# HEPATOPROTECTIVE EFFECT OF ETHYL ACETATE EXTRACT OF *Terminalia arjuna* root ON HCB INDUCED LIVER CARCINOGENESIS IN FEMALE ALBINO WISTAR RATS

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**ABSTRACT:** Cancer has been a dreadful and complex disease of present century. The use of various anticancer compounds from plants for human healthcare still remain the most widely used medication system in developing and least developed nation. Compounds possessing anticancer properties have been identified in fruits, vegetables, spices, and medicinal plants. *Terminaliaarjuna* is a widely used medical plant throughout India. The anticancer effect of *T. arjuna* were studied on albinowistar rats treated with *T. arjuna* crude and its extract from root. Therefore the root of *T. arjuna* were found to have anticancer activity. According to experiment, designed five groups of rats was evaluated the beneficial properties of *T. arjuna* consumption on liver marker enzyme regulation and lipid peroxidation in albino rats in HCB induced stage. For the study, the female albino rats were divided into five groups, normal, corn oil control, chemical treated, chemical along with the crude and its extract of plants treated groups for initiation phases. The treatment of HCB caused carcinogenesis for 30 days and the other group had plant crude and its extract treatment up to 30 days. Effects of *T. arjuna* consumption on LPO and liver marker enzymes were also evaluated. The plant treatment had remarkable effects on LPO and liver marker enzymes level in the female albino rats. An improvement in lipid peroxidation and liver marker enzymes were observed with lower lipid peroxidation and liver marker enzymes after 30 days of *T. arjuna*.

**Key words:** *Terminalia arjuna root*, female albino wistar rats, lipidperoxidation, liver marker enzymes, antioxidant effect, hepatocarcinogenesis.

**Introduction:**

Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases. Medicinal plants are the richest bio-resources of folk medicine, traditional systems of medicines, food supplements, nutraceuticals, pharmaceutical industries and chemical entities for synthetic drugs. Modern medicine has evolved from folk medicine and traditional systems, only after through chemical and pharmaceutical screening. According to the present scenario it is estimated that 40% of the world populations depends directly on plant based medicine for their health care. India is the birth place of renewed system of indigenous medicine such as Unani, Ayurveda, Homeopathy and Siddha. Traditional systems of medicine are prepared from a single plant or combinations of number of plants; the efficacy depends on the use of proper plant part and its biological potency which in turn depends upon the presence of required quantity and nature of secondary metabolites in raw drug.

According to the world Health Organization, over 80% of the world's population or 4.3 billion people realy upontraditional plant based systems of medicine for primary health care. In India, almost 95% of the prescriptions were plant based in the traditional systems. Knowledge of the chemical constituents/phytochemicals of plants is very important for the synthesis of complex chemical substances. Such phytochemical screening of plants is reported by many workers. Nowadays, cancer is widely recognized as one of the most formidable human afflictions. It exists in more than 100 forms and has many causes, from genetic factors to infection (WHO, 2005).

Hepatocellular carcinoma is one of the most common malignancies in the world. Hepatocellular carcinoma (HCC) in developing countries, accounts for 15% of total cancer mortality burden. Because the global pandemic of hepatitis-B and C viral infections, the incidence of HCC is rapidly increasing in Asian and Western countries and this trend is expected to continue for the next 50 years because of the long latency between infection and the development of HCC. The etiology of HCC in human is clearly multifactorial. In contrast to the situation in humans, the major hepatocarcinogens identified in mice and rats are chemicals. Numerous chemicals have been tested for their carcinogenic potentials in bioassays performed in mice and rats under standardized laboratory conditions. Carcinogenesis is a complex process that has been divided into three stages - Initiation, promotion and progression. These three stages of tumour formation have been characterized in many mammalian tissues, particularly in the liver. Similar patterns of development and expression of HCC in mice, rats, and human would support the use of rodents as substitute for identifying risk factors of HCC in human. Hepatocellular carcinoma can be induced in the livers of laboratory animals by variety of chemicals. Multistage carcinogenesis studies have extensively used for the analysis of cancer development. In particular, the two-stage initiation-promotion protocol has been widely used in systematic elucidation of the carcinogenesis.

