EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 5
152-157
Year: 2024

MODULATION OF ARSENIC INDUCED HEPATO-ENZYMATIC OXIDATIVE STRESS BY *CURCUMA AROMATICA* LEAF EXTRACT IN ALBINO RAT

Dr. Priya Bajaj* and Dr. Anjani Rani

Department of Zoology, Govt. PG College, Noida (GB Nagar) UP, India-201301.

***Corresponding Author: Dr. Priya Bajaj**
Department of Zoology, Govt. PG College, Noida (GB Nagar) UP, India-201301.

Article Received on 11/03/2024 **Article Revised on 01/04/2024** **Article Accepted on 21/04/2024**

ABSTRACT

Heavy metals have great ecological concern because of their bigger threat to mankind. Arsenic is one such well-known potent toxicant exposed naturally as well as anthropogenically. Groundwater arsenic contamination in exposed areas have groundwater arsenic content far away from WHO permitted maximum limit *i.e.* 10 ppb or 0.01 mg/ L. Arsenic contaminated water used for drinking, food preparation and irrigation of food crops poses the menacing threat to public health issues like keratosis, cardiovascular disease, diabetes and even cancers. The deleterious effects of arsenic toxicity and side effects of already available medicines led us to find out some dietary supplement cum herbal remedy against it. Leaf extract of *Curcuma aromatica* has been selected for this purpose on account of its renowned pharmacological activities. The LD_{50} of arsenic trioxide was estimated to be 14.98 mg/kg body weight. Acute (1 day) and sub-acute (7, 14 & 21 days) exposure of albino rats with sublethal doses of As_2O_3 resulted in damage of hepatocytes, which releases hepatic transaminases (AST, ALT), phosphatases (ALP, ACP) and dehydrogenase (LDH) in sinusoidal fluid. This significantly elevated the hepatic levels of aforesaid enzymes. Arsenic intoxication induces oxidative stress within hepatic tissues which is reflected by lower levels of antioxidant enzymes- SOD, CAT and GPx. Treatment with *Curcuma aromatica* leaf-extract (500 mg/kg body weight) following arsenic intoxication significantly reduced the hepatic enzyme levels to normalcy. Results reveal hepatoprotective effect of *Curcuma aromatica* in arsenic intoxicated rats due to its active ingredients terpenoids, exerting their potential as a future remedial agent against heavy metal toxicity.

KEYWORDS: Arsenic, *Curcuma aromatica*, Transaminases, Phosphatases, Dehydrogenases, Antioxidant enzymes, Heavy metals.

ABBREVIATIONS

ALT	-	Alanine Aminotransferase
AST	-	Aspartate Aminotransferase
ALP	-	Alkaline Phosphatase
ACP	-	Acid Phosphatase
LDH	-	Lactate Dehydrogenase
SOD	-	Superoxide Dismutase
CAT	-	Catalase
GPx	-	Glutathione Peroxidase
ANOVA-		Analysis of Variance
SNK Test-		Student Newman Keul's Test
ROS	-	Reactive Oxygen Species

INTRODUCTION

On account of industrialization, several heavy metals continue to be directly or indirectly discharged into the environment via wastewaters. Unlike most organic contaminants, metals are not biodegradable and tend to bioaccumulate or to be biomagnified in living organisms (Yang, 2011). Arsenic is one such ubiquitous metalloid occurring naturally, associated with rocks and soils. It has entered the food chain due to weathering and dissociation of rocks. Anthropogenic activities like pesticide application and discharge from industrial sources has added up its level significantly. Groundwater arsenic level has crossed the WHO permissible limit (0.01 mg/ L) at many places in India like West Bengal, Jharkhand, Bihar, and Uttar Pradesh. In Uttar Pradesh, which is the place of study as well, almost 40 districts are prone to risk with moderate to worst effect. The worst affected districts are Balia, Barabanki, Gorakhpur, Ghazipur, Gonda, Faizabad and Lakhimpur Kheri, situated on the floodplains of the Ganga, Rapti and Ghaghara rivers. Some other districts like Shahjahanpur, Unnao, Chandauli, Varanasi, Pratapgarh, Kushinagar, Mau, Balrampur, Deoria and Siddharthnagar are put with moderate risk of arsenic contamination. A new study has found that as many as 2.34 crore people in rural areas of Uttar Pradesh are exposed to high levels of arsenic in groundwater and over hundred million people worldwide

are exposed to arsenic contamination via water (Mishra, 2019). They are suffering from several ailments like hyperkeratosis, hyper-pigmentation, dermatitis, dermal lesions, and skin cancer. It has also been found to be prevalence of cardiovascular disease and diabetes (Ahsan etal., 2009). Human absorption of arsenic occurs mainly through gastrointestinal tract; however, inhalation and dermal absorption also occur in small amount. (Wester etal., 1993).

