Effect of Coleus Forskohlii Root Extracts on Liver Marker Enzymes

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***Abstract*—The roots of *Coleus forskohlii* were shade dried and powdered. The powder was extracted with different solvents such as petroleum ether, chloroform, acetone and methanol. The *Balb/C* mice weighing between 25-30g were induced with DLA cells and treated with the extracts of *Coleus forskohlii* roots to evaluate the activity of the liver marker enzymes. The liver marker enzymes such as alkaline phosphatase, acid phosphatase, aspartate transaminase, alanine transaminase, lactate dehydrogenase were found to have increased in the serum, indicating the liver damage and were reduced significantly in the methanol extract treated mice.**

***Index Terms*—*Coleus forskohlii*, liver marker enzymes, alkaline phosphatase, acid phosphatase, aspartate transaminase, alanine transaminase, lactate dehydrogenase**

I. Introduction

One of the major drawbacks of the current cancer therapeutic practices such as chemotherapy and radiation therapy is the suppression of the body's immune system. Both these conventional therapies of cancer produce several side effects like nausea, vomiting, mucosal ulceration, alopecia, pulmonary fibrosis, cardiac and hepatic toxicity etc. Drugs that could alleviate these side effects will be useful in cancer therapy [1]. Currently there is renewed interest in developing drugs from plant for cancer therapy. Many indigenous plants are being tested against a spectrum of experimental tumors with the perceived advantage of local availability, low cost and less side effects. Many plant derived products exhibit potent antitumor activity against several cancer cell lines [2].

Coleus forskohlii (Lamiacea) is an important plant in Indian Ayurvedic medicine. The roots of the plant produce the labdane diterpenoid, forskolin. It is distributed over the subtropical warm temperature climatic zones on the mountains of India, Nepal, Myanmer, Srilanka, Thailand and Africa. Forskolin from *Coleus forskohlii* has been identified as a potent activator of adenylate cyclase leading to an increase in the levels of cAMP affecting cardiac muscle contraction, blood and intraocular pressure, cancer, eczema, rheumatism and obesity [3].

Liver is the key organ in the metabolism, detoxification and secretary functions in the body and its disorders are numerous with no effective remedies, and however, the search for new medicines is still ongoing Many folk remedies from plant origin have been long used for treatment of liver diseases. Management of liver diseases is still a challenge to the modern medicine. In Ayurveda, various herbal and herbomineral preparations are extensively used for the treatment of various liver disorders. An indigenous system of medicine in India has a long tradition of treating liver disorder with plant drugs [4]. The presence of tumor in the humans or experimental animals is known to affect many functions of the vital organs especially the liver, even when the site of the tumor does not interfere directly with the organ functions. Liver plays an important role in the modulation of the process of carcinogenesis, as it is the primary site for biotransformation of xenobiotics including carciogens and anticancer drugs [5]. The efficacy of any hepatoprotective drug is essentially dependent on its capacity of either reducing the harmful effects or maintaining the normal physiologic function which has been disturbed by hepatotoxic agents. SGOT, SGPT and ALP are the most sensitive markers employed in the diagnosis of hepatic damage, because these are cytoplasmic in location and are released into the circulation after cellular damage. Measurement of the activities of serum marker enzymes, like aspartate transaminase, alanine transaminase, alkaline phosphatas and acid phosphatase can give an assessment of the liver function [6]. Hence a study has been carried out to evaluate the effect of *Coleus forskohlii* roots on the activity of liver marker enzymes in DLA tumour bearing mice.

II. Materials and Methods

The plant material *Coleus forskohlii* was collected from Tamil Nadu Agricultural University, Coimbatore

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and was duly authenticated by Dr. G. V. S. Murthy, Joint Director, Botanical Survey of India, Southern Regional Centre, Tamil Nadu Agricultural University, Coimbatore. The roots of the plants were cut into pieces and dried under shade for a week. The shade-dried roots were coarsely powdered and weighed. 100 g of the powder was extracted in 500 ml each of 70% petroleum ether, chloroform, acetone and methanol respectively using soxhlet apparatus. The extracts were concentrated to dryness in a rotary evaporator under reduced pressure and controlled temperature (40-50 ℃). The extracts were preserved in a refrigerator at 4 ℃ for further use. After the completion of the extraction, they were filtered and the solvent was removed by distillation under reduced pressure. A brown coloured fumy residue was obtained. It was then dissolved in 0.3% carboxy methyl cellulose and was used for the study.