The medium term liver foci bioassay developed by Ito *et al* involves the sequential administration of potent initiator, DEN followed by chemical treatment and mitogenic stimulation ofhepatocyte growth. This study is based on Cabral *et al* and smith and Cabral Study or initiation / promotion protocol. Experimental, clinical and epidemiological studies have provided evidences supporting the role of reactive oxygen species in the etiology of cancer. Diethylnitrasamine has been suggested to cause oxidative stress and cellular injury due to the enhanced formation of free radicals. Oxidative stress is considered as critical mechanism contributing to NDEA induced hepatotoxicity and carcinogenesis.

Since the increase in the use of synthetic chemicals in cancer therapy has led to many side effects and undesirable hazards, there is a worldwide trend to go back to natural resources (medical plants) which are therapeutically effective, culturally acceptable and economically within the reach of even the neediest people. Accumulating epidemiological, experimental evidence has revealed the influence of number of naturally occurring and synthetic compounds on drug-detoxification and HCC incidence (Premaladha and Sachuthaanatham, 2000).

Flavonoids are the polyphenolic phytochemicals with in- consistent phenolic structures; they consist of flavones, flavanone, flavanols, flavonols and flavanonols that com- prise a large group of secondary metabolites in plants. They are found in vegetables, fruits, flowers, grains, barks, roots and stems. Flavonoids have significant biological activities, such as anti-microbial, anti-oxidant, anti-cancer and anti-inflammatory activity.Being a developing country, in Bangladesh the rate of mortality due to infectious diseases is very high. Antimicrobial resistance of the variable drugs is the key reason for this. Inappropriate use of readily available antibiotics, prolonged hospitalization, and poor implementation of infection control measures is the main causes of drug resistance. Moreover, powerful drugs against which antimicrobial resistance has not yet been developed are unavailable and costly. So, poor people of our country cannot afford this. In this situation, there is a crying need for an alternative treatment for gut infection, which is both effective and inexpensive. Using of medicinal plant in healing this type of infection can be an important solution to this emerging problem.

Phytomedicine usually indicates to the use of plants or parts of any plant for the treatment and prevention of diseases. Natural products of plants are rich of different bioactive compounds which are used for the treatment of diverse diseases and also it is used as template for synthetic modification of the phytomedicine. According to World Health Organization (WHO) around 4 billion people, directly or indirectly use phytomedicine for treating and prevention of diseases. Now-a-days, natural products of plants and their analogs still represent a large portion of all drugs in clinical use.

In recent year some plant based new drugs have been introduced and commercialized in the international market during 2000-2007. These new drugs have been approved for the treatment of different genetic disorders and metabolic diseases. *Terminalia arjuna* is an only herb that helps uphold a healthy heart and decrease the effects of stress and anxiety. The bark of *T.arjuna* is useful as an antiischemic and cardio protective agent in hypertension (High blood pressure) and in ischemic heart illness (IHD), particularly in uneasy cardiac rhythm, angina or myocardial infarction. The bark powder possesses diuretic and a broadspectrum tonic effect in cases of cirrhosis of the liver, *T. arjuna* may also be useful in treating hypercholesterolemia by reducing LDL levels.

The recent explosion of interest in the bioactivity of the flavonoids of higher plants is due, at least in part, to the potential health benefits of these polyphenolic components of major dietary constituents. This review article discusses the biological properties of the flavonoids and focuses on the relationship between their antioxidant activity, as hydrogen donating free radical scavengers, and their chemical structures. This culminates in a proposed hierarchy of antioxidant activity in the aqueous phase. The cumulative findings concerning structure-antioxidant activity relationships in the lipophilic phase derive from studies on fatty acids, liposomes, and low-density lipoproteins; the factors underlying the influence of the different classes of polyphenols in enhancing their resistance to oxidation are discussed and support the contention that the partition coefficients of the flavonoids as well as their rates of reaction with the relevant radicals define the antioxidant activities in the lipophilic phase.

