Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(1): 89-94

Received: 12-11-2016
Accepted: 13-12-2016

Rajesh Prasad
Environmental Toxicology & Bioremediation Laboratory (ETBL), Department of Zoology, University of Lucknow, Lucknow, India

Manoj Kumar
Environmental Toxicology & Bioremediation Laboratory (ETBL), Department of Zoology, University of Lucknow, Lucknow, India

Sunil P Trivedi
Environmental Toxicology & Bioremediation Laboratory (ETBL), Department of Zoology, University of Lucknow, Lucknow, India

Correspondence
Sunil P Trivedi
Environmental Toxicology & Bioremediation Laboratory (ETBL), Department of Zoology, University of Lucknow, Lucknow, India

Antigenotoxic effect of turmeric powder extract curcumin against chromium trioxide induced genotoxicity in fish *Channa punctatus*

Rajesh Prasad, Manoj Kumar and Sunil P Trivedi

Abstract
Curcumin is a yellow colour main polyphenolic compound which is isolated from dry rhizome of the plant *Curcuma longa* (family Zingiberaceae). The present study was designed to evaluate the antigenotoxicity activity of curcumin against chromium induced micronuclei induction in fish *Channa punctatus*. After 15 days acclimatization, the fish were divided in six groups. In group III the induction of micronuclei increased significantly ($p<0.05$), when this group was exposed with only $LC_{50}/10$ (7.89mg/l) of chromium in comparison to group I for 24hr, 48hr, 72hr and 96hr. In groups IV, V and VI the increased induction of micronuclei were found reduced after simultaneous exposure of curcumin along with chromium in comparison to group I and group II. The outcome of this study shows that curcumin is a good antigenotoxic agent against chromium in time and dose dependent manner for fish *C. punctatus*. The finding of our study is also useful for aquaculture because curcumin is an excellent genoprotective agent against chromium toxicity.

Keywords: Chromium, curcumin, micronuclei, *C. punctatus*

1. Introduction
Chromium (Cr) is one of the most abundant heavy metal in the earth's crust and it is widely used by human in different manufacturing industries, mostly in leather tanning and wood treatment, it enters the aquatic environment through effluents discharged from printing industries, dyeing, electroplating, textiles, tanneries, pharmaceutical industries and photographic industries. In surface water naturally chromium content occurs 1-10 µg/l [1]. In the environment, mostly chromium is found in two valence states; Cr (III) and Cr (VI) out of which Cr (VI) is a highly toxic form because it rapidly crosses cell membrane and reduced to Cr (III) which complexes with intracellular macromolecules, genetic material and is ultimately responsible for the toxic and mutagenic capacities of chromium [2, 3, 4]. The fish is most suitable bioindicators because they occupy high trophic level in aquatic food chains [5]. For study of genotoxicity, fishes are mostly used as important genetic model due to aquatic pollution [6], because they are highly sensitive to minor alteration in their habitat [7, 8]. In present investigation fresh water fish *C. punctatus*, (Order-Perciformes, Family-Channidae) commonly known as Spotted Snakehead was selected because it has many important ecotoxicological characteristics such as wide distribution, easily available throughout year, easy handle and maintain in laboratory, survive at room temperature and commercial importance make this fish an excellent model for study of environmental toxicology [9]. Toxic effects of Cr (VI) induced DNA-protein cross-links resulting in genotoxic effects and also associated with intensification of free radical processes in aquatic organism. Many researchers have reported that due to the accumulation of chromium and its compounds lead to DNA damage through DNA single and double-strand breaks resulting in micronuclei, sister chromatids exchanges, chromosomal aberrations and DNA adduct formation as well as alterations in DNA replication & transcription [10, 11]. Many methods were used to study the genotoxicity in Fish like Micronuclei assay, CAT and Comet assay. Among all the assays, micronuclei assay has been broadly used for detection of aneugenic and clastogenic effects of harmful chemicals because it is formed at the cell division in anaphase stage when whole chromosome or fragment of chromosome breaks due to the lack of centromere. At present, interest is being given to the traditional India ethnomedicine due to their perceived fewer side effects, low price, easy and accessible to everyone [12]. Among these ethnomedicine *Curcuma longa* (curcuma or turmeric) is a herbaceous, monocotyledonous, rhizomatous, perennial plant which belongs to the ginger

Family (Zingiberaceae) and widely cultivated in tropical and subtropical regions of China, India, and South East Asia [13]. At present nearly all over the world including India, turmeric is mostly used in food as dietary spice, coloring agent and in food industry as additive, flavoring and preservative [14, 15]. The most active major photochemical of turmeric is curcumin which is an orange yellow crystalline powder, insoluble in water [16] and have multiple pharmacological properties such as antioxidant [17, 18, 19, 20], antimicrobial [21, 22], anti-inflammatory [23, 24], antiviral [25, 26], and anti-carcinogenic [27, 28, 29, 30, 31, 32]. Due to the antioxidant properties of curcumin *in vivo* exposure studies does not suggest genotoxicity or clastogenicity, investigated on the basis of micronuclei [33], chromosome aberrations [34, 35], and recombination assays [36]. Corona-Rivera *et al* [37] reported that *in vivo* study shows protective effect of curcumin against copper which induce genotoxicity in terms of micronuclei assay and comet in Balb-C mice. In human cultured lymphocyte cell Shafaghati *et al* [38] also reported protective effect of curcumin against 131-iodine induced micronuclei induction. Therefore, in the present study an attempt was made to explore mitigating potential of curcumin against chromium induced alterations in freshwater teleostean fish, *C. punctatus* in terms of Micronucleus assay to evaluate cytogenetic damage.