Healthy male Balb/C mice of approximately with the same weight (25-30grams) were procured from N.G.P. College of Pharmacy, Coimbatore. The mice were fed with normal laboratory diet and water *ad libitum* and acclimatized for a week under laboratory conditions. The study protocol was approved by the IAEC. The mice were divided into twelve groups of 6 each to determine the liver marker enzymes of the different extracts of the root of *Coleus forskohli*. The mice were given oral dose of extracts after the 1st day of induction of cancer.

The mice were made to fast overnight on the 21st day and were sacrificed on the next day after recording body weight by decapitation. Liver was excised, rinsed in ice cold normal saline, followed by cold 0.15M Tris-HCl buffer (pH 7.4) and blotted between filter paper to dry. A 10% w/v tissue homogenate was prepared in 0.15M, Tris-HCl buffer with a homogenizer fitted with a Teflon plunger and centrifuged at 1500 rpm for 15 min at 4 ℃. The supernatants were used for the estimation of liver marker enzymes.

A. Estimation of Acid Phosphatase (ACP) [7]

Pipetted out 0.5 ml of the buffered substrate (5.5mM P-Nitro phenyl phosphate in 0.05M sodium citrate buffer) into four clean, dry test tubes labelled as control, test, standard and blank. The volume of the blank was made up to 1.0ml by adding 0.5ml of distilled water. Mixed well and incubated at 37 ℃ for 3 min. Added 0.5 ml of the working standard phenol solution to the standard tube. 0.5 ml of the serum sample was added to the test. Mixed well and incubated at 37 ℃ for 60 min. 0.5 ml of 0.5N sodium bicarbonate was added to all the test tubes. To the control 0.5 ml of the serum was added. Added 0.5 ml of 4-aminoantipyrine (0.6 %) and 0.5 ml of potassium ferricyanide (0.24%) to all the test tubes. Mixed well after the addition of each reagent and measured the O.D at 510 nm against blank.

B. Estimation of Alkaline Phosphatase (ALP) [8]

Pipetted out 4.0 ml of buffered substrate (50 ml of 100mM disodium phenyl phosphate + 50 ml of 100mM sodium carbonate-bicarbonate buffer) in a test tube and placed in a water bath at 37 ℃ for few minutes. Added 0.2 ml of serum mixed, stoppered and left in the water bath for 15 minutes exactly. Removed, added 1.8 ml of diluted (1:3 dilution) phenol reagent of Folin-Ciocalteau. Set up control containing 4.0 ml buffer substrate and 0.2ml of serum to which added 1.8 ml of diluted phenol reagent. Mixed and centrifuged. To 4.0 ml of supernatant from each added 2.0 ml of sodium carbonate (150g Na_2Co3/L). To the standard (2-10 μg/ml) added 2.0 ml of sodium carbonate and varying concentrations of phenol reagent. Placed the tubes in a water bath at 37◦C for 15 minutes and read at 700 nm against a blank. The King-Armstrong unit corresponds to the liberation of 1.0 mg of phenol by 100 ml of serum under the assay condition.

C. Estimation of Serum Aspartate Transaminase (AST) and Alanine Transaminase (ALT) [9]

Added 2.0 ml of serum to 1.0 ml of buffered substrate (100mM phosphate buffer and 2 mM alpha-oxoglutarate with 100mM L-aspartate included for AST or 200 mM DL-alanine for ALT). Dissolved 15g K_2HPO4, 2.0g KH_2PO4 and 300 mg alpha oxo glutatarate in 700-800 ml of water and (a) for AST added 15.7 g L-Aspartate, monosodium salt or (b) for ALT added 17.8g DL alanine. In both cases the pH was adjusted to 7.4 with sodium hydroxide, mixed and incubated for 60 min for AST or 30 min for ALT at 37 ℃. Then added 1.0 ml of DNPH (1mM in 1M HCl), allowed to stand at room temperature for 20 min. Added 10 ml of 400 mM sodium hydroxide solution, mixed and after 5 min, read at 500-550 nm. For the blank take 0.2 ml of serum, 1.0ml of buffer substrate and 1.0 ml of DNPH, mixed and completed as for test. A set of pyruvate standards (10-100 μg) was run similarly and plotted the absorbance against concentration.

