

Elemental analysis of local galena based eyeliner and its effects on some enzymes of rat liver and brain

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The study was carried out to investigate the effect of galena-based eyeliner on the cellular and functional integrity of rat liver and brain by evaluating the activities of alkaline phosphatase (ALP), aspartate and alanine aminotransferases (AST and ALT respectively), lactate dehydrogenase (LDH) and malondialdehyde (MDA) concentration. 0.50, 0.75 and 1.00% (w/v) of the eyeliner were prepared in water. Analysis of the eyeliner was carried out to determine its elemental components. The experimental animals were grouped into four. Group 1 rats received 0.2ml of distilled water in drops to their eyes while groups 2-4 received 0.2ml of 0.50, 0.75 and 1.00% (w/v) eyeliner respectively for 14 days after which venous blood were collected for the determinations of lead (Pb) and cadmium (Cd). The results revealed a significant increase ($P<0.05$) in blood Pb and Cd concentrations. Also, there was a significant increase ($P<0.05$) in serum ALP, AST, ALT and LDH activities but a significant reduction in the liver and brain ($P<0.05$). The MDA concentration of the brain and liver were significantly elevated ($P<0.05$) in comparison to control. This pattern of results is suggestive of a possible alteration in the structural and functional capacity of the liver and brain by the eyeliner in a concentration and time dependent manner.

Key words: Elemental, galena, eyeliner, enzymes, liver, brain

INTRODUCTION

In Nigeria, the use of eyeliners such as 'tiro' (in Yoruba), 'Kwali' (in Hausa) and 'Uhie' (in Igbo) as a beautifying substance is an age long practice. Apart from lifestyle, living conditions and culture have influenced the use of eyeliners (Chukwuma, 1997). Galena-based Kwali is native eye liner which is majorly made from galena, the natural mineral form of lead sulphide (PbS); the principal ore of lead (Hardy *et al.*, 2002). The repeated and prolonged lead (Pb) exposure to the body as a result of the use of traditional cosmetic (eyeliners) in Asia, Africa and the Middle East has been a subject of debate to the scientific community (Hardy *et al.*, 2004). Eye cosmetics such as Kohl and Surma (Middle East Asian) have been identified as a suspected source of Pb exposure to the cellular system in humans (Sprinkle, 1995). This has been attributed to systemic delivery of drugs and other xenobiotics to the circulatory system via the ocular route

(Chiou 1991). Elevated blood Pb levels in Omani children indicative of subclinical lead intoxication, following treatment with Pb containing traditional medicines have been reported (Timms and Bold, 2000). The use of leaded eye cosmetics have been observed to be strongly correlated with elevated blood Pb levels (Sprinkle 1995; Al-Ashbau *et al.*, 2004).

Kohl and summar are composed of galena (PbO), amorphous carbon, zincite (ZnO), sassolite (H_3BO_3), minium (Pb_3O_4), magnetite (Fe_3O_4), goethite ($FeO(OH)$), cuprite (Cu_2O), and talc ($Mg_3Si_4O_{10}(OH)_2$) (Hardy *et al.*, 1998). There is a growing concern about the physiological and behavioural effects of environmental trace metals in human populations. Although the documented toxic effects of Pb and other trace metals in humans are well known, the increased vulnerability of children as well as adults to extremely low levels of Pb is increasingly becoming a major source of concern (Koller *et al.*, 2004). Chronic exposure to low levels of lead (10µg/dl or less) produces mental deterioration, lowered

intelligence quotients, learning disabilities and behavioural abnormalities in children. (Al-Hazza and Krahn, 1995). It has also been reported that Pb and cadmium (Cd) are particularly dangerous for the developing central nervous system due to lack of functional blood brain barrier and intense cellular proliferation (Antonio *et al.*, 2010). Lead and cadmium have also been reported to cause reduction in the activity of brain alkaline phosphatase while increasing oxidative damage in the central Nervous system (Antonio *et al.*, 2003).

