Original Article

Assessment Of Alpha-Fetoprotein Level After Treatment With Plectranthus Amboinicus Extract In Wister Albino Rats Induced By Carbon Tetra-Chloride

Vinotha M[1], Nithya V*

*[1]Department of Animal Health and Management, Pharmacognosy Lab, Alagappa University, Karaikudi-630 003, Tamil Nadu, India.; E-mail: nithyav@alagappauniversity.ac.in
Corresponding author: Nithya V* E-mail: nithyav@alagappauniversity.ac.in
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Abstract

Plectranthus Amboinicus, plant extract has indispensible beneficial agents in traditional Indian medicine and Ayurveda. Incidence of several diseases, including Hepatocellular carcinoma (HCC) is increasing gradually to a new peak. The major goal of this investigation was to identify and estimate defensive capability of Plectranthus Amboinicus extract on enzymatic functions of liver and to investigate attenuating potential on HCC biomarker. The analyses include, effects of Plectranthus Amboinicus, in serum level of AST,ALT,ALP, Total bilirubin, and alpha fetoprotein (AFP) fluctuations in carbon tetrachloride (CCL4), induced HCC rats. Toxicity was stimulated by CCL4 with olive oil as vehicle in the ratio of (1:1 v/v);(olive oil 5mL/kg i.p). Sylimarin was used as a reference (standard) for intoxicated animal groups. Moreover, observation of histological studies favours the biochemical changes observed. Results showed P.Amboinicus, is highly potential in depleting the level of liver enzymes and AFP biomarker, based on dose regimen. These might have been due to the action of enriched phenolic content of P.Amboinicus, to get rid of free radicals. Study concluded that P.Amboinicus is a universal medicine and modulating biochemical changes in hepatotoxicity of liver cells induced HCC rat model.

Keywords: HCC, Alpha fetoprotein, Carbon tetrachloride, P.Amboinicus, Anti cancer.

INTRODUCTION

Health provider of Ayurvedic medicine used cow plant extract as a remedy for chronic and acute diseases, including Cancer, AIDS, Diabetes and Dermatological diseases due to its non- toxicity, antiviral, antifungal, antibacterial and anti cancer properties [1-3]. Plant derived products have been endorsed with different extracts as a bio enhancer for the effectiveness of popular antibiotics and anti cancer therapeutic drugs[4-8]. The serum enzymes activities have been recognised as sensitive and critical biomarkers for liver diseases including Hepatocellular Carcinoma (HCC). They act as a good indicator for entire health concerns, specifically, metabolic syndromes and Obesity[9]. AST, ALT and ALP in serum are used as determinant factors and biomarkers to predict the health of the liver. The level of AST and ALT ratio (> 1.5) in vascular stream raised while the body tissues are damaged due to the discharge of excess serum enzymes. The alpha-fetoprotein represents a glycoprotein formed from the embryonic stage to adult, that involved in controlling of body, specifically in differentiation of cells and also participated in the metabolism of Fat and Glucose, indicating chronic damage of liver tissues, and crucial biomarker for HCC [10].

Plant extracts are also often used as a biological facilitator for anticancer therapy individually or synergistically with other anticancer molecules for cancer therapy (Khanuja et al., US patent [11-14]). The bio active fraction accelerates the action of antibacterial and anticancer actions from 2 to 80 folds and favours the antibiotics or other

compounds to exert their effectiveness[15]. For instance, Sulforaphane has been exhibited the inhibitory effects of cancer growth and categorized as Isothiocynate [16-17], Sulforaphane is a product of glucosinolate. Glucoraphanin, is a glucosinolate, enriched in Plectranthus Amboinicus and cauliflower [18-19]. Notably, Text of Ayurveda cited, plant products are considered to be universal, therapeutic potential, used to meet out the short come of elements in the human body and act as a reducing factor, if any increased abnormal appeared. However, still now, literature relevant to the cited is limited. Therefore, it is of great interest to demonstrate, the effects of Plectranthus Amboinicus with different extracts on, the level of Liver enzymes such as AST, ALT, ALP and Total bilirubin in CCL4 induced wistar albino rats, and to determine as enhancer for anticarcinogenic agents mediated from plant source.

