ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Studies on hepatoprotective effect of hexane extract of *Dillenia indica* against CCl_4 induced toxicity and its safety evaluation in wistar albino rats

Himakar Reddy K[1], Tharanath V[2], Nagi Reddy KB[1], Sharma PVGK[3], Reddy OVS[1]*

[1]Department of Biochemistry, Sri Venkateswara University, Tirupati-517502
[2]Department of Microbiology, Sri Venkateswara Veterinary University, Tirupati-517502, India
[3]Department of Biotechnology, Sri Venkateswara Institute of Medical Sciences, Tirupati-517502

ABSTRACT

The present study was carried out to evaluate the hepatoprotective effect and safety evaluation of hexane extract of *Dillenia indica* seed in wistar albino rats. The different groups of animals were administered with 30% CCl_4 (1 ml kg-1b wt) in olive oil intraperitoneally. The seed extract at the dose of 250 and 500 mg kg-1b wt were administered to the CCl_4 treated rats. The seed extract produced significant ($p<0.01$) hepatoprotective effect by decreasing the activity of serum enzymes, bilirubin, urea, creatinine and lipid peroxidation and significantly ($p<0.01$) increased the level of SOD, CAT, GPx, GR, GST, GSH, Vitamin C and E and protein. Sub-acute toxicity studies showed no alteration in biochemical parameters ($p<0.01$) compared to control groups. DNA damage of rat WBC caused by CCl_4 injection was considerably counteracted by treatment with the extract. From the results it was suggested that seed extract possess potent hepatoprotective antioxidant properties and no toxic effects.

Keywords: Antioxidant activity, CCl_4, *Dillenia indica,* Hepatoprotection, Safety evaluation.

Corresponding author
E-mail: ovsreddy@yahoo.com

ISSN: 0975-8585

INTRODUCTION

Free radical reactions have been implicated in the pathology of many disease conditions like atherosclerosis, ischemic heart diseases, aging process, inflammation, diabetes, immuno-suppresion and neurodegenerative disease [1]. Inadequate antioxidant defenses leads to disturbances in redox homeostasis causing damage to lipids, proteins, carbohydrates and DNA [2]. Drugs with multiple protective mechanisms, including antioxidant activity, may be one way of minimizing tissue injury. The free radical generated would lead to auto oxidation of the fatty acids present in the cytoplasmic membrane phospholipids and cause functional and morphological changes in the cell membrane [3].

A number of plants and plant isolates have been reported to protect free radical-induced damage in various experimental models [4]. Carbon tetrachloride (CCl_4) is a clear, heavy and non-flammable liquid widely used for experimental induction of hepatic cirrhosis [5]. Liver, an important organ actively involved in many metabolic functions is the frequent target for a number of toxicants [6]. In recent years there has been an increased interest in areas related to newer developments in the prevention of disease especially those involving natural compounds with antioxidant activities. These antioxidants neutralize free radicals or their activities. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infection and degenerative diseases [7]. Oxidative stress involving the enhanced generation of reactive oxygen species (ROS) including free radicals and other related reactive species has been implicated in a variety of toxicities and ailments [8].

Dillenia indica plant belongs to family Dilleniaceae, commonly called *Dillenia*. It is distributed in India, Nepal, Bangladesh and Sri Lanka. The fruit ripens in the month of November and December. Green calyx parts are eaten in various forms of pickles. Locally it is called *Uvya* or *Kalinga.* The main purpose of the present study was to elucidate the hepatoprotective effect of *D. indica* seed against CCl_4 induced toxicity and safety evaluation of hexane extract on rats.

MATERIALS AND METHODS

Preparation of seed powder and extraction

Seeds of *D. indica* were procured from Gautam Global, Dehradun (India) and the seeds were authenticated by the Herbarium keeper, Department of Botany, S.V.University, and Tirupati. Seeds were shade dried, powdered and subjected to solvent extraction. Hexane extract of *D. indica* (HEDI) seeds were prepared by taking 100g powder and soxhlated for 4 h with hexane and excess solvent was distilled off. The yield of the extract was 10.6% (v/w).