The enzymes like AST (EC 2.6.1.1), ALT (EC 2.6.1.2), ALP (EC 3.1.3.1), ACP (EC 3.1.3.2) and LDH (EC 1.1.1.27), SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), GPx (EC 1.11.1.9), closely intact with biological membranes of hepatocytes are released quickly as a result of membrane damage. Thus, assessment of these enzymes provides a simple but reliable tool to measure the protective activity of the target drug against hepatic damage (Hewawasam etal., 2004). Increased oxidative stress results from excess generation of reactive chemical species called free radicals from various sources and/or from decreased enzymic and nonenzymic antioxidant defenses. Free radicals and other reactive species have been implicated in the progression of more than hundred different diseases, thus the interest in free radicals and oxidative stress has grown in recent times.

Various indigenous systems of medicines such as Siddha, Ayurveda, Unani and sometimes allopath use several plant species for the treatment of several degenerative diseases. These medicines are commonly used to obviate the profound side effects of modern drugs. Hepatic injury due to metal toxicity is also a degenerative disease, which can be cured by herbal medicine extracts (Corns, 2003; Ramm & Ruddel, 2005).

In our course of study, we have used leaf extract of *Curcuma aromatic* Salisb (wild turmeric), a member of family Zingiberaceae, described as potent antioxidant (Remirez–Tortosa *etal.,* 1999). It is a traditional Indian herb, which has long been used as a dietary pigment and spice in Asian countries. Now it is widely cultivated and used as medicinal plant to treat gastrointestinal upset, arthritic pain, and cancer etc (Lev–Ari *etal.,* 2006; Shi *etal.*, 1981). It is a semi-erect, herbaceous plant exerting various pharmacological activities, viz. anti-inflammatory, antioxidant, and antimicrobial, and hepato-protective activities, etc. Toxicological evaluation has revealed that *Curcuma aromatica* is safe for prolonged treatment thus used in present investigation to assess its protectivity against arsenic toxicity using assay of transaminases, phosphatases & dehydrogenases. (Ahmed *etal.,* 2008; Chainani-wu, 2003; Masuda *etal.,* 2001; Deshpande *etal.,* 1998).

MATERIALS AND METHODS

Eighty adult male Wister albino rats of almost same age, weighing about 100 ± 10 g, were obtained from inbred colony. Rats were housed under standard environmental conditions (25ºC temp. and 12 hrs light/dark cycles) in well-ventilated cages, fed on standard rat chow and provided tap water *ad-libitum*. All the animal care and experiments were performed according to guidelines of *animal institutional ethical committee (360/01/9/CPSEA/2001)* under GLP.

The chemical selected for study was Arsenic trioxide, which was of analytical grade purity and obtained commercially from Merck, India. Its LD_{50} was estimated by Log dose/Probit regression line method and found to be 14.98 mg/kg body weight. Sub-lethal dose (LD_{50}/10) was dissolved in 1 ml distilled water. (Finney, 1971) Leaf extract of *Curcuma aromatica*, was taken for experimental protective studies. The leaves of *Curcuma aromatica* were grinded with the help of pestle-mortar and squeezed with muslin cloth to prepare the extract. Filtrate was then centrifuged at 2000 rpm for 20 minutes and supernatant was stored at 4ºC temperature. The extract was analyzed by Gas Chromatography/Mass Spectrometry. (Babu *etal.*, 2002)

All rats were assigned randomly into four groups with twenty rats each *viz.* acute (1d) and sub-acute (7, 14 and 21 ds). Sets were further divided into 4 groups – control (given water only), arsenic treated, curcuma + arsenic treated and curcuma treated. The designated sets/group and their respective doses are shown in table–1.

Table 1: Acute and Sub-acute doses of arsenic trioxide and *curcuma aromatic* leaf extract for *Rattus norvegicus*.