'Kwali' or 'tiro' has been applied for its beautifying and attractive effect on the eyes. It is also used for its protective or sunlight shielding effect on the eyes as well as in the treatment of ophthalmologic infections to cleanse the eyes (Chukwuma, 1997). Studies on traditional make-ups used in Nigeria have reported very high levels of trace metals in locally produced facial make ups (Ajayi *et al.*, 2002). However, there is paucity of information on the trace metal content and toxicological effects of repeated and prolonged exposure to galena-based Kwali or tiro.

The objective of the present study therefore is not only to emphasize the potential hazard of using traditional trace metals-containing eyeliners but also the need for toxicological information on organs such as the brain and liver since Cd and Pb neuropathy has been extensively studied (Thevenod and Friedmann, 1999). It is also aimed at sensitizing the general populace for strict caution in the use of such products.

Materials and Methods

Source of eyeliner and reagents

The galena-based eyeliner (tiro or Kwali) was purchased from Emir's market in Ilorin, North Central of Nigeria. All chemicals and solvent used were of analytical grades and were used without further purification. They were obtained from BDH Chemicals, England.

Source of experimental animals

A total of twenty four (24) female albino rats (*Rattus norvegicus*) with an average weight of 101.50 ± 4.42 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. Animal husbandry and experimentation were consistent with *Guiding Principles in the use of Animals in Toxicology* (Derelanko, 2000).

Elemental analysis of the eyeliner

The eyeliner was dried to constant weight in an oven at 80°C for 12hr. 1.0g of the dried sample was digested with HNO₃, evaporated to near dryness on a hot plate and then cooled. The procedure was repeated with HClO₄. More HClO₄ was added until evolution of white fumes (marking the end of the digestion process) before heating to near dryness. The digest was taken up in 1.0M HNO₃,

filtered with Whatman number 4 filter paper into 10ml volumetric flask and made up to mark with deionized water. This was subsequently analysed for Pb, Cd, chromium (Cr), zinc (Zn), nickel (Ni), and iron (Fe) using an air – acetylene flame atomic absorption spectrophotometer (UNICAM, 969) by the standard calibration technique. Batch precision and accuracy were successfully monitored with a 10% insertion rate of sample duplicates, blanks and spikes. Appropriate quality assurance procedures and precautions were carried out to ensure reliability of the results. Results from each batch were accepted if control samples were within 10% of the accepted value of the sample.

Preparation of different concentration of the eyeliner and administration

Three different concentrations of the eyeliner were prepared (0.5, 0.75 and 1.00% (w/v)) using distilled water. The 24 rats were randomly divided into four groups of six rats each. Group II rats (control) daily received 0.2ml of distilled water in drops to their eyes for 14 days while rats in groups 2-4 received daily 0.2ml of 0.5, 0.75 and 1.00% (w/v) eyeliner solutions respectively for 14 days in drops to their eyes.

Blood collection and preparation of tissue homogenates

At the end of the experimental period, approximately 5ml of venous blood was collected from each of the experimental animals according to the method of Narayanan *et al.* (1984). The serum was prepared by centrifuging the blood samples at 3000 rpm for 5min (Ogbu and Okechukwu, 2001) and serum collected with a Pasteur pipette. The animals were thereafter quickly dissected to obtain the liver. The brain was obtained also from the cranial cavity. They were kept separately in ice-cold 0.25M sucrose solution (1:5w/v) and homogenized.

Determination of blood lead and cadmium concentration

Lead and cadmium in blood of rats were determined by atomic absorption spectrometry (AAS) as described by (Corpas *et al.* 2002).

Assay of biochemical parameters

Activity of alkaline phosphatase (ALP) was determined in the serum, liver and brain of rats by the method of Wright *et al.* (1972). Activities of serum aspartate and alanine aminotransferases (AST and ALT) were determined by the method of Reitman and Frankel (1957) while lactate dehydrogenase (LDH) activity in the serum, liver and brain was determined by the method of Nielson *et al.* (1968). Lipid peroxidation in the liver and brain of rats was estimated by measuring the thiobarbituric acid reacting substance (TBARS) formation described by Varshney and Kale (1990). Protein content of serum and tissue homogenate was determined by the Biuret method (Gornal *et al.*, 1949).