Flavonoids are polyphenolic glycosides, water soluble and occur almostuniversally in higher plants. They impart color of flowers and fruits and their correlation between flower color and attaraction of insects for pollination is well known1. Over 6000 naturally occuring flavonoids have been described and many of them are common in higher plants (Harborne and Williams, 2000). Eleven of these are found commonly, Anthocyanidins-Pelargonidin, Cyanidin, Peoniodin, Delphinidin, Petunidin and Malvidin, Flavonols- Kaempferol, Quercetin and Myricetin; and Flavones-Apigenin and Luteolin. They have been found to own potent antioxidant and freeradical scavenging activities and also show biological effects such as anti-anginal, antiallergic, anti-ulcer, anti-hepatotoxic, anti-viral, anti-inflammatory and anti-spasmolytic. Flavonoids appear to play vital roles in defence against pathogens. However, very little work is reported with the leaves and fruits extract of *T. arjuna* and so far no work has been done on bioactivity of root of *Terminalia arjuna*. Present study was carried out the quantitative phytochemical analysis were done for the presence of various secondary metabolites present in ethyl acetate extract of *Terminalia arjuna* root.

**Materials and methods:**

**Plant materials collection:**

*Terminalia arjuna* roots were collected from Lagoon area of Muthupet, Thiruvarur district during the period of September – 2014 to October – 2014.

**Preparation of Plant material:**

*Terminalia arjuna* plant root was collected from Lagoon area of Muthupet and cut into small piece dried under the shed for 3 weeks at room temperature. The plant root was shaded and dried for grinding to get crude powder. **Preparations of plant extract using Soxhlet apparatus**:

10 g of crude powdered drug were taken and shifted into filter paper thimble. 250 ml of Ethyl acetate were poured into round bottom flask (1000 ml capacity) followed by fitting in on Soxhlet apparatus. The powdered drug was extracted with Ethyl acetate for 24 hours. A semisolid extract was obtained after completed elimination of ethyl acetate under reduced pressure. The extract was stored in refrigerator until use.

**Carcinogen**

Hexachlorobenzene or perchlorobenzene is an organo chloride with the molecular formula$C_6Cl_6$. Hexachlorobenzene is an animal carcinogen and is considered to be a probable human carcinogen. Animal carcinogenicity for hexachlorobenzene show increased incidences of liver, kidney and thyroid cancers. Chronic oral exposure in humans has been shown to give rise to a liver disease (porphyria cutaneatarda), skin lesions with discoloration, ulceration, photosensitivity, thyroid effects, bone effects and loss of hair.

### Animals

The study was carried out after getting permission from Institutional Animal Ethics Committee (CPCSEA Approval Number: 326/ SASTRA/ IAEC/ RPP) and CPCSEA regulations were adhered to during the study Albino wistar rats of female at an age of 15-20 weeks containing 120-150g weight were selected for this study. These animals were purchased from Animal and Veterinary Sciences University, Hyderabad, India. Female albinowistar rats were housed in polypropylene cages and maintained in controlled temperature with standard rat chow. The animals are acclimatized in laboratory condition and also divided into 5 groups (Group I, II, III, IV and V), thus each group contains 6 animals. Food and water were provided *ad –libitum* for one month.

### EXPERIMENTS SETUP:

### Group – I (Normal control):

Female albino rats are received (0.5 ml of normal saline/animal/day)up to $30^{th}$ days.

### Group – II (Vehicle control group):

Female albino rats received corn oil vehicle (1ml/animal/day)  up to $30^{th}$ days.

### Group – III (Carcinogenic group):

1 g/ litre HCB in corn oil vehicle from 3rd week to $30^{th}$ days.