ISSN: 0975-8585

Experimental animals

Male albino rats of wistar strain weighing 180-200g were used in the toxicological studies. The inbred animals were taken from Ragahavendra suppliers, Bangalore, India. The animals were maintained in well-ventilated room temperature with natural 12±1 h day-night cycle in the polypropylene cages. They were fed *ad libitum* with balanced rodent pellet diet and water through out the experimental period of 21 days. The animals were sheltered for one week and prior to the experiment they were acclimatized to laboratory temperature. Acute toxicity studies were carried out as per 'up and down stair case method [9]. The protocol was approved by Institutional Animal Ethics Committee constituted for the purpose as per CPSCEA guidelines.

Experimental grouping of rats CCl_4 induced toxicity studies

Healthy male albino rats were grouped in to four of six animals each and treated as follows: Group I rats were given a single administration of 0.5 ml vehicle (2% v/v) aqueous Tween-80 p.o for 21 days, which served as control. Group II rats were given a dose of 30% CCl_4 in olive oil of 1 ml/kg.b.wt intraperitoneally, on alternative days throughout the experiment. Group III rats were given orally a dose of 250 mg/kg.b.wt of HEDI after CCl_4 administration. Group IV rats were given orally a dose of 500 mg/kg.b.wt of HEDI after CCl_4 administration.

Biochemical assays

Serum and liver samples were collected from different group of rats and the following biochemical assays were carried out according to reported methods. Total protein content was estimated by Bradford [10]. Bilirubin content was analyzed according to the procedure of Malloy and Evelyn [11]. Blood urea nitrogen was evaluated by the method of Natelson [12]. Serum creatinine was estimated by the method of Owen [13]. Triglycerides were analyzed as described by Rice based on the method of Van Handel [14, 15]. Cholesterol content was estimated by the method of Parekh and Jung [16]. AST and ALT were assayed by the method of King [17]. The activity of alkaline phosphatase was assayed using disodium phenyl phosphate as substrate by the method of King [18]. Total sulfhydryl (TS), xanthine oxidase (XOD), glucose-6-phosphate dehydrogenase (G6PDH) and protein carbonyl (PC) were estimated according to reported methods [19-22].

Lipid peroxidation

Lipid peroxidation (LPO) in the tissue homogenate was measured by estimating the formation of thiobarbituric acid reactive substances (TBARS) [23]. Tissue homogenate (10%, w/v, in 50 mM phosphate buffer, pH 7.4) was boiled in TCA (10%) and TBA (0.34%) for 15 min, cooled and centrifuged. Absorbance of the supernatant was read at 535 nm. TBARS was calculated using tetraethoxypropane as the standard.

ISSN: 0975-8585

Antioxidants enzymes and non enzyme-antioxidants

Antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), reduced glutathione (GSH), vitamin C (Vit-C) and vitamin E (Vit-E) were estimated using standard methods [24-31].

DNA degradation analysis

Total DNA from White Blood Corpuscles was isolated by using Triazol reagent (Sigma Aldrich, USA) and isolated DNA was analyzed by running 1% agarose gel electrophoresis**.**

Experimental grouping of rats for safety studies of HEDI

The rats were divided into three groups with six animals in each group and treated as follows: Group I rats were given a single administration of 0.5 ml vehicle (2% v/v) aqueous Tween-80 p.o for 21 days, which served as control. Group II rats were given orally a dose of 250 mg/Kg.b.wt. of HEDI. Group III rats were given orally a dose of 500 mg/Kg.b.wt. of HEDI. Serum samples were collected followed by hematological and biochemical analyses were carried out.

Histopathology

These studies were performed to assess the tissue damage of liver to assess the protective effect against CCl_4 induced toxicity and safety evaluation of HEDI.

Statistical analysis

Values reported are the mean±SD. The statistical analysis was carried out using Analysis of Variance (ANOVA) followed by DMRT test. $p<0.01$ were considered as significant.

