. Serum bilirubin level was significantly (p<0.05) increased by 5 fold in benzidine alone treated group.
In ethanolic extract of *Coccinia indica* (CLEt) alone treated group levels of the above-mentioned biochemical parameters were restored at normal levels. In benzidine + CLEt cotreated group the levels of these parameters were shown to be restored in near normal levels.

Table 2 shows the activities of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (γ-GT) and serum AFP levels in control and experimental mice. Activities of all the enzymes were significantly increased (p<0.05) in benzidine alone treated group, while no significant elevation was observed in CLEt alone treated group. In mice treated with benzidine + CLEt, the levels of all enzymes were restored near to the normal levels. Tumour marker protein AFP level was significantly increased (p<0.05) by 4 fold in benzidine alone treated group, whereas in benzidine + CLEt co-treated group only an insignificant elevation was observed.

**DISCUSSION**

The present investigation was undertaken to evaluate the anti-tumour promoting potential of *Coccinia indica*, a small medicinal vine prescribed for a variety of ailments and liver diseases in particular. The benzidine alone treated group shows significantly reduced (p<0.05) levels of serum protein, albumin and liver protein when compared to the control mice. This finding is in accordance with the earlier ones of Kececi et al. [18]. Huff et al. [19] who reported that the most sensitive indicators of hepatocellular carcinoma are serum protein and albumin. This is due to the binding of benzidine to DNA and thereby impairing messenger RNA synthesis and selective inhibition of the enzyme activity of RNA polymerase, resulting in blockage of protein synthesis [20]. Protein waste implies underlying metabolic imbalance which is being expressed by an elevation in the apparent protein degradation rate with no changes in the apparent synthesis rate [21]. Reduced liver protein in Morris hepatoma bearing rats and walker 256 carcinoma has also been observed [22]. There is an increased protein degradation and recycling of amino acid has been decreased, resulting in enhanced efflux of these amino acids from the tissues. Thus the host responds to increased tumour load by increasing tissue protein breakdown [21].

The decreased levels of albumin present in most cases of hepatocellular disease result from loss into the extra vascular space and direct inhibition of synthesis. Several hepatic dysfunction, may also be due to increased catabolism. During hepatocellular damage, serum albumin level has been reduced and globulin level is increased, resulting in a decrease in A/G ratio [23]. A decrease in liver protein, serum total protein and albumin and an increase in globulin fraction were also observed during hepatic dysfunction in previous investigations [24]. Results of the present study correlate well the above findings. In mice treated with benzidine and *Coccinia indica* leaf extract, protein levels are near to normal one.
This could be due to the protective effect of *Coccinia indica* against benzidine induced hepatocellular dysfunction.

Determination of serum bilirubin serves as an index for the assessment of hepatic function and any abnormal increase in the level of bilirubin in serum indicates hepatobiliary diseases and disturbances in hepatocellular function [25]. In hepatic tumours hemolysis and deranged liver function leads to hyperbilirubinemia [26]. Mice treated with benzidine alone showed significantly increased levels of serum bilirubin which could result from an impairment of uptake or conjugation coupled with decreased excretion of the pigment [27]. In benzidine + CLEt co-treated group bilirubin level was restored in near normal level. The extract mediated suppression of the increased bilirubin level suggests the possibility of the extract being able to stabilize biliary dysfunction.

Liver damage caused by the carcinogens generally reflects an instability of liver cell metabolism which leads to distinctive changes in serum enzyme activities [28]. Serum transaminases (SGOT and SGPT), alkaline phosphatase and γ-glutamyl transpeptidase are representatives of liver functions; their increased levels are the indicators of liver damage. Increased activities of these enzymes are due to the leakage from the neoplastic cells into blood and may be due to the release of enzymes from normal tissue invaded by tumour. It may be due to the possible effect of tumour on remote tissue leading to loss of its enzyme and release into the blood [29].

The elevation of SGPT activity is repeatedly credited to hepatocellular damage and is usually accompanied by a rise in SGOT [2]. In hepatitis and HCC, SGPT is characteristically higher than SGOT and the SGPT/SGOT (De-Ritis) ratio which is normally lesser than 1, becomes greater than unity. In mice treated with benzidine alone this ratio is greater than unity. In mice treated with benzidine + CLEt, the SGPT/SGOT ratio was less than 1 and the enzyme activities were restored in near normal level. This suggests the protective effect of *Coccinia indica* against benzidine induced HCC.

An increase in ALP activity reflects pathological alteration in biliary flow [28]. The response of the liver to any form of biliary tree obstruction is to induce the synthesis of ALP. Activity of ALP is significantly increased (p<0.05) in benzidine alone treated group, which indicates the hepatocellular dysfunction caused by benzidine. ALP activity is restored near to normal level in group IV mice co-treated with benzidine + CLEt. γ-GT is an enzyme of hepatocyte plasma membrane, localized mainly in the canalicular domain. Liberation of this enzyme into serum too indicates damage to the liver cells. It is important to point out that γ-GT level is considered to be one of the best indicators of liver damage [2]. More elevations of GGT were observed in individuals with either primary or secondary (metastatic) neoplasms [23]. The levels of ALP and γ-GT are significantly elevated (p<0.05) in mice treated with benzidine alone and restored near to normal levels in benzidine + CLEt co-treated group. It could be suggested that *Coccinia indica* aids in parenchymal cell regeneration in liver, thus protecting membrane integrity, thereby decreasing enzyme leakage.

Alpha Feto Protein (AFP) an oncofetal protein, is progressively lost during development and virtually absent in healthy adult. It has long been recognized that exposure of mice to certain carcinogens causes an elevation of circulating AFP levels [2]. The reappearance of AFP in individuals with liver cancer demonstrates that certain genes are reactivated as a result of the malignant transformation of cells [23]. This corroborates with the results of the present study in which a significant increase in levels of AFP in benzidine alone treated mice and levels were restored near normalcy in group IV co-treated with benzidine + CLEt. The observed reduction in the levels of AFP in *Coccinia indica* co-treated mice was presumably due to decrease in the production rates of tumours.

Protective effect of *Coccinia indica* against benzidine induced hepatocellular carcinoma may be due to the presence of flavonoids and other phyto constituents in the ethanolic extract of *Coccinia indica*. Plant flavonoids were shown to have anticarcinogenic activity in certain experimental animals [30]. Cucurbitacin-B, a tri-terpenoid present in the leaves of *Coccinia indica* has also been shown to have anti-tumour activity. It inhibits the activation of
JAK/STAT 3 pathways which can contribute to oncogenesis [31]. It is concluded from the study that *Coccinia indica* suppressed the tumour formation by protecting the plasma membrane integrity of hepatocytes and inhibiting oncogenic pathways which can contribute to tumour development.

**List of abbreviations used:**
- HCC - Hepatocellular carcinoma
- CLEt - Ethanolic extract of *Coccinia indica* leaves
- SGOT - serum glutamate oxaloacetate transaminase
- SGPT - serum glutamate pyruvate transaminase
- ALP - alkaline phosphatase
- γ-GT - gamma glutamyl transpeptidase
- AFP - alpha feto protein

**REFERENCES**