**Group – IV (Treatment group I - Crude powder):** Female albino rats received HCB similar to that of Group – III along the treatment of*Terminalia arjuna* (200mg/kg) crude powder suspended in water/day upto $30^{th}$ days.

**Group – V (Treatment group II – Ethyl acetate extract):** Female albino rats received HCB similar to that of Group – III along the treatment of *Terminalia arjuna* (50mg/kg) ethyl acetate extract/day upto $30^{th}$ days.

### Collection of samples

After the completion of experimental regimen, the rats were fasted overnight and blood samples were collected by cervical decapitation with mild ether anesthesia and serum was collected.  Whole liver was immediately dissected out and washed in ice cold saline.  A known weight (1g) of liver was taken and homogenized with (10%) phosphate buffer (pH.7.4).  The serum, whole blood with EDTA, and liver homogenate were used for various biochemicalanalyses.  Liver preserved in salinized formalin was used for histochemical analysis.

### Biochemical analysis

The serum was used for the following estimations.
Lipidperoxidation and Antioxidant Enzymes (LPO, GSH, SOD, CAT), Antioxidant vitamins (Vit-C,Vit-E), Liver markers (AST, ALT, ALP, ACP, G-Glutathione, Bilirubin).

### Statistical analysis:

The data were presented as means (μ) ± standard errors of the means (S.E.M). Comparison between more than two different groups was carried out using the one way analysis of variance (ANOVA) where $p<0.05$ was considered statistically significant.  The data were analyzed using Origin Pro (version 8.5) was used to carry out the statistical tests.

**RESULTS**

**Table: 1 Effects of *Terminaliaarjuna* on body weight of rats**

| Duration | Group-I | Group-II | Group-III | Group-IV | Group-V |
|---|---|---|---|---|---|
| 1$^{st}$ week (g/rat) | 240±10 | 240±10* | 240±10* | 240±10* | 240±10* |
| 2$^{nd}$ week (g/rat) | 255±5.0 | 295±10* | 200±10* | 215±5.0** | 220±5.0** |
| 3$^{rd}$ week (g/rat) | 295±10 | 320±10* | 275±10* | 280±10** | 275±10** |
| 4$^{th}$ week (g/rat) | 305±10 | 345±5.0* | 225±10* | 245±10** | 260±10** |

Values are the mean ± SD of 6 animals in each group.
Group II and III compared with Group I
Group IV, V compared with Group III

**Table: 2 Effects of *Terminaliaarjuna* on Liver weight of rats**

| Duration | Organ | Group-I | Group-II | Group-III | Group-IV | Group-V |
|---|---|---|---|---|---|---|
| After 4$^{th}$ week | Liver | 5±0.6 | 6.8±0.3* | 7.8±1.8* | 6.3±0.5** | 5.92±0.6** |

Values are the mean ± SD of 6 animals in each group.
Group II and III compared with Group I
Group IV, V compared with Group III

**Table: 3 Effects of *Terminaliaarjuna root* on Lipid Peroxidation and Antioxidant enzymes**

| PARAMETER | Group-I | Group-II | Group-III | Group-IV | Group-V |
|---|---|---|---|---|---|
| LPO(mmole/ ml) | 8.24±0.24 | 11.9±0.45 | 53.08±1.10 | 21.83±0.5 | 23.4±0.20 |
| GSH(mmole/ dl) | 360±2.309 | 338±4.16 | 193±1.76 | 310±10.0 | 265.3±2.66 |
| SOD(U/mg) | 15.16±0.221 | 11.52±0.570 | 4.42±0.411 | 6.08±0.116 | 5.93±0.133 |
| CAT(U/L) | 147.66±1.008 | 128.2±0.01 | 98.266±0.176 | 120.73±0.437 | 102.93±0.133 |

Values are the mean ± SD of 6 animals in each group.
Group II and III compared with Group I
Group IV, V compared with Group III