D. Estimation of Serum Lactate Dehydrogenase (LDH) [10]

The serum Lactate dehydrogenase was determined by the method of Wrolewski *et al.* (1978). Measured 2.7ml of the phosphate buffer into a cuvette and added 0.1ml of serum and 0.1ml of NADH. Allowed to stand for 20minutes at 37 ℃ to reduce any keto acids already present in the serum. Then added 0.1ml of sodium pyruvate. Read the extinction for 5 minutes at intervals of 15-30 seconds at 340nm.

E. Statistical Analysis

Statistical significance was determined by One-way Analysis of Variance (ANOVA). 'P' value of 0.05 or less was considered as significant.

III. Results and Discussion

Changes in plasma enzyme activity are used as indicators of tissue injury, environmental stress or a diseased condition. The rate of increase of plasma enzyme activity depends on the concentration of an enzyme in the cells, the rate of leakage caused by injury and the rate of clearance of the enzyme from plasma [11]. In the assessment of liver damage by any hepatotoxin, the determination of enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatise (ALP) and acid phosphatase (ACP) are largely used [12].

Because of their intracellular location in the cytosol, toxicities affecting the liver pass subsequently to the membrane architecture of the cells leading to their spillage into plasma and their concentration gets increased [13].

A. Acid Phosphatase and Alkaline Phosphatase

Table I represents the influence of *Coleus forskohlii* root extracts on acid phosphatase and alkaline phosphatase in the DLA induced mice. Acid phosphatase and alkaline phosphatase activities in the DLA induced mice increased in the serum significantly compared to the untreated control mice indicating their leakage from the marker tissues to the serum due to stress and membrane damage created by the DLA cells. The petroleum ether, chloroform and acetone extracts of *Coleus forskohlii* showed a less significant decrease in the activity of ALP and ACP when compared to the methanolic extract. Methotrexate standard treated mice showed a significant decrease (p<0.05) in acid phosphatase activity. The increase in the enzyme activities is likely due to the leakage of enzyme from the cytosol of liver that might have entered into the blood stream resulting in high levels of activities of these enzymes. They were proved to be excellent indicators of DLA cells induced hepatocellular necrosis in mice [14].

TABLE I. ACTIVITIES OF ACID PHOSPHATASE AND ALKALINE PHOSPHATASE IN SERUM OF MICE

Treatment		Acid phosphatase #	Alkaline phosphatase#
Untreated control	UC	4.60 ±0.45^{e}	5.41 ±0.21^{f}
Vehicle control	VC	4.53±0.03	5.20 ±0.02^{f}
Petroleum ether	CPE	4.93 ±0.02	5.65 ±0.75^{f}
Chloroform	CCF	5.14 ±0.01	5.44 ±0.04^{f}
Acetone	CA	4.43 ±1.01	5.55 ±1.02^{f}
Methanol	CM	4.82 ±0.01	5.01 ±0.05^{f}
DLA Control	DC	11.52 ±0.08^{a}	9.92 ±0.31^{a}
Petroleum Ether extract	DPE	11.47 ±0.51^{a}	9.87 ±0.72
Chloroform extract	DCF	11.03 ±0.27^{b}	9.25 ±0.89^{b}
Acetone extract	DA	11.35 ±0.66^{a}	8.70 ±0.96^{c}
Methanol extract	DM	7.695 ±0.57^{c}	7.12 ±0.45^{d}
Methotrexate	DMT	6.48 ±0.52^{d}	6.53 ±0.46^{e}
CD (5%)		0.28	0.48

Values are mean ±SD of six replicates
Means followed by common superscripts do not differ significantly at 5% level
KA Units /100 ml serum

The increased activity of alkaline phosphatase in the DLA cells induced mice could be due to the damage to the cell membrane of tissues, where these enzymes are firmly attached. The damage releases these enzymes from the membrane, joining the binary canalicules and the sinusoidal border of parenchyma cells [15]. Alkaline phosphatase is a membrane bound enzyme found at the bile pole of hepatocytes and also found in the pinocytic vesicle and golgi complex. It is present on all cell membranes where active transport occurs. It is often employed to assess the integrity of plasma membrane, since it is localized predominantly in the microvilli of the bile canaliculi, located in the plasma membrane. Decrease in ALP activity may be taken as an index of hepatic parenchymal damage and hepatocytic necrosis [11].