RESULTS

The current data showed that administration of HEDI to rats for 21 days against CCl4 showed hepatoprotective effect. The levels of AST, ALT, ALP, bilirubin, urea and creatinine levels were significantly increased but protein content was significantly decreased in CCl_4 induced liver damage rats, when compared with control group ($P<0.01$). On the other hand, the treatment with HEDI at two different doses (250 and 500 mg/kg.b.wt) in CCl_4 induced liver damaged rats showed decreased activity of serum enzymes, bilirubin, urea and creatinine and increased the content of protein content when compared to the control group of rats with CCl_4 alone ($p<0.01$). The results were presented in Table 1.

The localization of free radical formation resulting in lipid peroxidation, measured in serum as shown in Table 1 and in rat liver homogenate (Table 2). Antioxidant enzymes such as

superoxidedismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione-S-transferase (GST) were estimated in the control, CCl_4 group and in experimental groups of CCl_4 and HEDI treated rats. There was significant decrease of all these enzymes in CCl_4 alone groups as compared to control ($p<0.01$), and significant increase in experimental groups of HEDI treated rats, shown in Table 2. Non-antioxidants such as, reduced glutathione (GSH), vitamin-C and vitamin-E were estimated in the control CCl_4 group and in experimental groups of CCl_4 and HEDI treated rats. There was significant decrease in CCl_4 alone groups of all these enzymes compared to control ($p<0.01$), and caused significant improvement in experimental groups of HEDI treated rats (Table 2).

The total sulfhydryl content was found to be significantly decreased ($p<0.01$ and $p<0.01$) in liver of Group II (CCl_4 treated) rats when compared to the control. A marked ($p<0.01$) increase in the activity levels of XOD was observed in the liver of CCl_4 treated (Group II) rats when compared with the control (Group I) rats. Administration of two doses of HEDI to rats caused a significant decrease in the levels of XOD in liver. The level of protein was found to be significantly decreased in CCl_4 treated rats. Administration of hexane extract of *D. indica* at a dose of 250mg/kg b.wt. and 500 mg/kg b.wt. (Group III and Group IV) reversed the changes. Administration of HEDI mitigated the reduced activity level of G-6-PDH in Group III and Group IV rats to normal level in dose dependent. It was observed that DNA damage caused by CCl_4 injection was considerably counteracted by treatment with the HEDI with the maximum level of protection being noted in those groups that received the treatment with the 500 mg/Kg b. wt. (Fig.1) of HEDI.

Toxicity related to traditional medicines is becoming more widely recognized as these remedies have become popular world wide. Biochemical parameters such as total protein, bilirubin, urea, uric acid, creatinine, triglycerides, and cholesterol were estimated in the serum of control and experimental animals (Table 3).

DISCUSSION

The extent of hepatic damage was assessed by the elevated levels enzymes such as AST, ALT and ALP, which is attributed to the generation of trichloromethyl free radicals which in turn cause lipid peroxidation. High levels of AST indicate the liver damage in the conditions such as viral hepatitis, cardiac infarction and muscle injury [32]. ALT catalyses the conversion of alanine to pyruvate and glutamate is released in a similar manner. This is a specific marker to the liver and thus a better parameter for detecting the liver damage [33]. CCl_4 damage to liver raises the serum level of ALP by releasing into the blood stream [34]. The results using the model of CCl_4 induced hepatotoxicity in rats demonstrated elevated levels of ALP and bilirubin. Increased levels of urea and creatinine in the serum indicate the damage of liver. The data obtained showed the increase levels of creatinine and urea in the liver of CCl_4 induced toxicity compared to control and treated rats.

ISSN: 0975-8585

Table No.1: Effect of *HEDI on liver marker enzymes and biochemical parameters of serum in control and experimental rats against CCl_4 induced toxicity.