Groups / Sets	Group I (Control)	Group II (Arsenic treated)	Group III (Curcuma treated)	Group IV (Curcuma + Arsenic treated)
Set : 1 Acute (1d)	Water	1.5 mg/kg b.wt	5000 mg/kg b.wt	5000 mg/kg b.wt + 1.5 mg/kg b.wt
Set : 2 Subacute (7ds)	Water	0.2mg/kg b.wt	5000 mg/kg b.wt	5000 mg/kg b.wt + 0.2mg/kg b.wt
Set : 3 Subacute (14ds)	Water	0.1 mg/kg b.wt	5000 mg/kg b.wt	5000 mg/kg b.wt + 0.1 mg/kg b.wt
Set : 4 Subacute (21ds)	Water	0.07 mg/kg b.wt	5000 mg/kg b.wt	5000 mg/kg b.wt + 0.07 mg/kg b.wt

At the end of 1st, 7th, 14th and 21st days of experiment, the rats of respective sets were sacrificed to dissect out their livers. All the livers were homogenized in 4°C cold physiological saline solution (pH = 7.4) in glass-Teflon potter homogenizer and centrifuged separately at 12000rpm for 1 hr. at 4°C. The clear supernatant was used for further experiments.

The marker metabolic enzymes – AST, ALT, ALP and ACP and LDH and antioxidant enzymes- SOD, CAT and GPx by standard laboratory methods. (Babson & Babson, 1973; Hilman, 1971; Reitmann & Franklin, 1957; Bessey etal., 1946; Johansson and Borg, 1988; Kakkar etal., 1984; Tappel, 1978).

The results were computed statistically using one way ANOVA followed by SNK test for intergroup comparison, and expressed as Mean ± SEM. The values were signified at 0.05 level. (Glantz, 1992)

RESULTS

Tables – 2 & 3 shows that hepatic levels of metabolic enzymes (transaminases, phosphatases, and dehydrogenase) increase significantly ($p < 0.05$) whereas that of antioxidant enzymes (SOD, CAT and GPx) decreases significantly ($p < 0.05$) after acute and sub-acute arsenic intoxication, when compared with control.

Table-2: Hepatic metabolic Enzyme Level of *Rattus norvegicus* after Arsenic trioxide and *curcuma aromatica* leaf extract Treatments.

Hepatic Enzyme (IU/L)	Treatment days	Control	Arsenic treated	*Curcuma*+ Arsenic treated	*Curcuma* treated
ALT	Acute (1 d)	74.33±1.20	84±1.00*	81±1.00*	73±0.58^{NS}
	Subacute(7 ds)	77.33±0.88	87±1.15*	80±1.00^{NS}	76±0.58^{NS}
	Subacute(14ds)	76±1.1	98±1.00*	79±1.15^{NS}	74±1.15^{NS}
	Subacute(21ds)	77±1.00	105±1.00*	79±0.58^{NS}	75±1.53^{NS}
AST	Acute (1 d)	111.33±1.45	119.33±1.20*	118.67±0.88*	109.33±1.20^{NS}
	Subacute (7 ds)	110±1.15	123±2.52*	115.33±1.77^{NS}	109±1.15^{NS}
	Subacute 14ds)	112.33±1.20	137.67±1.45*	116±1.53^{NS}	110±1.15^{NS}
	Subacute(21ds)	109±1.15	183±1.00*	113±1.33^{NS}	107±1.00^{NS}
ALP	Acute (1 d)	177.67±0.88	212.33±0.33*	202±0.58*	176±0.58^{NS}
	Subacute (7 ds)	174±0.58	208.33±0.88*	196±1.15*	171.83±0.44^{NS}
	Subacute(14ds)	174.4±0.76	217.2±2.31*	189.33±2.6*	174±1.15^{NS}
	Subacute(21ds)	181.67±0.88	221.67±1.45*	186±1^{NS}	183±.058^{NS}
ACP	Acute (1 d)	243.67±0.88	266±0.58*	254.33±1.10*	243±1.15^{NS}
	Subacute (7 ds)	241.33±0.88	268.67±0.67*	252.67±0.88*	240±0.58^{NS}
	Subacute(14ds)	242±1.53	273±0.58*	245.83±1.59^{NS}	239±1.00^{NS}
	Subacute(21ds)	244.33±0.88	288±0.58*	246.67±0.88^{NS}	242.33±0.88^{NS}
LDH	Acute (1 d)	83.33±0.61	142.73±1.46*	123.5±0.8*	81.83±0.9
	Subacute (7 ds)	83.67±0.67	148.66±1.45*	109.7±1.2*	83.33±0.8
	Subacute(14ds)	85.67±0.67	152.67±1.45*	96.83±0.6*	84.90±0.6
	Subacute(21ds)	87.53±0.52	156.1±1.10*	89.67±0.88	87.33±0.88

