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ANTIHEPATOTOXIC ACTIVITY OF *COLOCASIA ESCULENTA* LEAF JUICE

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ABSTRACT

Influenced by the ancient literature of various communities and reports presented by modern authors regarding the medicinal uses of *Colocacia esculenta.*, present study was conducted to investigate the antihepatotoxic efficacy associated with *Colocacia esculenta* whole leaf juice. The antihepatotoxic and hepatoprotective studies were carried against two well known hepaotoxins paracetamol and CCl_4 using *in vitro* liver slice method. The free radicals generated by CCl_4 and paracetamol cause oxidative stress as well as damage various cell organellaes consequently resulting in injury to the hepatocytes. The extent of damage caused by these free radicals as well as evaluation of antihepatotoxic and hepatoprotective efficacy associated with the *Colocacia esculenta* leaf juice was measured using the leakage of marker enzymes of liver function *viz* AST, ALT and ALP in the incubation medium. In presence of CCL_4 as well as paracetamol there was increase in the levels of marker enzymes indicating hepatotoxicity of these compounds. At one and two hours interval insignificant alterations were observed in the enzymes levels. Marked elevations of toxicity marker enzymes were noted at four hours in presence of CCl_4 as well as paracetamol. However the leaf juice of *Colocacia esculenta* remarkably declined the leakage of AST, ALT and ALP in the medium indicating hepatocyte integrity. The investigation is supportive to conclude that the *Colocacia esculenta* leaf juice as a whole possesses antihepatotoxic and hepatoprotective efficacy when tested *in vitro* using rat liver slice model.

Keywords: *Colocacia esculenta*, hepatoprotecitve *in vitro*, liver, CCl_4, Paracetamol AST, ALT, Alkaline phosphatas

[I]INTRODUCTION

In vivo studies on hepatotoxicity are limited by animal welfare/ethical concerns and difficulties to distinguish primary and secondary toxic effects, *in vitro* liver preparations are increasingly used as they offer different approaches on all levels of investigational toxicology [1]. The use of liver slices is an addition to the battery of *in vitro* models to evaluate the metabolism of xenobiotics. Liver slices have been used as an alternative *in vitro* method for the assessment of hepatic drug metabolism. Use of liver slices provides decided advantage over previous *in vitro* techniques because this preparation allows for maintenance of the functional acinar architecture of the liver and has displayed drug metabolism over a span of hours to days. Advantages like maintenance of the functional architecture have made liver slice method a very versatile method for the study of drug disposition *in vitro* [2]. The *in vitro* isolated hepatocyte [3, 4], cell line [5] as well as liver slice [6] model is earlier used to assess toxicity and understand the underlying mechanisms.

Liver and herbal medicines: Acute and chronic liver diseases constitute a global concern, but medical treatments for these diseases are often difficult to handle and have limited efficacy. Therefore, considerable efforts to obtain useful herbal medicines from documented medicinal plants for a wide variety of clinical conditions are currently underway. Developing therapeutically effective agents from natural

products may reduce the risk of toxicity when the drug is used clinically [7]. The search for newer natural antioxidants, especially of plant origin is increasing. Recently, natural plants have received much attention as sources of biologically active substances including antioxidants. Numerous studies have been carried out on plants, vegetables and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavonoids which prevent free radical damage, reducing risk of chronic diseases. Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. They exert their effect by scavenging reactive oxygen species, activating a buttery of detoxifying proteins or preventing the generation of reactive oxygen species [8].