**Table: 4: Effects of *Terminaliaarjuna* root extracton antioxidant Vitamins**

| PARAMETER | Group-I | Group-II | Group-III | Group-IV | Group-V |
|---|---|---|---|---|---|
| Vit-C (mg/dl) | 4.02±0.016NS | 5.68±0.065 | 3.66±0.016 | 5.81±0.016 | 4.12±0.46 |
| Vit-E (mg/dl) | 3.3±0.076 | 5.6±0.19 | 2.94±0.164 | 4.48±0.80 | 5.22±0.083 |

Values are the mean ± SD of 6 animals in each group.
Group II and III compared with Group I
Group IV, V compared with Group III

**Table: 5: Effect of *Terminaliaarjuna* root on Liver markers**

| PARAMETER | Group-I | Group-II | Group-III | Group-IV | Group-V |
|---|---|---|---|---|---|
| AST (IU/L) | 18.833±0.202 | 37.733±0.622 | 61.066±0.666 | 31.433±0.120 | 38.6±0.01 |
| ALT (IU/L) | 10.866±0.037 | 17.733±0.352 | 21.266±0.240 | 10.466±0.133 | 10.116±0.196 |
| ALP(IU/L) | 10.21±0.055 | 14.4±0.10 | 19.4±0.20 | 13.066±0.266 | 14.2±0.20 |
| ACP(IU/L) | 10.2±0.01 | 11.66±0.166 | 18.466±0.133 | 14.46±0.133 | 12.6±0.115 |
| g-GT(IU/L) | 33.8±1.30 | 37.266±1.333 | 49.93±1.33 | 32.533±0.333 | 30.933±0.533 |
| Bilirubin  (mg/dl) | 0.29±.06 | 0.48± .06* | 1.36± .06* | 0.61± .06[NS] | 0.711± 0.07[NS] |

Values are the mean ± SD of 6 animals in each group.
Group II and III compared with Group I
Group IV, V compared with Group III

**Discussion and Summary:**

The concept of multi-stage carcinogenesis was first proposed by Berenblum and Schubik in 1948 and supported by later studies. Present day oncology recognizes three main phases, Initiation, promotion and progression.

**Effects of *T. arjuna* crude and ethyl acetate extracton Physical observation**

Table: I shown the body weights of rats. The level of body weight was significantly decreased in II week of HCB group (1 mg/rat) as compared with normal group.  The level of body weight of 4th week of HCB group was lower (g/rat) when compared with normal and also other groups of this week. Likewise the 4th week of oil control's body weight was significantly higher (g/rat) as compared with normal as well as other groups. The body weight of HCB group had higher in third week than other weeks.

Table: II provides the value of liver weight changes in the different groups of rats. The HCB treated group of rats with I week decreased (g/liver) as compared with normal and other groups. Likewise, HCB group in 4th week liver weight was significantly (p<0.05) increased (g/liver) as compared with normal as well as other groups.

The body weight and liver weights of HCB slightly increased. The body weight and liver weight increment were responsible for HCB activity because, the HCB accelerate the DNA and RNA synthesis. Therefore HCB effect may be responsible mainly for weight increment and reduction reaction. Plant treated groups had low level of these changes; indicate its metabolic control activity.

**Effects of *T. arjuna* crude and ethyl acetate extract on lipidperoxidation**

LPO is regarded as one of the basic mechanism of cellular damage, caused by free radicals. Free radicals reacts with lipid causing peroxidation, resulting in the release of product such as malanodialdehyde, hydrogen peroxide ($H_2O_2$) and hydroxyl radicals (OH).  Lipidperoxidation is one among these and it is a process, which is formed by means of the oxidation of polyunsaturated fatty acids.  MDA is one of the final products of lipidperoxidation (Bast*et al.,* 1991). An increase in lipid-peroxides and hydroxyl radical indicates serious damage to cell membranes, inhibition of several important enzymes, reduced cellular function and cell death.