Statistical Analysis

All data are presented as mean $\pm$ standard deviation. Statistical analyses were carried out using Duncan Multiple Range test (Montogonery, 1976). In all cases probability level of 95% was taken as significant.

Table 1. Trace metal contents¹ of galena –based eyeliner

Metal	µg/g dry weight
Fe	198.6±19.9
Ni	112.7±12.1
Pb	134.5±14.8
Zn	96.4±10.5
Cr	39.3±4.8
Cd	1.6±0.2

Values are means (n=3) ± S.D

Table 2. Blood lead and cadmium levels¹ of rats following the administration of galena based eyeliner for 14 days

Concentration of galena-based eyeliner(%w/v)	Concentration of Lead (µg/dl)	Concentration of cadmium (µg/dl)
Control	10.1±0.94 ^a	N.D
0.5	31.20±2.82 ^b	0.6±0.05 ^a
0.75	33.50±2.97 ^b	0.8±0.06 ^a
1.00	59.80±4.31 ^c	1.5±0.20 ^b

Values are mean (n=6) ± S.D. Values with different superscripts along the column are significantly different

RESULTS

The result of the elemental analysis of galena-based eyeliner is presented in Table 1. Six elements were recorded with their concentrations ranging from 198.6µg/g for Fe to 1.6µg/g for Cd. Table 2 shows the Pb and Cd concentrations in the blood of experimental and control animals. Significant differences ($P<0.05$) between the control and the groups were observed for Pb while for Cd, significant increase ($P<0.05$) in concentration was only observed in the group administered 1.00% (w/v) of the eyeliner solution when compared with the control.

Figures 1-3 show the effects of the fourteen days administration of galena based eyeliner on the activities of ALP, AST, ALT and LDH in the tissues studied. Serum ALP and LDH activities were significantly increased ($P<0.05$) in all the groups when compared with the controls. Also serum AST and ALT activities of the groups administered 1.00% (w/v) of eyeliner were significantly elevated ($P<0.05$) when compared with the control.

However, there was a significant reduction ($P<0.05$) in ALP activity of the liver and brain in all the treatment groups when compared with control. The significant reduction ($P<0.05$) observed in both the liver and brain LDH activity was observed in the groups administered 0.75% (w/v) and 1.00% (w/v) concentration of eyeliners for 14days.

The concentration of malondialdehyde (MDA) in the liver and brain of rats administered varying concentration of galena-based eyeliner for 14 days is presented in Figure 4. The data obtained revealed a significant

increase ($P<0.05$) in the concentration of MDA in all the groups in both the liver and brain when compared with the controls.

DISCUSSION

The environmental and public health implication of exposures to metals in Africa have been reviewed and the use of local herbal remedies and cosmetics have been indicated as sources of exposure (Chukwuma, 1997). Eye cosmetics have been reported as sources of toxic metals such as Pb and Cd to the cellular system in humans (Sprinkle, 1995), this may not be unconnected to the reported systemic delivery of drugs through the ocular route. (Chiou 1991).

The high concentration of Fe in the sample reflects the natural source of the galena-based eyeliner (Funtua and Oyewale, 1997). The Iron and zinc are not of toxicological significance. Iron compounds have an established role as colourants in many cosmetic products. Apart from its importance as an essential nutrient necessary for oxygen metabolism and mitochondrial function, Fe exhibits a functional importance as a trace metal in the normal growth and functional maturation of the skin cells (Landsdown, 2001). Zinc also have been reported to serve as cofactors for more than 200 enzymes such as superoxide dismutase isoenzymes (Kunikowska and Jenner, 2002) and those involved in the synthesis and repair of DNA and RNA. The presence of toxic metals such as Pb, Cr, Ni and Cd suggests the eyeliner may possess certain degree of deleterious effects since some