Table-3: Hepatic antioxidant Enzyme Level of *Rattus norvegicus* after arsenic Trioxide and *curcuma aromatica* leaf extract Treatments.

Hepatic Enzyme (IU/L)	Treatment days	Control	Arsenic treated	*Curcuma* +Arsenic treated	*Curcuma* treated
SOD	Acute(1 d)	24.9±1.4	13.7±1.3*	17.2±1.1*	25.8±1.6^{NS}
	Subacute(7 ds)	24.7±1.2	13.1±1.2*	22.8±1.2^{NS}	25.4±1.1^{NS}
	Subacute(14ds)	25.1±1.0	12.8±1.3*	23.9±1.4^{NS}	26.2±1.3^{NS}
	Subacute(21ds)	24.5±1.1	12.2±1.0*	25.1±1.1^{NS}	26.8±1.1*
CAT	Acute (1 d)	0.36±0.4	0.23±0.2*	0.32±0.3*	0.38±0.2^{NS}
	Subacute (7 ds)	0.33±0.2	0.20±0.4*	0.34±0.4^{NS}	0.39±0.4^{NS}
	Subacute 14ds)	0.39±0.6	0.19±0.1*	0.38±0.2^{NS}	0.41±0.1^{NS}
	Subacute(21ds)	0.42±0.9	0.20±0.2*	0.41±0.3^{NS}	0.45±0.5^{NS}
GPx	Acute (1 d)	0.49±0.4	0.28±0.3*	0.45±0.2*	0.50±0.3^{NS}
	Subacute (7 ds)	0.51±0.2	0.31±0.2*	0.51±0.3^{NS}	0.53±0.1^{NS}
	Subacute(14ds)	0.49±0.3	0.25±0.3*	0.50±0.4^{NS}	0.52±0.6^{NS}
	Subacute(21ds)	0.53±0.4	0.22±0.4*	0.53±0.2^{NS}	0.54±0.2^{NS}

Administration of *C. aromatica* leaf extract before As_2O_3 intoxication, significantly ($p < 0.05$) reduces the elevated level of transaminases within 7 days, while that of ALP within 21 days, ACP within 14 days and LDH within 21

days of treatment respectively. In case of antioxidant enzymes (SOD, CAT & GPx), the levels were retained within 7 days of exposure. Further no changes were exhibited by the extract alone.

DISCUSSION

Arsenic inside the body first interacts with liver xenobiotics metabolizing system, where it is methylated for excretion through urine (Roy & Saha, 2002). When methylation capacities of liver are exceeded by arsenic concentration, it starts to create cellular toxicity. The toxicity is mediated by production of free radical (Poli & Parola, 1997). Antioxidant defense mechanisms of body can protect cells from oxidative injury, including enzymatic and nonenzymatic systems. Antioxidant enzymes include catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), and peroxiredoxin III (PrxIII).

Arsenic exposure has implicated in alterations of enzymes, carbohydrate, proteins, and lipid content of liver along with serious histological changes (Straub *etal.,* 2008; Khudabakhsh *etal.,* 2005). Hepatocytes contain a well-developed enzymatic system related to metabolism of proteins, fats, carbohydrates, and xenobiotics. These enzymes are either present in cytosol or attached with biological membranes viz. plasma membrane, mitochondrial membrane, and lysosomal membrane (Martini, 1989). ALT is a cytosolic enzyme, while AST has two isozymes one being cytoplasmic and the other being bound to mitochondrial membrane. ALP is found firmly attached to plasma membrane and ACP is present within lysosomes (Luxton, 1999). ALT appears to reflect hepatic disease more specifically than AST and others, because of biological location of the enzyme. However, the assessment of AST and ALT along with ALP may reflect injury to liver. (Ayalogu *etal.,* 2001)