Parameters	Group I	Group II	Group III	Group IV
AST (IU/L)	46.11±0.03	147.10±1.23	87.33±1.01	54.32±0.58
ALT (IU/L)	46.89±0.32	151.27±0.52	77.01±0.60	59.45±0.54
ALP (IU/L)	74.95±0.41	176.07±1.30	114.46±1.42	86.78±0.43
Bilirubin (mg/dl)	1.19±0.06	2.39±0.02	1.89±0.02	1.32±0.07
Urea (mg/dl)	21.74±0.58	42.76±1.58	36.23±0.44	29.02±0.19
Creatinine (mg/dl)	1.00±0.01	1.94±0.00	1.73±0.00	1.34±0.01
Total protein (mg/dl)	7.42±0.01	3.32±0.65	5.55±0.05	7.14±0.01
γGT (IU/L)	13.01±0.10	45.22±0.68	24.74±0.15	18.64±0.23
LDH (IU/L)	149.33±1.27	442.5±2.32	240.74±0.47	157.63±1.11
LPO (µmoles of MDA/mg protein)	3.34±0.07	4.27±0.00	3.97±0.00	3.68±0.02

*HEDI=Hexane extract of *D. indica* seed; Group I=Control; Group II= CCl_4 treated; Group III= CCl_4+HEDI treated (250 mg/kg b.wt); Group IV= CCl_4+HEDI treated (500mg/kg b.wt). Values are expressed as mean ± SEM of six rats in each group.

Table No.2: Effect of HEDI on enzymatic and non-enzymatic antioxidants in liver of control and experimental rats against CCl_4 induced toxicity.

*Parameters	Group I	Group II	Group III	Group IV
SOD	0.49±0.00	0.20±.00	0.35±0.00	0.45±0.00
CAT	70.82±0.42	49.64±0.40	55.33±4.26	66.06±1.08
GPx	57.91±0.83	35.59±0.47	46.32±0.20	51.62±0.46
GR	10.32±0.17	8.75±0.30	9.43±0.02	11.18±0.04
GST	1.34±0.02	0.71±0.01	0.87±0.00	1.14±0.04
GSH	7.85±0.07	3.00±0.04	5.43±0.04	7.21±0.09
Vit-C	2.30±0.08	1.35±0.04	1.95±0.04	2.17±0.02
Vit-E	1.77±0.00	0.68±0.00	1.31±0.00	1.67±0.01
LPO	1.73±0.17	2.85±0.04	2.07±0.07	1.55±0.18
TS	2.68 ± 0.07	1.41 ± 0.01	1.72 ± 0.02	2.16 ± 0.05
XOD	0.89± 0.00	1.33± 0.01	1.07 ± 0.00	0.95± 0.01
G6PDH	2.89 ± 0.00	1.88 ± 0.00	2.14 ± 0.01	2.58 ± 0.02
PC	4.13 ± 0.04	17.33±0.06	12.90±0.17	6.97 ± 0.20

Values are expressed as mean ± SEM of six rats in each group. *Parameters expressed as SOD-units/min/mg protein; CAT-µmoles of H_2O_2 utilized/min/mg protein; GPX-µmoles of GSH oxidized/min/mg protein; GR-µmoles of GSH utilized/min/mg protein; GST–units/min/mg protein; GSH-µg of reduced glutathione/mg protein; Vit-C-µg/mg protein; Vit-E-µg/mg protein; LPO-µmoles of MDA/mg protein; TS–µg/mg protein; XOD–µg of urate formed/h/mg protein; G6PDH–units/min/mg protein and PC–mg/dl.

ISSN: 0975-8585

Table No.3: Effect of HEDI on hematological and biochemical parameters (marker enzymes of serum and liver) in control and treated rats during safety evaluation studies.