B. Aspartate Transaminase, Alanine Transaminase and Lactate Dehydrogenase

Aspartate aminotransferase (AST) is a mitochondrial enzyme involved in the transfer of an amino group from 2-amino - to 2-oxoacid found in the heart, liver, skeletal muscle and kidney and is normally present in the plasma. The activities of these enzymes in the serum are raised when any of these tissues are damaged and are clinically useful in the diagnosis of hepatobiliary disease, myocardial infarction, skeletal muscle necrosis and pulmonary infarction [16]. Serum glutamate oxaloacetate transaminase (SGOT or AST) and serum glutamate pyruvate transaminase (SGPT or ALT) activities are known toxicity markers in the study of hepatotoxicity caused by chemicals. An increase in the activity of these enzymes is termed as the early recognition of toxic hepatitis [11].

The influence of *Coleus forskohlii* root extracts on serum aspartate transaminase, alanine transaminase and lactate dehydrogenase activity in the DLA induced mice is given in Table II.

TABLE II. ACTIVITIES OF ASPARTATE TRANSAMINASE, ALANINE TRANSAMINASE AND LACTATE DEHYDROGENASE IN SERUM OF MICE

Treatment	Aspartate transaminase #	Alanine transaminase #	LDH#
UC	30.30 ±0.90^{f}	34.57 ±1.79^{e}	20.40 ±0.92^{e}
VC	35.55 ±0.41^{f}	33.45 ±0.07^{e}	23.15 ± 0.75^{e}
CPE	33.23 ±3.00^{f}	33.05 ±0.05^{e}	21.57 ±0.08^{e}
CCF	29.01 ±3.03^{f}	35.12 ±0.03^{e}	23.74 ±0.93^{e}
CA	34.02 ±0.02^{f}	34.13 ±0.07^{e}	23.15 ±0.14^{e}
CM	32.43 ±0.05^{f}	33.14 ±0.02^{e}	20.20 ±0.10^{e}
DC	55.51 ±3.64^{b}	51.48 ±2.65^{a}	46.81 ±5.53^{b}
DPE	59.76 ±1.46^{a}	47.30 ±1.85^{b}	43.14 ±0.56^{c}
DCF	52.23 ±3.23^{c}	51.73 ±0.49^{a}	49.02 ±3.32^{a}
DA	39.62 ±0.68^{d}	40.08 ±1.84^{c}	41.23 ±0.08
DM	34.02 ±2.03^{e}	31.99 ±1.69^{f}	20.19 ±0.89^{e}
DMT	35.62 ±3.47^{e}	37.96 ±1.53^{d}	24.06 ±0.08^{d}
CD (5%)	2.94	1.68	3.52

Values are mean ±SD of six mice
Means followed by common superscripts do not differ significantly at 5% level; # IU /100 ml serum

Asparte transaminase and alanine transaminase activities showed a marked increase (p<0.05) in the DLA control mice compared to the untreated control mice. The high levels of AST and ALT in the serum are usually indicative of liver damage in animals. Among all the extracts, methanolic extract caused the most significant decrease in these serum marker enzymes showing the efficiency of this extract for healing the membrane

damage. Antihepatotoxic effect of *Cichorium intybus* extract against CCl_4 toxicity in albino rats was observed [17]. He observed the tendency of the extract to serum levels of ALT, AST and ALP to return towards near normal level in CCl4 intoxicated rats. The reduction in AST and ALT activities towards normal might be due to the regeneration of hepatic cells by active constituents like flavonoids, alkaloids and terpenoids in the plant extract.

Effective control of ALP activity indicated an early improvement in the secretary mechanism of the hepatic cell [18].

LDH is an enzyme associated with the soluble portion of the cell. LDH catalyses the conversion of lactate to pyruvate using NAD+ as coenzyme of NAD [25]. The increase in LDH activity in serum (46.81 IU/100ml serum) may be due to leakage of the enzyme from the tissues into the blood on account of cellular injury. The increase in serum LDH activity in the control mice was supported by the suggestion [19] of possible leakage of cytosolic enzyme from the tissues into the serum. Administration of the methanolic extract of *Coleus forskohlii* attenuated the increased activity of LDH in serum (20.19 IU/100ml of serum). A subsequent recovery towards normalization of these hepatic markers strongly suggests the possibility of the extract being able to protect the hepatocyte against membrane fragility and maintaining the functional status of the liver.