Materials and Methods

The extraction protocol for Plectranthus Amboinicus as extracted from aqueous, ethanolic, methanolic and distillated cow urine with followed in this experiment, as described by Sucharitha et al.,[20]with slight modification.

Chemicals and Reagents

Analytical grade chemicals and reagents were purchased from Sigma Chemical Company, MO, USA. for analysing and estimating ALT, AST, ALP and total bilirubin kits were procured from Apollo Diagnostic Medical Center, Chennai, Tamil Nadu. The silymarin was obtained from the Swastic scientific co., at Karaikudi, Tamil Nadu, India.

Animal study

About 180-250 gm weighing, male Wister rats were procured from the King Institute of Preventive Medicine and Research, Guindy, Chennai, Tamil Nadu and acclimatized in 12 hour light/dark cycle, fed with diet and water in our laboratory condition. Institutional Animal Ethical Committee Guidelines at PRIST University, Thanjavur, Tamil Nadu (743/21/abc/CPCSEA dt. 3.3.21) approved the protocol of the experiment and adhered the guidelines of the committee and followed.

Determination of dose

To assign the effects of aqueous extract of P.Amboinicus on biochemical changes in the liver enzymes, three types of dose regime were fixed. The rat dose was derived and calculated from human dose (usually 60 ml/day) and multiplied by a constant factor 0.018 x 5 is similar to 5.4 ml/kg body weight (First dose) [20]. The second dose was determined as double the first dose, i.e. 10.8 ml/kg body weight. Third dose was fixed as 50% of first dose, i.e. 2.7 ml/kg body weight.

Animal Grouping

As per the protocol of the experiment, the procured Wister rats were grouped into 6 numbers (n=6) Group-A was maintained independently as control, and administered saline alone (10ml/kg P.O.) for a week. Group-B received a vial of CCl4, Single dose with Plectranthus Amboinicus, used as vehicle in the ratio of 1:1, 5ml/kg i.p.) as treatment control group (Positive control) on the 7th day of the experiment. Group-C animals were injected with Silymarin (100mg/kg p.o.) once a day, for a week's time, as a reference group, next to a vial of CCl4 with aqueous extract of Plectranthus Amboinicus on the 7th day of experiment. Group D& E were orally administered with aqueous extract of Plectranthus Amboinicus distillate with three types of dosage pattern such as 2.7ml. 5.4ml and 10.8 ml. per kg body Wt., once a day for a week period, subsequently with a single dose of CCl4 with olive oil (1:1, 5ml/kg i.p.) on the 7th day of experiment.

After 24 hours of CCl4 treatment blood was collected from the retro orbital plexus of experimental animals, and conceded to clot for 1 hour at laboratory temperature. The supernatant serum was isolated by using centrifugation for evaluating the level of ALT, AST, ALP and Total bilirubin contents [21]. A couple of rats from each group were sacrificed on the day of blood collection. Liver was quickly segregated and washed well with saline in the cold, followed by fixed in 10% Formalin. Liver was degraded in analytical alcohol and fixed in paraffin wax. A fine section

of 5 Um thicknesses was stained with Haematoxylin and Eosin stains for light microscopic observations and photomicrographs for the examined tissues and were documented.

Biochemical studies of serum AST, ALT, ALP and Total bilirubin [21]

Biochemical fluctuations in clinical view, the level of enzymes such as Aspartate aminotransferase (AST) Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP) and Total bilirubin was estimated to determine the enzymatic activities of the liver cells of control and plant extract treated groups. The action of serum enzymes was determined by using kits and performed based on the instructions on the overleaf.

Alpha fetoprotein(AFP) [15]

Estimation of Alpha fetoprotein, a crucial biomarker for liver diseases, found in normal and aqueous extract of Plectranthus Amboinicus treated rats, was performed, by using commercial medical kits (Elabsciece, made in China) for Enzyme linked immunosorbent assay, specifically for rat models, along with micro litter plate and required elisa reader for the analysis of alpha fetoprotein level fluctuations.

Measurement of AFP

The level of alpha fetoprotein,in HCC induced rats, was measured,by following earlier study, which revealed that 250 mg/kg and 500mg/kg of phenolic extract was used their study, to assess AFP. Therefore, in our study, a similar quantity of aqueous extract of Plectranthus Amboinicus was chosen. All doses of aqueous extract of Plectranthus Amboinicus were administered orally, for the period of 60 days, prior to the administration of CCl4.The results were expressed as Mean values.