Colocasia esculenta: *Colocasia sp* is an ancient crop grown throughout the humid tropics and is widely used throughout the world; Africa, Asia, the West Indies, and South America. Its edible corms and leaves are traditionally used for hepatic ailments [9]. *Colocasia esculenta* (L) Schott of the family Araceae is an herbaceous perennial plant cultivated as annuals. The large green leaves often described as 'elephant ear' and they can reach up to 1-2 m high during growth. The starchy, tuberous root is the main edible part of the crop; however the leaves are also used as a leafy vegetable. *Colocasia esculenta* leaves have been reported to be rich in nutrients including minerals and vitamins such as calcium, phosphorours, iron, vitamin C, thiamine riboflavin and niacin [10]. Among various edible aroids commercially cultivated in India, *Colocasia esculenta* assume note-worthy dietary significance having multiple uses in the form of various culinary preparations of its corm and edible stem. Fresh edible leaves of *Colocasia esculenta* form rich source of protein, ascorbic acid, dietary fibre and some nutritionally important minerals. Tender leaves of *Colocasia esculenta* are used as vegetable. Leaf juice is applied over scorpion sting or in snake bite. It is also given in food poisoning of plant origin. Plant pacifies vitiated (Ayurveda identified ailments *viz*.) vata and pitta, constipation, stomatitis, alopecia, hemorrhoids and general weakness [11, 12]. *Colocasia antiquorum* is reported to possess hepatoprotective activity against experimentally induced liver injury in rats [9]. *Colocasia esculenta* is reported to possess hypoglycemic efficacy due to the presence of cyanoglucoside [13]. Hypolipidemic and antihyperlipidemic activity has been reported due to the presence of arabinogalactan [14] and mono and digalactocyl diacylglycerols [15]. Also it possesses antifungal activity due to presence of cystatin [16]. Antibacterial activity of *Colocasia esculenta* has been mentioned by Ravikumar and co-workers [17]

Paracetamol and CCl_4: Carbon tetrachloride is a well-known hepatotoxin that is widely used to induce toxic liver injuries in laboratory animals. The hepatic necrosis caused by CCl_4 is thought to involve bioactivation by cytochrome P450 2E1 (CYP2E1) resulting in the formation of trichloromethyl free radicals and reactive oxygen species (ROS), which initiate lipid peroxidation and protein oxidation and damage the hepatocellular membranes [18] Paracetamol is available over the counter and over dosage of paracetamol leads to the saturation of conjugation pathway leading to glutathione depletion and increase in the formation of toxic reactive metabolites. A high level of reactive metabolites increases the level of hepatotoxicity, with increased level of protein adducts formation, mitochondrial dysfunction and oxidative stress [19]. In cases of paracetamol overdose and related toxicities, N-acetylcysteine (NAC) is used as an antidote, however its efficacy is still in question in treatment of acute paracetamol poisoning [5]. It is found that paracetamol attenuates mast cell and peripheral

blood mononucleocyte cell histamine release induced by this antidote. Hence there is a necessity to find a safer and better antioxidant, and hepatoprotective agent against paracetamol as well as a general anti-hepatotoxic.

[II] Materials and Methods

2.1 Plant material: *Colocacia esculenta* plants were collected locally, from Kolhapur, MS India. The plant identification was done by an expert in Botany, Dr A. R. Jadhav, from Department of Botany, Yashavantarao Chavan College, Warananagar, Kolhapur, MS India. After the careful removal of the leaves, they were washed thoroughly using distilled water and blotted briefly prior to the preparation of crude juice. The juice of whole leaves was prepared and filtered through Whatman filter paper. The filtrate was collected in sterilized and aseptic conditions and was refrigerated till further use.

2.2 Chemicals: Highly pure and of analytical grade chemicals were utilized for the present studies. Paracetamol or the acetaminophen and CCl_4 were purchased from S D fine chemicals, Mumbai while the culture media was obtained from Himedia (M199). Pathological diagnostic kits were procured from Pathozyme Diagnostics, India.

2.3 Experimental Animals: Healthy Wistar strain Albino male rats weighing 175 to 225 gm, bred and reared under standard housing conditions were obtained from the registered animal house of Tatyasaheb Kore College of Pharmacy, Waranangar, Dist. Kolhapur, Maharashtra, India. The animals were kept in standard plastic animal cages with a 12 hours light and dark cycle and fed on standard rat chow and provided pure water *ad libitum*. The experiments were carried out according to guidelines of 'Committee for Prevention and Control of Scientific Experimentation on Animals' (CPCSEA) New Delhi. Animals were sacrificed giving deep ether anesthesia.