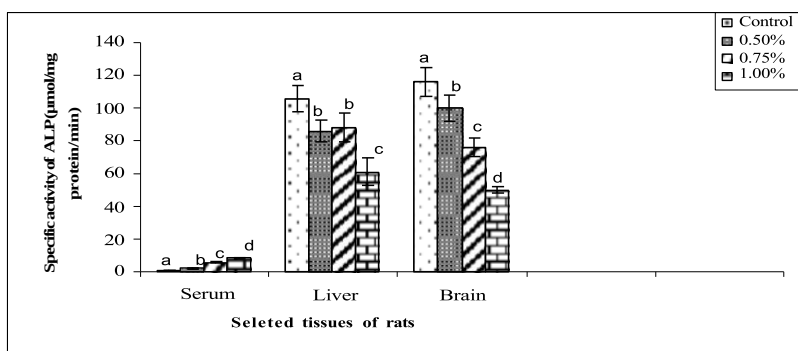


Figure 1. Specific activity of ALP in selected tissues of rats following the administration of galena-based eyeliner for 14 days.
¹Values are means (n=6) $\pm$ S.D. Values with different superscripts a, b, c.... in each bar are significantly different.

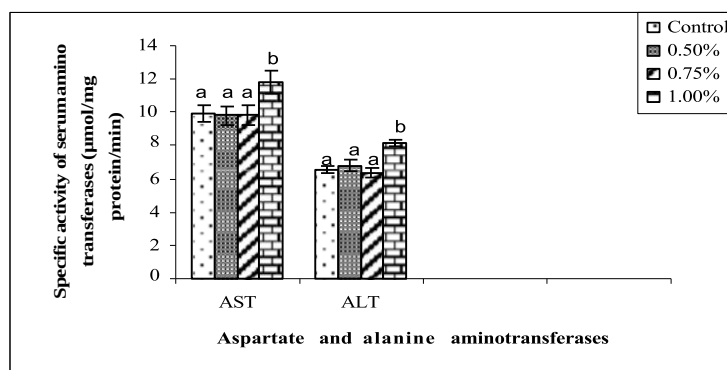


Figure 2. Specific activity of serum aminotransferases following the administration of galena-based eyeliner for 14 days.
 Values are means (n=6) $\pm$ S.D. Values with different superscripts a, b, c.... in each bar are significantly different.

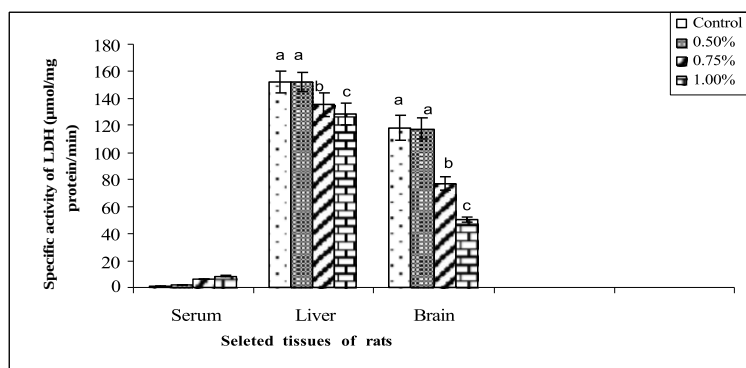


Figure 3. Specific activity of LDH in selected tissues of rats following the administration of galena-based eyeliner for 14 days.
 Values are means (n=6) $\pm$ S.D. Values with different superscripts a, b, c.... in each bar are significantly different.