Statistical Analysis

The illustrated data were expressed as Mean values and statistically analysed using ANOVA. $P < 0.05$ was indicated significant variance.

RESULTS

In the present investigation, HepG-2 was treated with sulforaphane, an anticancer agent, extracted from Plectranthus Amboinicus [22] to detect and evaluate the anti cancer enhancing potentials. HepG-2 was maintained with sulforaphane as standard (100 µl, 200 µl) and also sulforaphane anticancer causative agents(ACCA) with plant extract in progressive elevating concentrations such as 100 Ul ACCA + 100 µl aqueous extract of P.Amboinicus, 100 Ul ACCA + 200 µl aqueous extract of P.Amboinicus and 100 UL ACCA + 300 µl aqueous extract of P.Amboinicus. Subsequently the ACCA of 100 µl was added. Results showed that as increased concentration of aqueous extract of P.Amboinicus, the rate of deterioration of HepG-2 cells was observed increasingly, and progressively (Fig 1). The optimum degeneration of HepG-2 cells was noticed in 100 Ul ACCA +300 µl aqueous extract of P.Amboinicus. Therefore it was evident that there was a strong effect exerted by the aqueous extract of P.Amboinicus (Fig 1).

Fig -1 Effect of aqueous extract of P.Amboinicus on the against HepG-2 assessment as Enhancer against cancer cells.

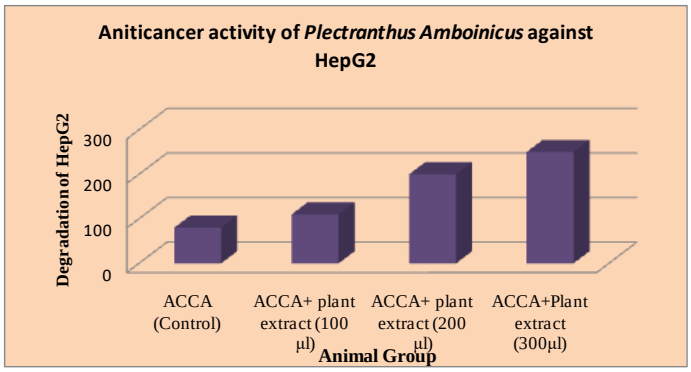

Table-1 Biochemical fluctuation on the enzyme level of liver cells, treated with three concentrations of aqueous extract of P.Amboinicus and standard silymarin in CCl4 induced HCC induced rats.

Sl. No.	Animal Group	AST (UL)	ALT (UL)	ALP (UL)	Total bilirubin (Mg/dL)
1.	Group-A	69.11	45.78	79.44	147
2.	Group-B	230.48 **	137.95**	126.51**	3.25**
3.	Group-C	74.64*	57.61*	75.90*	0.64*
4.	Group-D	124.20*	94.61*	116.52*	0.72*
5.	Group-E	115.27*	85.92*	97.41*	0.85*
6.	Group-F	119.02*	77.63*	100.79*	0.92*

***P < 0.05 against Group-B; **P < 0.05 against Group-A; A-Control Group; B-CCl4 Treated Group; C-Silymarin administered group; D-F aqueous extract of P.Amboinicus treated groups of 3 doses. Enzymes: AST-Aspartate Aminotransferase; ALT-Alanine Aminotransferase; ALP-Alkaline phosphatise**

Biochemical analysis: Administration of CCl4 remarkably enhanced the levels of AST, ALT and ALP and total bilirubin, which were depicted in Table-1 at (P< 0.05) significant level.

Histological studies

The reported findings of serum enzymes analysis have shown to be good agreement and supportive evidence for histological observations. The section of normal control animal liver exhibited typical hepatocytes filled with cytoplasm, and a conspicuous nucleus associated with a clear central vein. Sections of lever, from CCl4 inebriated rats exhibited wide alteration in the fat deposition, well defined necrosis, disintegration and invading of lymphocytes at the every side of the central vein and showed detrimental effects on cellular boundaries. The structural architecture of liver cells of aqueous extract of P.Amboinicus treated animals manifested a soft fatty changes unpredictable necrosis and less infiltration of lymphocytes, when compared with normal control and standard silymarin served groups (Fig-2-(i)-(iv))

Fig-(i). Liver section showing a typical sinusoides with central vein and depicted hepatocyte coordination.