2.4 Experimental Procedure: Surgical procedures were carried out on fed rats under deep ether anesthesia to obtain whole liver. The liver slices (LS) were prepared from the whole liver as described earlier [6] The slices were transferred to experimental vials with combinations of hepatotoxins i.e. paracetamol and CCl_4 and/or hepatoprotectants *Colocacia esculenta* leaf juice. These vials also contained fresh medium M199 and supplemented with CCl_4 or paracetamol (PA) concentration of 1.0×10^{-3} M, with or without *Colocacia esculenta* leaf juice. Based upon preliminary studies carried in our laboratory, *Colocacia esculenta* leaf juice concentrations decided to be used in the present study were 5µl /ml (CE1) and 10 µl/ml (CE2) of medium. After transferring the LS to these vials containing different concentrations of paracetamol/CCl_4 and CE1/CE2 the vials were incubated for 1, 2 and 4 hours in standard incubation conditions. Unsupplemented slices were used as control/s. Table 1 presents the experimental design in a comprehensive manner

Table 1: Experimental Design for testing of antihepatotoxic effects of *Colocacia esculenta* leaf juice against paracetamol and CCl_4

Sr No	Test	CCl_4	PA	CE1	CE2
1	Control	-	-	-	-
2	CCl_4 Control	√	-	-	-
4	CE1 control	-	-	√	-
5	CE2 control	-	-	-	√
6	CCl_4 + CE1	√	-	√	-
7	CCl_4 + CE2	√	-	-	√
8	PA Control	-	√	-	-
9	PA+CE1	-	√	√	-
10	PA+CE2	-	√	√	-

This experimental procedure was repeated for one, two and four hours to understand time dependent toxicity, if any, associated with the xenobiotics used in this experiment.

2.5 Assessment by Biochemical parameters: At the end of one, two and four hours of incubation, the surrounding media were used to

test the amount of leakage of ALT/alanine transaminase [20], AST/aspartate transaminase [20] and ALP/alkaline phosphatase [21].

2.6 Statistical Analysis of the data: Statistical analysis of the results obtained from the experiments was carried out using ANOVA. The values with their respective units are expressed as mean of 6 sets ± SE. Value of $p < 0.05$ was considered as significant

RESULTS:

Results of the present study are represented in Fig.1, 2 and 3, obtained at the end of one, two and four hours respectively

Fig 1. : *Colocasia esculenta* influenced *in vitro* alterations in AST, ALT and ALP activities in presence of CCl4 and paracetamol at the end of one hour incubation