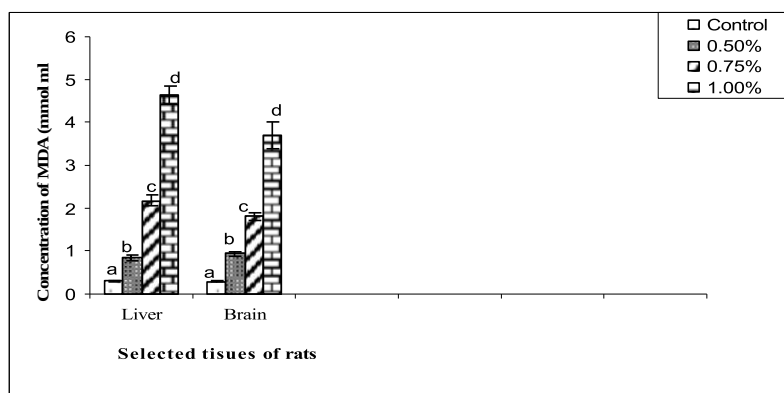


Figure 4. Concentration of MDA in selected tissues of rats following the administration of galena-based eyeliner for 14 days.

Values are means (n=6) $\pm$ S.D. Values with different superscripts a, b, c.... in each bar are significantly different.

of the metals detected have been implicated in carcinogenesis (DeSwart and Shioff, 1987).

Lead and cadmium are recognized as the two most important heavy metal contaminants in the environment (Garcia and Corredor, 2004). Thus, the significant increase ($P < 0.05$) in blood Pb concentration as the concentration of the eyeliner increased and the significant detection ($P < 0.05$) of blood Cd concentrations at 1.0% w/v concentration of eyeliner after 14 days administration may be attributed to systemic delivery of constituents of the eyeliner through the ocular route (Chiou, 1991), which may consequently affect the metabolism of Fe and Zn. Pb appears to produce relative Zn deficiency while Cd mainly affects the distribution of Zn in the body (Peterson and Oskarsson, 2000), because Zn is required for optimum activity of more than 200 enzymes, including those involved in the synthesis and repair of DNA and RNA, the related protein synthesis and tissue repair responses, this may have multiple adverse consequences. Studies of the effect of blood Pb on children's mental development have shown intelligence quotient deficits of an estimated 0.25 points for every $\mu\text{g/dl}$ increment in blood Pb level (Wang *et al.*, 2002). It has been suggested that Pb may have this effect by interfering with the role of calcium in brain cell development (Driscoll *et al.*, 1992).

Alkaline phosphatase has been employed to assess the integrity of plasma membrane and endoplasmic reticulum (Akanji *et al.*, 1993). Result of ALP activity from this study suggests that the integrity of the various membrane systems has been compromised by the administration of the galena-based eyeliner for 14 days. Therefore, the increase in ALT and AST activities of the serum as a result of the ocular administration of 1.00% (w/v) galena-based eyeliner for 14 days may have resulted from leakage into the extracellular fluids. The significant increase ($P < 0.05$) in serum LDH activity and

the corresponding reduction in both the liver and brain LDH activities of the groups administered 0.75% (w/v) and 1.00% (w/v) may also be as a result of plasma membrane derangement by the constituents of the eyeliner leading to excessive leakage of cytosolic materials including LDH into extracellular fluids (Huang *et al.*, 2009). This may be due to possible tissue plasma membrane labilization by the constituents of the eyeliner singly or in combination, inhibition of the enzyme molecule by these trace metals or probably the inactivation of the enzyme molecule *in situ* (Copeland, 2005). Moreover, ALP is a metalloenzyme and zinc dependent, and it has been described that the administration of Cd and Pb or their compounds could induce changes in the metabolism of essential metals like Zn, Fe, and Cu, thereby altering the activity of metal dependent enzymes (Tandor *et al.*, 2001).