Although the exact molecular mechanisms for metal toxicity are not completely understood, the potential of metals to generate reactive oxygen species (ROS) and thus to alter cellular redox states is considered the most important (Qian etal., 2003). Toxic effect of arsenic on hepatocytes induces oxidative stress producing reactive oxygen species (ROS). ROS leads to production of free radicals and subsequent depletion of antioxidant cell defense, resulting in imbalanced oxidant/antioxidant in mammalian tissues (Banua *etal.,* 2009). It causes the peroxidation of unsaturated fatty acids in bio-membranes. The metal atom donates an electron to fatty acid, which in turn releases a hydrogen ion and converts into free radical. Whenever these radical releases its electron, a second radical is formed, which then turns around and does the same thing to a third molecule, continuing to generate more unstable products. Oxidation of any substance in presence of arsenic also generate superoxide free radical. Due to peroxidation the hepatocyte membranes get disrupted. (Radhika *etal.,* 2007). It is thought that reactive oxygen free radicals could inactivate and reduce hepatic CAT, SOD, and GPx activities. This speculation agrees with the findings of Wohaieb and Godin, 1987.

Besides peroxidation, disruption of thiol proteins is also a crucial part of cell injury, since arsenic attaches to sulfhydryl group containing proteins and damages them (Mallick *etal.,* 2003). Inhibition of sulfhydryl group containing enzymes also enhances the lipid peroxidation (William *etal.,* 1999). Increased lipid peroxidation causes oxidative inactivation of both membranous and soluble proteins (Mantle & Preedy, 1999). These damages release the enzymes from the membranes joining the biliary canaliculus and sinusoidal border of parenchymal cells into the sinusoidal fluid, Causing the elevation of liver enzyme levels. Increased acid phosphatase in liver may be due to the metallic nature of arsenic which alters the cell membrane permeability and loss of lysosomal stability. (Srivastav etal., 2007; Gray & Micheal, 2002).

LDH is a bifunctional enzyme of glycolytic pathway, catalyzing the pH dependent interconversion of lactate into pyruvate. Liver damage results in significantly elevated hepatic LDH, so it has been widely used for *in vitro* studies related to hepatotoxicity caused by arsenic and many other toxicants. These studies have shown a time and dose dependent increase in percent of total LDH release from hepatocytes. Arsenic reacts with key sulfhydryl groups of membrane $Na^+ – K^+$ ATPase pump inducing cell-swelling and lysis of hepatocytes, this results in LDH release which is an indicator of cell death. (Fierro *etal.,* 1999; Levinsky *etal.,* 1970).

Turmeric has earlier been found to exhibit anti-hepatotoxic activity. Phyto-chemical. analysis reveals that terpenoids are the active ingredients of leaf extract. These terpenoids include monoterpenes like 4-terpineol, borneol, 1,8-cineol, linalool and sesquiterpenes like nerolidol, ar-curcumenean zingiberene etc. which are either hydrocarbon or phenolic in nature. They are found to show various pharmacological activities. Synergistic antioxidant and antiperoxidative effects of these terpenoids probably contribute for protection against reactive oxygen species induced by arsenic stress. They modulate hepatocytes against deleterious effect of arsenic toxicity either by quenching singlet oxygen or by reducing the free radical species or by increasing phase II activities. Reduction of free radical species converts it into non-radical product and involves abstraction of H-atom from antioxidant molecule. Some of the terpenoids like ocimene, limonene and their alcohol derivatives donate their H-atom at the level of phase II detoxification by enzymes like GST, GPx and CAT etc. (Grassmann, 2005; Hakino, 1985)

Intake of leaf extract reduces the peroxidation injury by hepatocyte membrane stabilization, thus reducing the leakage of hepatic enzymes, which is reflected by lower levels of transaminases, phosphatases, and dehydrogenase. The restoration of oxidant/antioxidant balance is indicated by elevated levels of antioxidant

enzymes SOD, CAT and GPx. In some subacute groups-III and IV the levels of these enzymes are quite higher than control groups confirming the high antioxidant potential of *Curcuma aromatica* leaf extract. It successfully ameliorates the toxic effect of arsenic on hydrolytic, amino acids catabolizing and dehydrogenases and antioxidant enzymes, which merits it to recommend as an excellent hepato-protective drug.