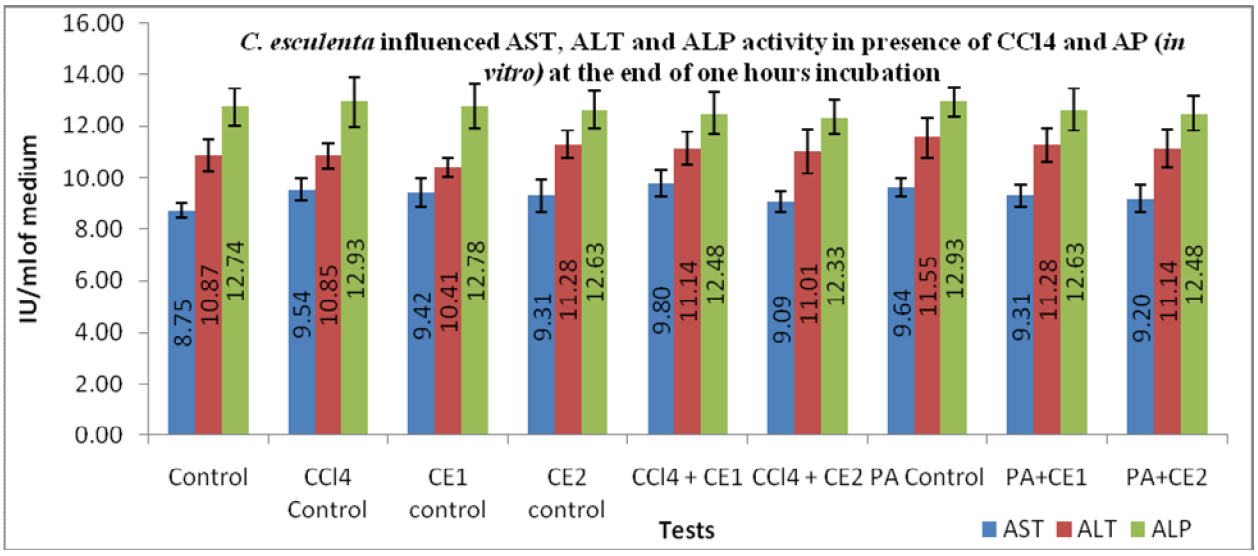


Fig 2. : *Colocasia esculenta* influenced *in vitro* alterations in AST, ALT and ALP activities in presence of CCl4 and paracetamol at the end of two hours incubation

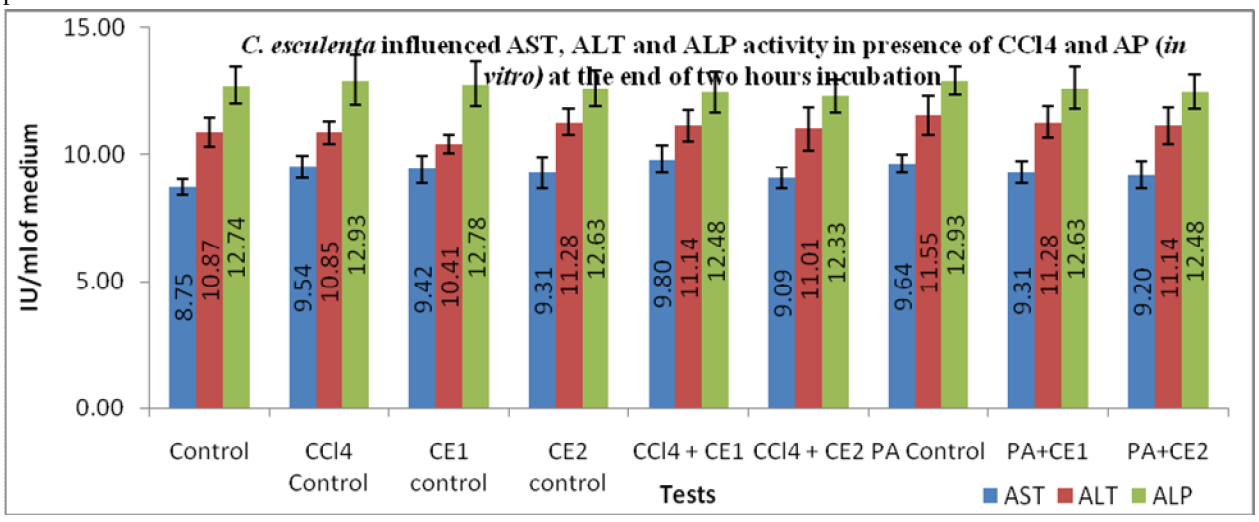
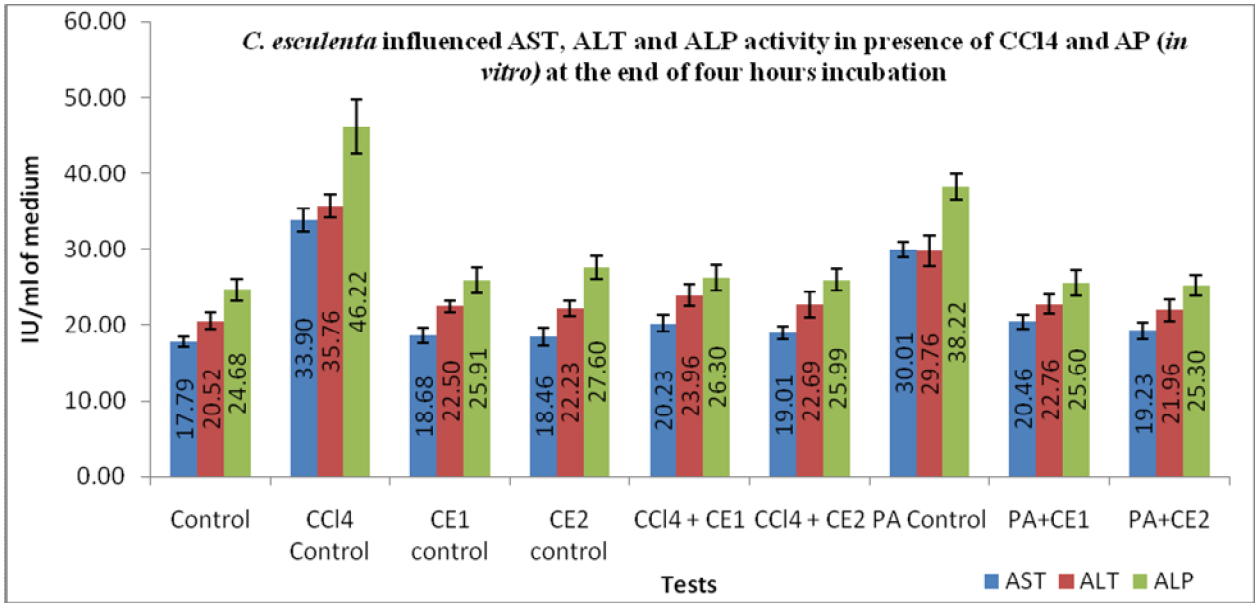