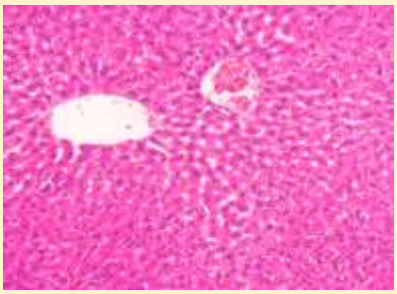

Fig- (ii). CCl 4 induced liver section exhibiting abnormality in the fat deposition around portal track and central vein. Hepatocytes showing vacuoles and distrubed nuclei.

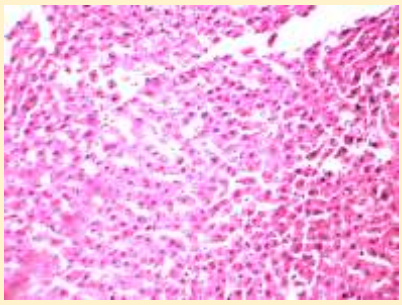

Fig -(iii). aqueous extract of P.Amboinicus **pretreated liver section showing normal appearance of hepatocytes a very little fat vacuoles exhibited in the few hepatocytes.**

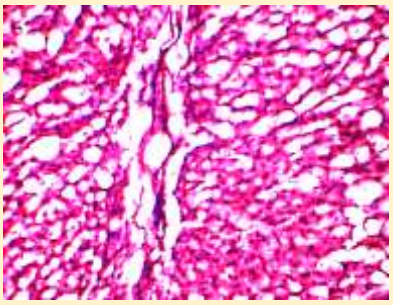

Fig -(iv) This section of liver pretreated with silymarin depicting normal architecture of hepatocytes and no changes were observed in the fat accumulation.

Fig -2. The liver cells of aqueous extract of P.Amboinicus treated animals manifested when compared with normal control and standard silymarin served groups.

Measurement of Biomarker (AFP)

The outcome of the experiment reported that a significant increase was observed in the level of AFP, in the treated group with CCL4 (19.71 ± 0.76) while compared with the control group of animals (8.36±0.32). These results confer an evident of the protective role of both concentration of aqueous extract of P.Amboinicus, prior and post administration of CCL4. It also exhibited a significant slowdown of AFP level at 5% p value, as compared with CCL4 treatment group.

In our investigation, depicted in Table -2 , showed a significant decrease was noticed in AFP level in Plectranthus Amboinicus extracted animal groups. It was noted that, in previous studies, phenolic compounds conciliated from varied plant extracts, reduced AFP level of CCL4 , attributable to the antioxidant potential. The values obtained, were presented to analyse fluctuations.

Table -2 Effect of aqueous extract of P.Amboinicus **on the AFP Level of induced HCC treated rats with ccl$_4$**

s.no	Trial	Level of AFP(ng/mL)

1	control	8.46 ± 0.22
2	ccl_4	*19.82 ± 0.67
3	aqueous extract of P.Amboinicus treated (250mg/Kg)	*(B)9.22 ±0.75(A)9.10 ±0.5
4	aqueous extract of P.Amboinicus treated (500mg/Kg)	*(B)9.17 ±0.14(A)9.01 ±0.15

(B)-Prior to ccl_4, administration (A)-After treating ccl_4,
Mean value ≤ 0.05 level, n=6