REFERENCES

1. Ahmed J, Ansari SH, Ali M, Bhatt D, Ansari F. Phytochemical and biological investigations on *Curcuma aromatic*. Pharmcog Rev., 2008; 2(3): 151–156.
2. Ahsan T, Zehra K, Mushi A, Ahsan S. Chronic arsenic poisoning. J Pak Med Assoc, 2009; 59(2): 105–7.
3. Ayalogu E, Igboh NM, Dede EB. Biochemical changes in the serum and liver of albino rats exposed to petroleum samples (gasoline, kerose and crude petroleum. J Appl Sci Environ Mgt., 2001; 5(1): 97–100.
4. Babson AL, Babson SR. Kinetic colorimetric measurement of serum lactate dehydrogenase activity. Clin Chem, 1973; 19: 766 – 769.
5. Babu S, Seethraram K, Kumar PN, Johnson I. Fungitoxic properties of some plant extract against *Alternaria solani*. The tomato leaf blight pathogen. J.Ecotoxicol Environ. Monitoring, 2002; 10(2): 157 – 159.
6. Banua GS, Kumarb G, Murugesanb AG. Effects of leaf extract of *Ocimum sanctum* L. on arsenic – induced toxicity in wistar albino rats. Food Chem Toxicol, 2009; 47(2): 490– 495.
7. Bessey OA, Lowery OH, Brock MJ. A method for determination of alkaline phosphatase with five cubic millimeters of serum. J Biol Chem, 1946; 164: 321 – 329.
8. Chainani-Wu N. Safety and anti-inflammatory activity of curcumin: a component of tumeric (Curcuma longa). J Altern Complement Med, 2003; 9(1): 161-8.
9. Corns CM. Herbal remedies and clinical biochemistry. Ann Clin Biochem, 2003; 40(5): 489 – 507.
10. Deshpande WR, Gadre SG, Raste AS, Pillai D, Bhide, SV, Samuel AM. Protective effect of turmeric (*Curcuma longa* L.) extract on carbon tetrachloride induced liver damage in rats. Indian J Exp Biol., 1998; 36: 573–577.
11. Fierro FA, Barber DS, Rad LT, Carter DE. *In vitro* tissue specificity for arsenic and arsenic toxicity in the rat. Toxicol Sci., 1999; 52: 122 – 129.
12. Finney DJ. Probit Analysis, 3rd ed. Cambridge: Cambridge University Press, 1971; 8 – 80.
13. Glantz SA. In: Primer of Biostatistics. 3rd ed. NewYork: McGraw Hill, 1992; 32–100.
14. Grassmann, J. Terpenoids as plant antioxidants. Vitam Horm, 2005; 72: 505 – 535.
15. Gray MW, Michael JI. In: Toxicology Pathology. ed. Taylor S, Francis D. 2nd ed, London: Elsevier, 2002; 41.
16. Hakino H. Antiheptotoxic activity of crude drugs. Yakugaku Zasshi, 1985; 105(2): 109–118.
17. Hewawasam RP, Jayantilaka KAPW, Pathirana C, Mudduwa LKB. Hepatoprotectective effect of Epaltes divericata extract on carbon tetra chloride induced hepatotoxicity in mice Ind J Med Res 2004; 120: 30–4.
18. Hillman GJ. Continuous photometric measurement of prostate acid phosphatase activity. Clin Chem Clin Biochem, 1971; 9: 273 – 277.
19. Johansson L H, Borg L A. A spectrophotometric method for determination of catalase activity in small tissue samples. Anal Biochem, 1988; 174: 331–336.
20. Kakkar P, Das B, Viswanathan P N. A modified spectrophotometric assay of superoxide dismutase, Ind. J Biochem Biophys, 1984; 21: 130–132.
21. KhudaBukhsh AR, Pathak S, Guha B, Karmakar SR., Das TA, Banerjee P, Biswas SJ, Mukherjee P, Bhattcharjee N, Choudhary SC, Banerjee A, Bhadra S, Mallick P, Chadrabarti J, Mandal B. Can Homeophathic arsenic remedy combat arsenic poisoning in humans exposed to groundwater arsenic contamination?: 4 preliminary report on first human trial. eCAM, 2005; 2(4): 537 – 548.
22. Lev–Ari S, Malmen Y, Strier L, Kazanov D, Arber N. Down-regulation of prostaglandin E2 by curcumin is correlated with inhibition of cell growth and induction of apoptosis in human colon carcinoma cell lines. J Soc Integr Oncol, 2006; 4: 21–6.
23. Levinsky WJ, Smalley RV, Villyer AN Shindler RL. Arsine hemolysis. Arch Environ Health, 1970; 20: 436 – 440.
24. Luxton R. Liver Function In: BMS Explained Clinical Biochemistry. Oxford: Butterworth – Heinemann Press, 1999; 228–240.
25. Mallick P, Mallick JC, Guha B, Khudabukhsh AR. Ameliorating effect of microdoses of a potentized homeopathic drug arsenicum album. Altern Med, 2003; 3(7): 3–7.
26. Mantle D, Preedy VR. Adverse Drug Reaction and Toxicology Review, 1999; 18: 235–252.
27. Martini F. In: Fundamentals of Anatomy and physiology. New Jersey: Printice Hall Englewood Cliffs, 1989; 944.
28. Masuda T, Mackwa T, Hidaka K, Bindo H, Takedo Y & Yamaguchi H. Chemical studies on antioxidant mechanism of curcumin: analysis of oxidative coupling products from curcumin and linoleate. J Agric Food Chem, 2001; 49(5): 2539–2547.
29. Mishra U High levels of arsenic found in Groundwater in Uttar Pradesh. Environment, In: The wire, 31/05/2019.
30. Poli G, Parola M. Oxidative damage and fibrogenesis. Free Rad Biol Med, 1997; 22: 287–305.