Fig 3. : *Colocasia esculenta* influenced *in vitro* alterations in AST, ALT and ALP activities in presence of CCl4 and paracetamol at the end of four hours incubation



AST activity noted in the control at the end of one, two and four hour was 8.75±0.30, 9.83±0.67 and 17.79±0.77 IU/ml of medium. Activity of ALT in control was recorded as 10.87±0.59, 11.84±0.91 and 20.52±1.18 IU/ml of medium at the end of one, two and four hours respectively. Similarly ALP exhibited 12.74±0.76, 13.63±0.73 and 24.68±1.33 IU/ml of the medium after one, two and four hours of incubation of control liver slices.

AST, ALT and ALP activity in the medium were noted as 9.54±0.43, 10.85±0.47, and 12.93±0.93 after incubation of one hour in presence of CCl_4, similarly after incubation of one hour in presence of paracetamol the activity recorded in AST, ALT, ALP were 9.64±0.34, 11.55±0.75 and 12.93±0.56 IU/ml of medium.

At the end of two hours (fig 2), the medium surrounding the LS treated with CCl_4 showed 10.44±0.58, 12.58±0.86 and 14.49±0.62 IU/ml activity in AST, ALT and ALP respectively. The paracetamol influenced activities in paracetamol control liver slice medium were recorded as 10.44±0.64, 12.58±0.87 and 14.49±0.65 in AST, ALT and ALP respectively.

Conspicuous alterations were observed in the medium of liver slices incubated in presence of CCl4 upto four hours (fig. 3) where the AST activity was recorded as 33.90±1.20 IU/ml, ALT activity was recorded as 35.76±2.58 IU/ml, and ALP activity was recorded as 46.22±3.98 IU/ml of medium. Paracetamol containing medium exhibited 30.01±1.34 IU/ml AST, 29.76±1.37 IU/ml ALT and 38.22±1.78 IU/ml ALP activity.

DISCUSSION

The duration dependent increased enzyme activity in the medium of control LS was indication of hepatocyte activity/viability at the end of two and four hours. Insignificant alterations ($p>0.05$) in AST, ALT and ALP activities in the medium containing the toxicants (paracetamol and CCl_4) and *C. esculenta* leaf extract were observed (against the control) after one and two hour incubation. These alterations were not much deviated from that of the respective control/s and it is an indication that during this period intracellular biotransformation of xenobiotics was turned on, however the plasma membrane was still stable, hence no leakage of enzymes was noted. These data are

consistent with the hypothesis that paracetamol induced toxicity occurs by two phases, a metabolic phase and an oxidative phase [3]. A similar mechanism may be existing for CCl_4. During first two hours or maximum upto four hours the first phase i.e. metabolic phase may be occurring with glutathione depletion and protein binding however without elicit of lipid peroxidation and consequent damage of plasma membrane. [3].