Parameters	Group I	Group II	Group III
Hematological			
Hemoglobin	10.63 ± 0.32	10.45 ± 0.47	10.85 ± 0.48
RBC count	4.56 ± 0.20	4.55 ± 0.30	4.33 ± 0.15
WBC count	6.34 ± 0.05	6.385± 0.07	6.38 ±0.14
PCV	34.21 ± 2.31	35.92 ± 1.43	36.16 ± 1.94
Serum			
Protein (mg/dl)	7.25±0.33	7.53±0.10	7.28±0.26
Bilirubin (mg/dl)	0.07±0.00	0.07±0.00	0.07±0.00
Urea (mg/dl)	15.81±0.63	15.74±0.42	15.48±0.19
Uric acid (mg/dl)	4.28±0.08	4.19±0.08	4.28±0.10
Creatinine (mg/dl)	0.77±0.02	0.73±0.026	0.75±0.02
Triglycerides (mg/dl)	123.83±5.98	122.33±9.28	126.17±6.55
Cholesterol (mg/dl)	102±5.36	102.17±4.40	106.5±4.89
AST (IU/L)	41.63±0.44	41.22±0.53	35.35±12.37
ALT (IU/L)	22.07±0.86	21.10±0.54	20.50±0.67
ALP (IU/L)	0.17±0.002	0.15±0.01	0.17±0.01
Liver			
AST (IU/L)	31.31±0.57	30.01±0.08	31.34±0.38
ALT (IU/L)	96.66±0.88	96.42±0.26	97.21±0.36
ALP (IU/L)	0.02±0.001	0.02±0.000	0.02±0.000

Group I=Control; Group II=HEDI treated (250 mg/kg b.wt); Group III=HEDI treated (500 mg/kg b.wt). Values are expressed as mean ± SEM of six rats in each group.

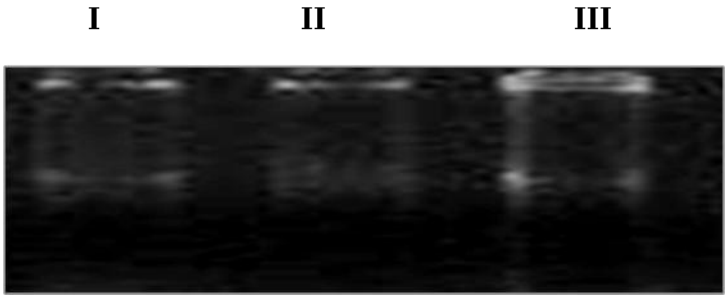

Fig No. 1: DNA degradation analysis in WBC (rat) of control (Lane I), CCl_4 treated (Lane II) and CCl_4+HEDI treated with 500 mg/Kg.b.wt (Lane III)

ISSN: 0975-8585

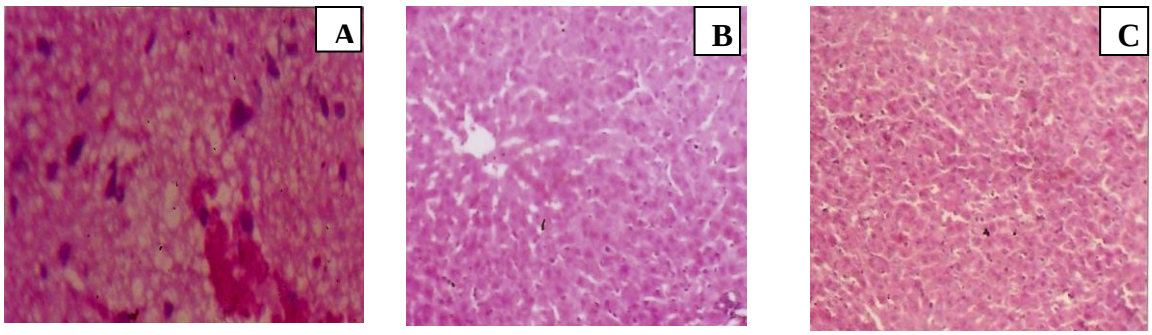

Fig No. 2: Histopathological changes in HEDI treated (500 mg/kg.b.wt) rats in normal and CCl_4 (30% in olive oil) induced toxicity conditions. a. Liver section treated with CCl_4; b. Liver section treated with CCl_4+HEDI (500 mg/kg b.wt); c. Liver section treated with HEDI (500 mg/kg b.wt).

MDA (malondialdehyde) content in rat liver homogenate and in serum was increased in the CCl_4 group compared to control group. HEDI treated groups showed a decrease in lipid peroxidation levels ($p<0.01$). The increase in MDA level in liver suggests that enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive radicals. Treatment with HEDI significantly reversed these changes.

Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical scavengers and chain reaction terminators, enzymes such as SOD, CAT and GPx systems [35]. The SOD superoxide radicals O_2- in to H_2O_2 plus and O_2, thus participating, with the other antioxidant enzymes, in the enzymatic defense against oxygen toxicity. Catalase is a key component of the antioxidant defense system. Inhibition of these protective mechanisms risks in enhanced sensitivity to free radical-induced cellular damage [36]. GPx is an equally important antioxidant enzyme, which reacts with hydrogen peroxide thus preventing intracellular damage caused by the free radicals [37].

Glutathione reductase is one of the GSH-related enzymes which play detoxifying and antioxidant roles in metabolizing xenobiotics through the conjugation with glutathione or reduction of free radicals [38]. Deficiency of this enzyme will affect the redox status of GSH in biological system and could not protect tissues from oxidative damage. Vitamin C can protect cell membranes and lipoproteins particles from oxidative damage by regenerating the antioxidant form of vitamin E. Thus vitamin C and E act synergistically in scavenging a wide variety of ROS [39].

Living systems contain large amounts of sulfhydryl compounds which must be maintained in the proper oxidation state to retain their biological activity [40]. Xanthine oxidase is a flavoprotein, which catalyses the oxidation of hypoxanthine to xanthine and generates superoxide, hydroxyl radicals and uric acid [41]. Proteins are the macromolecular substances present in all living cells. They serve as major structural component in animal tissues. HMP shunt is the major path way in which a major portion of the cell's NADPH is produced and glucose-6-phosphate dehydrogenase is the important enzyme in this pathway which converts

NADP to NADPH. The activity of G-6-PDH is an index for determining the efficiency of HMP shunt [42].

Toxicity of medicinal plants may be related to the mixtures of active compounds and their interactions with other herb and drugs. Plants have complex mixtures of terpenes, alkaloids, phenols and other chemicals [43]. Many medicinal herbs and pharmaceutical drugs are therapeutic at one dose and toxic at another. Histopathological studies also supported the data (Fig. 2).

CONCLUSION

Our data clearly showed that the extract possess hepatoprotective effect when rats induced with CCl_4. When HEDI was given orally to the normal rats there are no significant alterations in hematological and biochemical parameters exhibiting. Further investigations on isolation and identification of compounds responsible for hepatoprotective effect are under progress in our laboratory.

ACKNOWLEDGEMENTS

We thank Dr.S.C.Basappa, former Deputy Director and Scientist, Central Food Technological Research Institute (CFTRI), Mysore (India), for his suggestions, encouragement and critical comments on the manuscript. We also thank Dr. Kumar Vadlapudi of Kuvempu University, Dhavangere (India) for providing fine chemicals.

REFERENCES

[1] Droge W. Physiol Rev 2002; 82:47-95.
[2] Sundaram R, Mitra SK. Ind J Pharmacol 2007; 39:33-38.
[3] Pandit S, Sur TK, Jana U, Debnath PK, Sen S, Bhattacharyya D. Ind J Pharmacol 2004; 36:312-313.
[4] Scartezzini P, Speroni E. J Ethnopharmacol 2000; 71:23-43.
[5] Lin WC, Wu YW, Shih CC. Phytomedicine 2005; 12 (6/7):453-460.
[6] Meyer SA, Kulkarni AP. Hepatotoxicity. In: Introduction to biochemical toxicology. 3rd Edn. Newyork: John Wiley & Sons, 2001;487.
[7] Himakar Reddy K, Sharma PVGK, Reddy OVS. Pharmaceutical Biology 2009 (Accepted).
[8] Yoshikawa T, Toyokuni S, Yamamoto Y, Naito Y. Free Radicals in Chemistry. Biology and Medicine, (Eds) OICE International Saint Lucia, 2000.
[9] Ghosh MN. Toxicity studies, In Fundamentals of Experimental Pharmacology, Scientific Book Agency, Calcutta, 1984; 153–158.
[10] Bradford M. Anal Biochem 1976; 72: 248-254.
[11] Malloy HT, Evelyn KA. J Biol Chem 1937; 119: 481.
[12] Natelson S, Scott ML, Beffna C. Am J Clin Pathol 1951; 21: 275-281.
[13] Owen JA, Iggo TB, Scandrett FJ, Stemart IP. Biochem J 1954; 58: 426-437.