31. Qian Y, Castranova V, Shi X. New perspectives in arsenic-induced cell signal transduction. J Inorg Biochem, 2003; 96(2): 271–8.
32. Radhika R, Geetha S, Lakshmi R, Kannan M. Antioxidants are boon to life. Agrobios Newletter, 2007; VI(6): 11–12.
33. Ramm, GA, RG Ruddell. Hepatotoxicity of iron overload: mechanisms of iron induced hepatic fibrogenesis. Semin Liv Dis., 2005; 25(4): 433 – 49.
34. Reitman, S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol, 1957; 28: 56-63.
35. Remirez–Tortosa MC, Mesa MB, Aguilera MC, Quiles JL, Baro L, Renirez–Tortosa CL. Oral administration of a turmeric extract inhibits LDL oxidation and has hypocholesterolemic effects in rabbits with experimental atherosclerosis. Atheroscl, 1999; 147(2): 371 – 78.
36. Roy P, Saha A. Metabolism and toxicity of arsenic: A human carcinogen. Curr Sci., 2002; 82(1): 38–45.
37. Shi JH, Li CZ, Liu DL. Experimental research on *Curcuma aromatica* volatile oil. Zhongyao Tongbuo., 1981; 6: 36–8.
38. Shrivastav S, Jadon A, Shukla S. Effect of Tiron and it's combination with nutritional supplements against vanadium intoxication in female albino rats. J Toxicol Sci., 2007; 32(2): 185–192.
39. Straub AC, Clark KA, Ross M.A, Chandra AG, Li S, Gao X, Pagano PJ, Stolz DB, Barchowsky A. Arsenic stimulated liver sinusoidal cappillarization in mice requires NADPH oxidase – generated superoxide. J Clin Invest, 2008; 118(12): 3980–3989.
40. Tappel A L. Glutathione peroxidase and hydroperoxides. Methods Enzymol, 1978; 52: 506–513.
41. Wester RC, Wingren HI, Sedik L, Melendres J, Wade M. Percutaneous Absorption of Pentachlorophenol from Soil. Fundam Appl Toxicol, 1993; 2: 336–40.
42. Williams F, Robertson R, Roworth M. In: Detailed profile of 25 major organic and inorganic substances. Glassow : SCEIH, 1999; 12–19.
43. Wohaieb S A, Godin D V. Alterations in free radical tissue defense mechanisms in streptozotocin-induced diabetes in rat: effect of insulin treatment. Diabetes, 1987; 36: 1014–1018.
44. Yang M A current global view of environmental and occupational cancers. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev., 2011; 29(3): 223–49.