The table: III represents the level of LPO in experimental animals in this study. The level of lipid per-oxidation of HCB group was significantly higher in the serum level (53.8 mole/ml) as compared with normal groups. But in treated groups the serum LPO was significantly lower (23.4, 21.83 mole/ml) in *T.arjuna* treated group than control group as well as other treatment groups.

HCB is still of concern as an environmental contaminant, precise evaluation of its hepato carcinogenic potency is very important factor. The half-life of HCB was found to be 24 days in rats and 32 days in rabbits (Scheufler and Rozman, 1984). The metabolism of HCB in humans resembles that of HCB in rodent. Meabolites found in rats after exposure to HCB, include pentachlorophenol, tetrachloro -1, 4- dihydroxyquinone and diverse tetra and trichlorophenol (Koss *et al.,* 1979).  In the present study, Ito medium- term rat liver bioassay was selected to assess the level of induction effects of HCB on rat liver carcinogenesis.

HCB also cause LPO in this study because reactive oxygen species (ROS) have been proposed to play an important role in the pathogenesis caused by poly-halogenated aromatic hydrocarbons like HCB. Several authors had shown an increase of lipidperoxidation  in the liver of rats treated with different doses of HCB.

However, the activities of catalase (CAT) and reduced glutathione (GSH) were significantly lower andassociated with enhanced oxidative stress and lipid peroxidation, and supposed that this might lead to the development and progression of atherosclerosis. Reactive oxygen species (ROS) have been reported to induce oxidative damage to membrane lipids, proteins, and DNA, and might in cell death by necrosis or apoptosis (Gamaley and Klyubin, 1999). Both glutathione peroxidase and catalase are major defenses against harmful effects of ROS  in cells, and in cultured thyrocytes, both have a high capacity to degrade exogenous hydrogen peroxide ($H_2O_2$) (Bjorkman and Ekholm, 1995).

Liver injuries may be caused byexcessive exposure to toxic chemicals and drugs. These toxicants mainly damage the liver by producing highly reactive oxygen species (ROS). The injury of these organs can result in many disorders, including fibrosis, liver cirrhosis, renal failure, and even hepatocellular and renal carcinoma (Srivastava&Shivanandappa, 2010).

In the study, the level of LPO was increased in HCB administered group's serum than normal group. The increase in LPO level in the animal induced by HCB suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals.  Therefore the levels of antioxidant enzymes were decreased in chemical control groups than the normal. These results were modified after administration of the crude powder and the ethanol extract of *Terminalia arjuna*.  These results indicated that these plants had anti-oxidative  effect in carcinogenesis.

In this experiment, the antioxidant enzymes SOD and CAT were given in the table – III. The level of SOD and CAT were significantly decreased in HCB groups as compared with normal groups. The decreased value of SOD in serum in Initiation was (4.42 U/mg) Likewise; the plant treatment increased the value of SOD significantly than control groups. The higher value was present in *T. arjuna* crude treated group (6.08 U/mg).  Similarly the CAT in crude treatment with increased values was (120.7 U/dl). The decreased activity of SOD and CAT were found increased by the supplementation of the root extract of *Terminalia arjuna*.

## Effects of *Terminalia arjuna* root on antioxidative vitamins

The present results also revealed that, the levels of vit-C and Vit-E were decreased in HCB group than the normal group.  Generally the natural antioxidants strengthen the endogenous antioxidant defense from ROS ravage and restore the optimal balance by neutralizing the reactive species.  Therefore the levels of vitamin  -C, E were decreased in HCB group.  Similarly the plant extracts *Terminalia arjuna* revealed its anti-lipidperoxidative  effect by incrementing of these vitamins (E and C) in initiation  phase.

ALT, AST, ALP and GGT which were known altogether as cholestatic liver enzymes.  Elevation of these enzymes can indicate the presence of liver disease.  AST and ALT (SGOT/SGPT) were jointly known as transaminases. They are associated with inflammation or injury to liver cells, a condition which is known as hepatocellular liver injury. Damage to the liver typically results in a leak of AST and ALT into the bloodstream. High levels of GGT and ALP hint at a blockage of the bile ducts (or) possible injury (or) inflammation of bile ducts.  It characterized by an impairment of bile flow, which were known as cholestasis.  When a blockage or inflammation of the bile ducts occurs, the GGT and ALT can overflow into the bloodstream (Melissa Palmer, 2004).