[14] Rice EW. Triglycerides in serum. In: Standard methods of clinical chemistry Roderick, P. and MacDonald (Ed) Academic press, New York, 1970; 6: 215-222.
[15] Van Handel E. Clin Chem 1961;7(3): 249-251,
[16] Parekh AC, Jung DH. Anal Chem 1970; 42: 1423-7.
[17] King JC. The transferases-alanine and aspartate transaminases. In: Practical Clinical Enzymology. King, J.C. (ed.), Van D Nostrand Co, London, 1965a; 83-93.
[18] King JC. The phosphohydrolases-acid and alkaline phosphatases. In: Practical Clinical Enzymology. King, J.C. (ed.), Van Nostrand Co, London 1965b; 191-208.
[19] Srikanthan TW, Krishnamurthy C. J Sci Ind Res 1955; 14: 206.
[20] Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shultiel S, Stadtman ER. Methods Enzymol 1990; 186: 464–478.
[21] Sedlack J, Lindsay RH. Anal Biochem 1968; 25: 7826-7833.
[22] Ellis HA, Kirkman HN. Proc Soc Exp Biol Med 1961; 106: 607-609.
[23] Okhawa H, Ohishi N, Yagi K. Anal Biochem 1979; 95: 351–358.
[24] Misra HP, Fridovich I. J Biol Chem 1972; 247: 3170-3175.
[25] Aebi H. Methods Enzymol 1984; 105: 121-126.
[26] Wendel A. Glutathione peroxidase. In: Methods in Enzymology by Jacoby.W.B (Ed), Academic press, New York, 1981; 77: 325-333.
[27] Staal GEJ, Visser J, Veeger C. Biochim Biophys Acta 1969; 185: 39-48.
[28] Habig W, Pabst MJ, Jacob WB. J Biol Chem 1974; 249: 7130-7139.
[29] Moron MS, Defierre JW, Mannervik B. Biochim Biophys Acta 1979; 582: 67-78.
[30] Omaye ST, Turnbull JD, Sauberlichm HE. Methods Enzymol 1979; 62: 3-11.
[31] Quaife MC, Dju MY. J Biol Chem 1948; 180: 263-272.
[32] Aniagu SO, Nwinyi FC, Akumka DD, Ajoku LA, Dzanma S, Lzebe KS, Ditse M, Nwaneri PEC, Wambebe C, Lamaniel K. Afr J Biotechnol 2005; 4:72–78.
[33] Shanmugasundaram P, Venkataraman S. J Ethnopharmacol 2006; 104:124–128.
[34] Asha VV. Ind J Pharmacol 2001; 33:276–279.
[35] Semiz A, Sen A. Afr J Biotechnol 2007; 6 (3): 273-277.
[36] Young IS, Woodside JV. J Clin Pathol 2001; 54: 176-186.
[37] Lee CP, Shih PH, Hsu CL, Yen GC. Food Chem Toxicol 2007; 45: 888-895.
[38] Yang Y, Liu W, Han B, Liu B, Fu C. Hepato Res 2006; 35(3):178-184.
[39] Kamalakkannan N, Rukkumani R, Aruna K, Suresh Varma P, Viswanathan P, Padmanabhan Menon V. Iranian J Pharmacol. Therapeu 2005; 4 (2):118-123.
[40] Isik A, Koca SS, Ustundag B, Selek S. Tohoku J Exp Med 2007; 212: 133-141.
[41] Cheng HY, Lin TC, Yu KH, Yang CM, Lin CC. Biol Pharm Bull 2006; 26: 1331–1335.
[42] Oh SI, Kim CI, Chun HJ, Park SC. J Nutr 1998; 128(4): 758-763.
[43] Azaizeh H, Fulder S, Khalil K, Said O. Fitoterapia 2003; 74: 98-108.