Malondialdehyde is a major product of lipid peroxidation. During oxidative stress, MDA and / or other aldehydes are formed in biological systems. These can react with amino acids and DNA to introduce cross linkages between proteins and nucleic acids, resulting in alterations in replication, transcription and leading to tumour formation (Perchellet and Perchellet, 1989). Higher level of MDA suggests a higher degree of lipid peroxidation. The increased level of MDA in the liver and brain of rats administered the eyeliner is suggestive of oxidative stress. This may be ascribed to alteration in the cellular redox status of the animals as a result of increased lipid peroxidation. It may also be that the level of antioxidant enzyme is not sufficient to cope with the level of oxidant influx caused by the eyeliner and its constituent heavy metals which have been reported to interact with membranes, membrane enzymes, disrupt energy production, calcium metabolism, glucose homeostasis and ion transport processes (Tsuruoka *et al.* 2000).

In conclusion, this study provides added information on the consequences of continuous use of galena-based eyeliners by revealing that this facial eyeliner could result into increase in trace metal levels in the blood and cellular system of which Pb and Cd are of much toxicological concern. Also, users and the general public should be strictly cautious on the use of these toxic eyeliners.

REFERENCES

- Ajayi SO, Oladipo MOA, Ogunsuyi HO, Adebayo AO (2002). Determination of the minor and trace elements in Birinwa tin pyrite and ornament lead /zinc ore using neuron activation analysis. *Bull. Chem. Soc. Ethiopia* 16(2): 207-211.
- Akanji MA, Olagoke OA, Oloyede OB (1993). Effect of chronic consumption of metabisulphite on the integrity of the rat kidney cellular system. *Toxicology* 81: 173-179.
- Al-Ashban RM, Aslam M, Shah AH (2004). Kohl (Surma): A toxic traditional eye cosmetic study in Saudi Arabia. *Public Health* 118(4): 292-298.
- Al-Hazza SA, Krahn PM (1995). Kohl: hazardous eyeliner. *Int. Ophthalmol* 19(2): 83-88.
- Antonio MT, Peinado V, Gonzalez JC, Leret ML (2010). Effect of maternal cadmium administration on development of monoaminergics, GABAergic and glutamergic systems. *Environ. Toxicol and Pharmacol* 29 (1): 87-90.
- Chiou GCY (1991). Systemic Delivery of Polypeptide Drugs through traditional Route. *Ann. Rev. Pharmacol. and Toxicol.* 31: 457-467.
- Chukwuma C (1997). Environmental lead exposure in Africa. *Ambio* 26 (6): 399-403.
- Copeland RA (2005). Evaluation of enzymes inhibitors in drug discovery: A guide for medicinal chemists and pharmacologists. Illustrated edition. John Wiley and Sons pp. 271.
- Corpas I, Benito MJ, Castillo M, Lopez, and Antonio MT (2002). Gestational and lactational lead intoxication produces alterations in the hepatic system of rat pups. *Ecotoxicol. Environ. Saf.* 51: 35-43.
- De Zwart D, Shioff WC (1987). Toxicity of mixtures of heavy metals and petrochemicals to *Xenopus laevis*. *Bull. Environ. Contam. Toxicol.* 38: 345-351.
- Derenlanko MJ (2000). Guiding Principles in the use of Animals in Toxicology In: *Toxicologist's Pocket Handbook*. CRC Press Boca Raton, London pp. 1-7.
- Driscoll W, Mushak P, Garfias J, Rothenbery SJ (1992). Reducing lead in gasoline: Mexico's experience. *Environ. Sci. Technol.* 26(9): 1702-1705.
- Funtua II, Oyewale AO (1997). Elemental composition of traditional eye make-up (Kwali) in Nigeria. *J. Chem. Soc. Nig.* 22: 160-163.