**Effects of *Terminalia arjuna* on Hepatic marker enzymes**

AST, ALT, GGT and ACP exhibit high levels in the abnormally functioning of liver. The administration of carcinogenic substances may bring changes in enzyme levels arising from cellular proliferation. So, it is of some importance to analyze the enzyme activity variation quantitatively, in order to understand the process involved.

In this study, the liver marker enzymes levels were given in the Table: IV. The marker enzymes ALT, AST, ALP, ACP and GGT were significantly increased in HCB groups as compared with normal as well as treatment groups. The increased levels of ALT and AST in initiation phase were (61.066, 21.266 IU/L). Likewise, the increased level of ACP and ALP were (18.466, 19.4 IU/ L). The peptidase enzyme GGT levels increment was (49.93 IU/ L). From the observation, revealed that the GGT increment in phase was higher than other values. It indicated that, the liver damage was induced by chemicals.

The increased level of AST, ALT, ALP and serum bilirubin were indicative of cellular leakage and loss of functional integrity of liver cell membrane (Drotman and Lowhorn, 1978). In the study, elevated serum level of AST, ALT and ALP and total bilirubin were indicative of poor hepatic function in HCB treated animals. All these indicate an induction of hepatocellular carcinogenesis by HCB. Consequently, elevated activities of ALT and AST were observed in the current study in response to HCB administration could be a common sign of impaired liver function to a group of enzymes catalyze the hydrolysis of phosphomonoesters at alkaline $P^H$ ALP is found present in cell surface in most human tissues. The highest concentrations were found in the intestine, liver, bone, spleen and kidney (Moss and Handeson, 1999). Acute cell necrosis liberates ALP in the circulation. GGT was found predominantly in liver. GGT was a sensitive marker of certain hepatotoxic drugs.

Exposure of Hexachlorobenzene in humans altered the level of hepatic marker enzymes AST, ALT and bilirubin were observed in Queiroz Study (1998). It indicates that the HCB was involved in liver cell damage. In the present result, the level of GGT also significantly found increased in HCB groups, than the normal rats (Table: IV). The administration of the plant extracts diminished these four enzyme increment level may be due to liver cell regenerating capacity. More over the remarkable activity of regeneration was observed from ethyl acetate extract of *Terminalia arjuna* and crude powder of *Terminalia arjuna* which observed from enzyme AST, ALT decrement (31.433, 10.116 IU/dl) level by these plant activities.

**Bilirubin**

In the result, the level of total bilirubin was found increased and it indicates that the unconjugated bilirubin accumulation was increased due to the deficiency of conjugating enzyme or decrement of elimination due to hepatic cell damage. Therefore HCB treated group had increased the total bilirubin than the normal rats group. But the administration of the plant *Terminalia arjuna* crude powder and the ethyl acetate extract near normalized this bilirubin level by its liver cell regenerating activity. Crude powder of *Terminalia arjuna* leads better effect on removal of bilirubin. Likewise hyperbilirubinemia observed in DEHP (di-2-ethylhexyl phthalate) treated rats indicates impairment of glucuronyltransferase system leading to inhibition of bilirubin elimination (Sjoberg*et al.,* 1991).

**CONCLUSION**

From this study, the selected plant *Terminalia arjuna* root crude powder and ethyl acetate extract showed the hepatoprotective activity, antioxidant and anticancer activity. These positive results proved/ indicates that, the presence of bioactive compounds in the plant were active. The plant root of *Terminalia arjuna* screened for bioactive compounds seemed to have the potential to act as a source of useful drug. This plant can be, further subjected to isolation of the hemotherapeutic agent and carry out for further pharmacological evaluations.

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