The significant increase ($p<0.05$) of ALP in the serum of experimental mice treated with DLA cells may be associated with possible leakage of the enzyme from the liver into the serum. Normally, enzyme will not always be found in the serum except there is damage to one or more organs or tissues of the body. Therefore, enzymes from diseased tissues or organs (e.g. cardiac, hepatic and neoplastic diseases) and from drug assault or other xenobiotics may become manifested in the serum resulting in increased activity since they must have leaked from the diseased tissue. Increased activity of serum enzymes have been reported in conditions of tissue damage due to such disease conditions and from the use of several chemicals and drugs [20]. This is often accompanied by a corresponding decrease in enzyme activity in the effected tissues or organs. It is well known that the transaminases play a paramount role in amino acid metabolism and in providing necessary intermediates for gluconeogenensis. It has also been reported that these enzymes assist in differential diagnosis of cardiac diseases. The decrease in the specific activity of AST and ALT of the liver and the significant increase in activities of the two enzymes in the serum suggest that there may be a leakage of the enzymes from the liver to the serum which may be interpreted to mean damage to the liver [21]. AST and ALT are cytoplasmic in location and are released into circulation after cellular damage. Assessment of liver function can be made by estimating the activities of serum AST, ALT and ALP, which are enzymes originally present in higher concentration in cytoplasm, when there is hepatopathy, these enzymes leak into blood stream in confirmity with the extent of liver damage [4]. ALT is frequently included in biochemical profiles for the purpose of assessing hepatic injury [23] and is also regarded as indicative of liver effects in dogs, nonhuman primates, rats, mice and hamsters [24], [25]. The elevation of alkaline phosphatase indicates the disturbed excretory function of liver. Assay of serum ALP activity has been recognized as a suitable marker of skeletal and hepatobiliary disorder. Moreover, an elevated serum level of ALP activity is frequently associated with various pathological conditions. ALP is a membrane bound enzyme, which is released unequally depending on the pathological phenomenon. The elevation of serum ALP concentrations is regarded as one of the most sensitive indices of hepatic damage. The garlic supplementation significantly lowered the activities of ALT and AST demonstration reduced liver damage following garlic administration (26). The leaf extracts of *Moringa oleifera* also showed hepatoprotective activity against mice fed with high fat diet (27). Thus the liver marker enzymes such as ALP, ACP, AST, ALT and LDH were found to have increased in the serum, indicating the liver damage and it is reduced significantly in the methanol extract treated mice which are on par with the standard and the untreated control.

IV. Conclusion

The study indicates that the serum enzymes, acid phosphatase, alkaline phosphatase, aspartate transaminase, alanine transaminase and LDH activities were found to be increased in the DLA treated mice and the activities reverted to almost normal in animals treated with the methanolic extract of roots of *Coleus forskohlii.* The outcome of this study confirms the protective effects of the *Coleus forskohlii* roots on liver function and its antitumor activity.

References

[1] C. Guruvayoorappan and G. Kuttan, "Immunomodulatory and antitumor activity of biophytum sensitivum extract," *Asian Pacific J. Cancer Prev.*, vol. 8, pp. 27-32, 2007.

[2] S. Madhuri and G. Pandey, "Some dietary agricultural plants with anticancer properties," *Archieves*, vol. 8, pp. 13-16, 2008.

[3] J. A. Duke and F. Mary, *Handbook of Medicinal Herbs*, Boca Raton-Florida: CRC Press, 2002, pp. 210.

[4] N. Mohandoss, S. Indra, and S. Sethupathy, "Antihepatotoxic efficacy of Indigofera tinctoria (Linn.) on paracetamol induced liver damage in rats," *Int J Pharm Biomed Res*, vol. 1, no. 1, pp. 13-18, 2010.

[5] A. Koul, A. R. Ghara, and S. C. Ganger, "Chemomodulatory effects of Azadirachta Indica on the hepatic status of skin tumor bearing mice," *Phytother Res.*, vol. 20, pp. 167-177, 2006.

[6] E. Porchezhian and S. H. Ansari, "Hepatotprotective effect of Abutilon indicum on experimental liver damage in rats," *Phytomed*., vol. 12, pp. 62-64, 2005.

[7] P. R. N. Kind and E. J. King, "Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine," *J. Clin. Pathol.*, vol. 7, no. 4, pp. 322-326, 1954.

[8] O. H. Lowry, N. J. Rosebrough, A. L. Fair, and R. J. Randall, "Protein measurement with the phenol-folin reagent," *J. Biol. Chem.*, vol. 193, no. 1, pp. 265-275, 1951.

[9] S. Reitman and S. Frankel, "A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases," *Am. J. Clin. Pathol*., vol. 28, pp. 56-63, 1957.