When compared with the control there was marked increase ($p>0.05$) in the leakage of enzymes in the surrounding medium where the liver slices were incubated in presence of the toxicants by the end of fourth hour of incubation. This may be due to the outset of second phase of toxicity known as oxidative phase. During this phase there is increased oxidative stress, loss of mitochondrial membrane potential and toxicity. The significant increase in the leakage of marker enzymes AST, ALT and ALP in the surrounding medium of the liver slices incubated in presence of CCl_4 and paracetamol at the end of four hours of incubation indicates the second phase of toxicity.

Damage of liver cell is reflected by an increase in the levels of hepatospecific enzymes, the transaminases and alkaline phosphatase. These are cytoplasmic and are released into circulation after cellular damage [22]. In this study significant increase in the enzyme levels in the medium by hepatocytes incubated in presence of CCl_4 and paracetamol indicate severe hepatocyte injury. These activities in the CCl_4 and paracetamol treated LS were taken as an index of hepatocyte damage.

The hepatotoxic effects of CCl_4 are largely due to the generation of free radicals [23]. CCl_4 is biotransformed by the cytochrome P450 system to produce the trichloromethyl free radicals, which in turn covalently bind to cell membranes and organelles to elicit lipid peroxidation [24].

Both the concentrations of *C. esculenta* in the medium were found effective in reducing all the elevated levels of AST, ALT and ALP towards the levels noted in control. This is an indication of stabilization of plasma membrane as well as repair of hepatocyte damages caused by hepatotoxins. The data of protein level study (not presented in this work) suggests the stabilization of endoplasmic reticulum, leading to protein synthesis. It can be postulated that the *Colocasia esculenta* leaf juice may be protecting the hepatocytes against the injurious effects of CCl_4 and paracetamol that may result from the interference with cytochrome p450 system, resulting in the hindrance of the formation of hepatotoxic free radicals eliciting the lipid peroxidation and consequent damage to macromolecules and membrane leading to the leakage of cytoplamsic contents (including enzymes) in the surrounding medium.

The possible mechanism of action underlying the antitoxic effect of *Colocasia esculenta* leaf juice against CCl_4 may be due to its interference with the cytochrome p450 system involved in biotransformation of CCl_4 and responsible to produce the free radical of trichloromethyl. The possibility of antioxidant activity of leaf juice as a whole or some of its component cannot be eliminated where it may be scavenging the trichloromethyl free radicals produced by the biotransformation of CCl_4 by the cytochrome p450 systems. Similarly hepatoprotection against cytotoxic concentrations of paracetamol by *Colocasia esculenta* may be due to its interference with the cytochrome p450 system. Also it may be scavenging the free radicals formed during the biotransformation of paracetamol. Additionally it is possible that the leaf juice may be enhancing the synthesis of glutathione in the hepatocytes, and hence eliminating the possibility of free radical mediated damage to the macromolecules and due leakage of membrane and cytoplasmic contents in the surrounding medium.

The crude juice of *C. esculenta* was also tested for toxicity to hepatocytes, the data of CE1 and CE2 tests is suggesting that the leaf juice itself did not exerts any toxicity. However any dose dependent results were not found for the concentrations of leaf juice of *C. esculenta* tested in this *in vitro* study.

Several plants have been tested for their efficacy in controlling the CCl_4 and paracetamol induced liver damage. Further it has been evident that several phytoconstituents have the ability to induce microsomal enzyms either by accelerating the excretion of toxicants or by inhibition of lipid peroxidation induced by the toxin. Phytoconstituents like flavonoids and triterpenoids are known to possess hepatoprotective activity [25, 26]. Phytochemical investigations on the *Colocasia* extracts have shown the presence of anthocyanins such as cyanidin-3-glucoside, pelargonidin-3-glucoside and cyanidin-3-rhamnoside, which have antioxidant activities as evident from previous studies [27, 28, 29]. Therefore, anthocyanins may be responsible for the hepatoprotective activity which was observed associated with the leaf juice of *Colocasia esculenta*. However, the results obtained in the present studies may be a synergic action of all the components present in *Colocasia esculenta* leaf juice hindering various stages of the toxicity development.