DISCUSSION

Metabolic alterations coupled with hepatic factors are associated with the development of HCC disease and profound toxicity [23]. Serum AST, ALT and ALP are significant biomarkers to evaluate the structural integrity of the liver cells and served as aids in the prediction of liver toxicity [24]. The effectiveness of aqueous extract of P.Amboinicus on the level of serum AST, ALT, and ALP were depicted. The ALT, AST, ALP and total bilirubin levels in the serum was recorded and tabulated in Table No.1. The reference range of AST, ALT and ALP enzymes are 50-150 UL, 10-40 UL, and 30-130 UL separately detected [25]. In the present study, the mechanism of liver injury induced through generation of trichloromethyl (CCl3) by liver at the time of CCl4 metabolism [26]. Free radicals showed they were highly capable of finding cell molecules with covalent bonds, resulting in cell necrosis. The increased level of AST, ALT, ALP and Total bilirubin was attributable to the enzymatic actions of free radicals from CCl3 [27-28]. These facts were supportive evidence for enhanced level of the tested biomarkers in our findings and correlated with findings of Gururaja et al.,[29]. Consequently, pretreated with aqueous extract of P.Amboinicus (Distillate) altered the enhanced enzyme levels, based on the dosage regime, symptomatic that it interrupts the mechanism of action that emerged by CCl3, free radicals. Such a unique feature enhanced the importance of biomarker enzymes as a screening tool for detecting liver associated diseases.

In normal conditions, Hepatic cells involved in different enzymatic metabolic actions and CCl4 induce a significant liver injury with a determined dose regime. Administration of CCl4 in heavy dose, caused liver necrosis after indulging bioactivation to the toxic factor, N-Acetyl-p-benzoquinone-imine (NAPQI) by cytochrome P-450 monooxygenase. The NAPQI factor binds with cellular biomolecules and activates the oxidation process on fatty acids which in turn disturbed the homeostasis of calcium, after exhaustion of Glutathione. Whereas treating cows distillates during pre-treatment, lowering down the elevated level of enzymes involved. Refurbishment of such biochemical changes, attributable to the down regulation effects on cytochrome p-450 [30-35].

HCC is a chief component for malignancy in liver tissues carcinoma and associated death worldwide. The obtained results of the study, showed a remarkable fluctuation, increased in AFP level, after treating with CCL4. In tumor pathology, alteration or disease affected liver cells can express, AFP elevation in HCC was reported. The range between 20-200 ng/mL in serum AFP, indicating non-specific criteria in HCC prediction [36-40]. Similarly, findings of several studies speculated that, level of AFT sensitivity is high in specific HCC [41-47]. However, in another study, carcinogenesis due to CCL4, was considered to be secondary to its toxicity effects of hepatic cells [48]. Similar studies have suggested the progression of AFP, is linked with the severity of the disease[49-54].

Our findings can be assumed that higher content of phenol present in aqueous extract of P.Amboinicus contributed efficacy, to get rid of free radicals to deplete CCL4 effects. Similarly, in another study, it has exhibited that, inter linking of phenols and antioxidant potential, to stabilize carcinogenesis, and lipid peroxidation,resulting in a decline of AFP level [55-59].

As a bio enhancer

The present investigation is ample evidence that plant extract is believed to cure several diseases including HCC and an effective bio enhancer for anti cancer activities. In earlier studies, [60-65] demonstrated that plant extract distillate is a potential enhancer and facilitator for biomolecules and served as antibiotic, antifungal and anticancer agents. In a recent study, plant extract distillate was treated with increasing concentration of 20 UL, 40UL 60UL and 80 UL and outcome showed that plant extract was more effective enhancing property to the several antimicrobial drugs within 48 hours of incubation [66-69]. Interestingly, earlier studies have deciphered that extraction of anti cancer agents derived from plant species particularly, cruciferous vegetables can bring down the risk of developing lung cancer HCC, colorectal and prostate carcinomas [70-74]. In another study Plectranthus Amboinicus seeds are potential and enriched with Glucosinolates (or) Isothiocyanate [75]. In our work, sulforaphane, a glucosinolate exhibited from Plectranthus Amboinicus was evidenced as a bioenhancer for anticancer therapeutic agents.

CONCLUSION

The present investigation has endorsed that Plectranthus Amboinicus extract has played a crucial role in modulating vital biochemical alterations in the liver enzymes, for the hepatocytes protection and also functions as enhancer for effectiveness to the anti cancer agents, that was being tested in HepG2 cell lines. Decline of AFP biomarker has favoured the evidence for plant extract potential in treating HCC in early detected diseases. It can be concluded that plant extract imparts dual protective role in depleting liver enzymes and AFP,an important biomarker for HCC diseases, as observed in the study and need a further research to explore or validate its mechanism of actions in treating Hepatocellular carcinoma clinically.

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