[10] H. Varley and Arnold, *Practical Clinical Biochemistry*, 4th Ed. India: Heinemann Publishers (India) Private Limited, 1998, pp. 472-473.
[11] O. Siakpere, R. B. Ikow, and M. G. Ogbe, "Variations in acid phosphatase and alkaline phosphatase activities in the plasma of the African cat fish: Clarias garie pinus exposed to sublethal concentrations of potassium permanganate," *Asian J. Exp. Biol*., vol. 1, pp.170-174, 2010.
[12] N. A. Dobbs, C. J. Twleves, W. Gregory, C. Curickshanka, M. A. Richards, and R. D. Rubens, "Epirubicin in patients with liver dysfunction: Development and evaluation of a novel dose modification scheme," *Eur. J. Cancer*, vol. 39, pp. 580-586, 2003.
[13] G. Jagadeesan and A. V. Kavitha, "Recovery of phosphatase and transaminase activity of mercury intoxicated *Mus musculus* (Linn.) liver issue by Tribulus terrestris (Linn.) (Zygophyllaceae) extract," *Tropical Biomedicine*, vol. 23, pp. 45-51, 2006.
[14] R. M. Johnson, G. Gerard, R. Yaddanapudi and H. Ye-Shih, "Oxidation of glutathione peroxidase-deficient red cells by organic peroxides," *Blood*, vol. 100, pp. 1515-1516, 2002.
[15] M. W. Gary and J. L. Michael, "Alteration of liver cell function and proliferation: Differentiation between adaptation and toxicity," in *Toxicologic Pathway*, S. C. Taylor, D. Francis, Eds. 2nd ed. London PA: Elseviver, 2002, pp. 41.
[16] M. W. Whitehead, N. D. Hawkes, I. Hainsworth, and J. G. C. Kinghorn, "A prospective study of the causes of notably raised aspartate aminotransferase of liver origin," *Gut*, vol. 45, pp. 129-133, 1999.
[17] H. Sedeghi, M. R. Nikbakht, I. Ghaitasi, and S. Sabzali, "Hepatoprotective effect of *Chichorium intyus* on CCl_4 induced liver damage in rats," *African Journal of Biochemistry Research*, vol. 2, pp. 141-144, 2008.
[18] O. O. Ojo, M. S. Nadro, and I. Tella, "Protection of rats by extracts of some common Nigerian trees against acetaminophen-induced hepatotoxicity," *Afri. J. Biotechnol*., vol. 5, no. 9, pp. 755-760, 2006.
[19] B. T. Ayorinde, M. A. Akanji, and M. T. Yakubu, "Alterations in some marker enzymes of liver and kidney damage following chronic administration of aqueous extract of Tapinanthus globiferus leaves to rats," *Phcog. Mag*., pp. S9-S14, 2008.
[20] K. S. Hanley, E. Schmidt, and F. M. Schmidt, *Enzymes in Serum, Their Volumes in Diagnosis*, Illinois: Charles Thomas Springfield, 1986, pp. 79-81.
[21] O. B. Oloyede and T. O. Sunmonu, "Decrease in activities of selected rat liver enzymes following consumption of chemical effluent," *J. Appl. Sci. Environ. Manage*., vol. 12, no. 2, pp. 95-100, 2008.
[22] G. S. Smith, R. L. Hall, and R. M. Walker, *Handbook of Toxicologic Pathology*, W. M. Haschek, C. G. Rousseaux, M. A. Walling, Ed., San Diego, Ca: Academic Press, 2002, pp. 681-771.
[23] A. J. Lenaerts, C. M. Johnson, K. S. Marrieta, V. Gruppo, and I. M. Orme, *Int J Antimicrobial Agents*, vol. 26, 152-158, 2005.
[24] R. J. Kothavade, S. N. Joylekar, and S. A. Barodavalla, *Indian J Pharm Sci*, vol. 58, pp. 142-146, 1996.
[25] L. A. Burtis and E. R. Ashwood, *Textbook for Clinical Chemistry*. W.B. Saunders Company, Philadelphia, Pennsylvania, 1986.
[26] S. Mirunalini, V. Arulmozhi, and T. Arulmozhi, "Curative effect of garlic on alcoholic liver diseased patients," *Jordan Journal of Biological Sciences*, pp. 147-152, vol. 3, no. 4, 2010.
[27] N. Das, K. Sikder, S. Ghosh, B. Fromenty, and S. Dey, "Moringa oleifera lam leaf extract prevents early liver injury and restores antioxidant status in mide fed with high fat diet," *Indian Journal of Experimental Biol.*, vol. 50, pp. 404-412, 2012.

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