CONCLUSION:

The results of the present study support the traditional claim in both Indian and other ethnic medicinal systems that the leaves of *Colocasia esculenta* possess antihepatotoxic and hepatoprotective efficacy. The exact constituent(s) responsible for this effect cannot be explained with the present data. It is speculated that antihepatotoxic and hepatoprotective as well as antioxidant effects of the crude filtered juice of the *Colocasia esculenta* may be due to the presence of anthocyanins or some flavonoids.

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REFERENCES:

1. Gronberg D. A., Grosse-Siestrup Christian and Fischer Axel (2002), 'In vitro models to study hepatotoxicity', Toxicological pathology, Vol. 30 (3) 394-399
2. Thohan Sanjeev, Zurich Marylynn C., Chung Ho, Weiner Myron, Kane Andrew S., Rosen Gerald M., (2001), 'Tissue slices revisited: Evaluation and development of a short-term incubation for integrated drug metabolism' *Drug Metabolism and disposition,* 10 *(29)* 1337-1342
3. Reid Angela B. , Kurten Richard C. , McCullough Sandra S. , Brock Robert W. and Hinson Jack A. (2004), "Mechanisms of Acetaminophen-Induced Hepatotoxicity: Role of Oxidative Stress and Mitochondrial Permeability Transition in Freshly Isolated Mouse Hepatocytes" from http://jpet.aspetjournals.org/content/312/2/509.full, doi:10.1124/jpet.104.075945
4. Neyrinck Audrey M., Gomez Cristina and Delzenne Nathalie, (2004), 'Precision-cut liver slices in culture as a tool to assess the physiological involvement of kupffer cells in hepatic metabolism', *Comparative Hepatology*, 3*(I);*545
5. Coulson J, Thompson JP (2010) 'Paracetamol (acetaminophen) attenuates in vitro mast cell and peripheral blood mononucleocyte cell histamine release induced by N-acetylcystein', *Clin Toxicol (Phila)* , 48(2) 111-114
6. Patil Bhagyashree (2010) '*In vitro* study of cytoprotection by *Aloe vera* leaf gel against APAP (AAP) mediated oxidative stress in rat liver slices' *Int J Adv Biotech and Research,* 1 (*2*) 73-86