- Garcia TA, Corredor L (2004). Biochemical changes in the kidneys after perinatal intoxication with lead and/or cadmium and their antagonistic effects when coadministered. *Ecotoxicol and Environ Safety* 57: 118-189.
- Gornal AG, Bardawill CJ, David MM (1949). Determination of Serum protein by means of Biuret reaction. *J. Biol. Chem* 177: 571-576.
- Hardy AD, Walton R, Vaishnav R (2004). Composition of eye cosmetics (Kohl) used in Cairo. *Int. J. Environ Health Res.* 14: 83-91.
- Hardy AD, Sutherland HH, Vaishnar, R (2002). A study of the composition of some eye cosmetics (Kohl) used in United Arab Emirates. *J. Ethnopharmacol.* 80: 137-145.
- Hardy AD, Vaishnar R, Al-Kharusi SS, Sutherland HH, Worthing MA (1998). Composition of eye cosmetics (Kohl) used in Oman. *J. Ethnopharmacol.* 6(3): 223-234.
- Huang T, Lii C, Chou M, Kao C (2009). Lactate dehydrogenase leakage of hepatocytes with AH26 and AH plus sealer Treatments. *J. Endodont.* 216: 509-511.
- Koller K, Brown T, Spurgeon A, Levy L (2004). Recent development in low level lead exposure and intellectual impairment in children. *Environ Health Perspect* 112 (9): 987-994.
- Kunikowska G, Jenner P (2002). The distribution of copper, zinc- and manganese- superoxide dismutase, and glutathione peroxidase messenger ribonucleic acid in rat based ganglia. *Biochem. Pharmacol.* 63: 1159-1164.
- Lansdown ABG (2001). Iron: A cosmetic constituent but an essential nutrient for healthy skin. *Int. J. Cosm. Sci* 23(3): 129.
- Montgomery DC (1976). Design and analysis of experiment. John Wiley, New York pp. 48-50.
- Narayanan CR, Joshi DD, Mujumbarr AM (1984). Hypoglycaemic activity of *Boungavillea spectrabilis* leaves. *Curr. Sci.* 53: 579-581.
- Nielson UK, Kemp E, Laursen T (1968). Lactic dehydrogenase in kidney and renal disease: adaptive changes of the synthesis in acute renal failure. *Acta. Med. Scand.* 184: 109-115.
- Ogbu SI, Okechukwu EI (2001). The effect of storage temperature prior to separation on plasma and serum potassium. *J. Med. Lab. Sci* 10: 1-4.
- Perchellet JP, Perchellet M (1989). Antioxidant and multistage carcinogenesis in mouse skin. *Free Radical Biol. Med.* 7: 377-408.
- Peterson GK, Oskarsson A (2000). Cadmium in milk and mammary gland in rats and mice. *Arch. Toxicol* 73: 519-527.
- Reitman S, Frankel S (1957). Determination of Serum transaminase. *Am. J. Clin. Path* 28: 56-59.
- Sprinkle RV (1995). Leaded cosmetics: A cultural cause of elevated lead levels in children. *J. Family Practice* 40: 358-362.
- Tandon SK, Singh S, Prasad S, Mathur N (2001). Hepatic and renal metallothionein induction by an oral equimolar dose of zinc, cadmium or mercury in mice. *Food Chem. Toxicol.* 39: 571-577.
- Thevenod F, Fredman, JM (1999). Cadmium mediated oxidative stress in kidney proximal tubule cells induces degradation of Na⁺/K⁺ - ATPase through proteasomal and endo- lysosomal proteolytic pathways. *FASEB J.* 13: 1751-1761.
- Timms PM, Bold AM (2000). Alternative medicine elevate blood leads in Omani children referred for extensive investigation. *J. Trop. Paediatr* 46: 245-246.
- Tsuruoka S, Sugimoto K, Muto S, Nomiya K, Fujimura A, Imai M (2000). Acute effect of cadmium-metallothionein on glucose and amino acid transport across the apical membrane of the rabbit proximal tubule perfused in vitro. *J. Pharmacol. Exp. Ther.* 292(2): 769-777.
- Varshney R, Kale RK (1990). Effect of calmodulin antagonist on radiation induced lipid peroxidation in microsome. *Int. J. Rad. Biol* (58): 733-743.
- Wright PJ, Leathwood PD, Plummer DT (1972). Enzymes in rat urine. Alkaline phosphatase. *Enzymologia* (42): 317-327.