7. Kim Sung-Hwa, Cheon Ho Jun, Yun Nari, OhSun-Tack, Shin Eunju, ShimKyu Suk and Lee Sun-Mee (2009), 'Protective Effect of a mixture of Aloe vera and Silybum marianum against carbon tetracholoride-induced Acute Hepatotxicity and liver fibrosis' *J Pharmacol sci*, 109, 119-127
8. Miladi Sonia and Damak Mohamed, (2008) 'In vitro antioxidant activities of aloe vera leaf skin extracts' *J de la Soc Chimique de Tunisie*10, 101-109
9. Tuse T.A., Harle U.N., Bore V.V., (2009) 'Hepatoprotective activity of Colocasia antiquorum against experimentally induced liver injury in rats' *Malyasian J pharma sci, Vol 7, No. 2, 99-112*
10. Lewu, M.N., Adebola, P.O. and Afolayan, A. J. (2009) ' Effect of cooking on the proximate composition of the leaves of some accessions of *Colocasia esculenta* (L.) Schott in KwaZulu-Natal province of South Africa' *African J of Biotechnology* 8(*8*), 1619-1622
11. Awasthi CP and Singh AB, (2000) 'Nutritional quality evaluation of edible leaves of some promising Colocasia and Alocasia collections', *Ind J Agric Res.*34*(2)*: 117-121
12. Devarkar V.D., Marathe V.R., Chavan D.P., (2011) 'Dietary and medicinal significance of wild vegetables from Osmanabad region, Maharashtra (India), *Life Sciences Leaflets* 11:317-332
13. Phillip, B. A., Grindleya, O. F., Asemotaa, H. N., Errol, Y. & Morrisona, A. (2002) Carbohydrate digestion and intestinal ATPases in streptozotocin-induced diabetic rats fed extract of yam (Dioscorea cayenensis) or dasheen (Colocasia esculenta), Nutrition Research, 22: 333–341.
14. Boban, P., Nambisan, B. and Sudhakaran, P. (2006) Hypolipidaemic effect of chemically different mucilages in rats: A comparative study, British Journal of Nutrition, 96: 1021–1029.
15. Tanaka, R., Sakano, Y., Nagatsu, A., Shibuya, M., Ebizukab, Y. and Goda, Y. (2005) Synthesis of digalactosyl diacylglycerols and their structure–inhibitory activity on human lanosterol synthase, Bioorganic and Medicinal Chemistry Letters, 15: 159–162.
16. Yang, A. H. and Yeh, K. W. (2005) Molecular cloning, recombinant gene expression and antifungal activity of cystatin from taro (Colocasia esculenta), Planta Medica, 221: 493–501.
17. Ravikumar S., Gracelin N, Anitha Anandha, G Selvan Palani, A Kalaiarasi (2011) '*In vitro* antibacterial activity of coastal medicinal plants against isolated bacterial fish pathogens', *Int J Pharma Research and Devel*., 3(4) 109-116
18. McCay PB, Lai EK, Poyer JL, DuBose CM, Janzen EG (1984) 'Oxygen- and carbon-centered free radical formation during carbon tetrachloride metabolism. Observation of lipid radicals in vivo and in vitro' *J Biol Chem*, 259:2135–2143.
19. Payasi A., Chaudhary M, Singh BM, Gupta A., Sehgal R., (2010) "Sub Acute Toxicity Studies of Paracetamol Infusion in Albino Wistar Rats", Int J. Pharmaceutical Sciences and Drug Research 2(2), 142-145
20. Reitman, S., and Frankel, S.A. (1957). A calorimetric method for the Determination of serum glutamate oxaloacetate and glutamate pyruvic transaminase. *Am J Clinical Pathology*, 28, 56
21. Bergmeyer, U. H. (1965). "Phosphatases" in Methods of Enzymatic Analysis, Pub by: *Academic Press, London*, 779.
22. Sallie R. J., M. Tredger and R. William (1991) 'Drug and the liver' *Biopharm. Drug Disp*. 12, 251-259
23. Shenoy; K. A., S. N. Somayaji, K. L. Bairy (2001) 'Hepatoprotective effects of Ginkgo biloba against carbon tetrachloride induced hepatic injury in rats' *Indian J. Pharmacol* 33, 260-266.
24. Recknagel R. O., E. A. Glende, J. A. Dolak Jr and R. L. C. Waller (1989). Mechanism of carbon tetrachloride toxicity. Pharmacol. Ther. 43, 139-154.
25. Baek N. L., Y. S. Kim, J. S. Kyung and K. H. Park (1996). Isolation of anti-hepatotoxic agent from the roots of Astragalus membranaceous. Korean J. Pharmacog. 27, 111-116
26. Hesham R., El-Seedi and Shgeru N., (2007), 'Chemistry of Biofaavonoids, *Indian J Pharm Educ, 39, 172-175*

27. Noda, y., kaneyuki, T., Mori, a. & Packer, l. (2002) Antioxidant activities of pomegranate fruit extract and its anthocyanidins: Delphinidin, cyanidin, and pelargonidin, *Journal of Agricultural Food Chemistry*, 50: 166–171
28. Cambie, R. C. & Ferguson, L. R. (2003) Potential functional foods in the traditional Maori diet, Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis, 523–524: 109–117.
29. Kowalczyk, E., Kopff, A., Fijalkowski, P. Niedworok, J., Blaszczyk, J., Kedziora, J. (2003) Effects of anthocyanins on selected biochemical parameters in rats exposed to cadmium, *Acta Biochimica Polonica*, 50: 543–548.