2. Materials and methods

2.1 Experimental animals and acclimatization: In the present investigation Live and healthy specimens of a common pond Murrel fresh water fish, *C. punctatus* (10-12 cm in length and 40-50gm an average weight) family: Channidae, were purchased from the local fish market and transported to the laboratory in the appropriately aerated plastic tank. The specimens were transferred in a big glass aquarium and wash with tap water followed by with 0.05% potassium permanganate ($KMnO_4$) for 2 minutes to avoid dermal infection. The specimens were acclimatized to the laboratory conditions for 15 days, before they were used for experiments. During acclimatization, fish were maintained in all optimal conditions, according to APHA *et al* [39]. The fish were fed with highly nutritious aquarium food (OPTIMUM, produced & transported by Bangsaothong district, Thailand, its nutritional combination is 3% crude fat, 4% crude fiber, 10% moisture max and 28% crude protein), minced Goat liver and boiled egg. The fecal matter and other waste materials were siphoned out daily from aquaria to reduce the content of ammonia in the water.

2.2 Test chemicals: Chromium trioxide Batch NO: T-8371997, Mfg. Date: 03/2013, were purchased from Sisco Research Laboratories Pvt. Ltd. and Giemsa Batch No 39382/01 was obtained from Qualigens Fine Chemicals of Glaxo India Limited (Mumbai). Curcumin Batch No: 5883165, Mfg. Date: 01/2016 was also purchased from SRL. All other used chemical were of analytical grade.

2.3 Determination of LC_{50} of Chromium trioxide: The value of 96 h-LC_{50}/10 of chromium trioxide was determined by using Trimmed Spearman-Karber Method [40].

2.4 Experimental design: After 15 days, acclimatized fish were divided into six groups having 12 fishes in each group. The I group serves as control without any treatment, group II and group III were treated separately with polyphenolic compound curcumin (3mg/l) and sub lethal test concentration of chromium trioxide (LC_{50}/10, 7.89mg/l) for 24hr, 48hr, 72hr and 96hr respectively. Groups IV, V and VI fishes were treated with sub lethal test concentrations of LC_{50}/10 of chromium trioxide (7.89mg/l) along with three different concentrations of curcumin 1mg/l/, 2mg/l, and 3mg/l for 24hr, 48hr, 72hr and 96hr. After stipulated duration, three fishes of each treated group and control were utilized on each sampling day for Micronuclei assay**.**

2.5 Micronuclei assay: On each sampling day peripheral blood sample was taken with 1 ml heparinized syringe by heart puncture and immediately make a thin smear on pre-cleaned slide with the help of a clean glass slide. After fixation in absolute methanol for 5-10 min, the slides were air dried for at least1hr at room temperature. After air drying the slides were stained with May-Grunewald's solution I & II for 3-6 min and washed with D.D water and dried. Finally, slides were stained with 6-10% Giemsa stain in phosphate buffer for 30 min and thoroughly washed with D.D water for removing all Giemsa particles. Then the slides are air dried overnight at room temperature in a dust and moisture free environment. For preparation of permanent slide, the slides are mounted with DPX and air dire over a hot plate at 60 ^{0}C for overnight.

2.6 Scoring of micronuclei: Overnight air dried permanent slides mounted with DPX are observed under oil immersion microscope (Nikon Corporation K 12432) using eyepiece 40X and objective lens of 100X. At the time of micronuclei scoring under the microscope a drop of immersion oil was used and micronuclei were identified according to the criteria described by Fenech *et al* [41]. Minimum 1000 cells were scored for each specimen slide. Small, ovoid or circular, non-refractive, not exceeding 1/3 part of the main nucleus diameter, chromatin bodies clearly separate from main nucleus, same color as the main nucleus were examined as micronucleus [42].

2.7 Calculation of scoring micronuclei

$$\text{MNi (\%)} = \frac{\text{No. of observed cells with micronucleus}}{\text{Total no. of observed cells}} \times 100$$

3. Result

The observed frequency of MN induction in erythrocytes of fresh water fish *C. punctatus* after exposure of sub lethal test concentrations of chromium trioxide (LC_{50}/10; 7.89mg/l) along with three concentrations of curcumin for 24hr, 48hr, 72hr and 96hr are summarized in given Table 1. Therefore in comparison to control the frequency of micronuclei induction is significantly increased (p>0.05) in group III treated with sub lethal test concentration of chromium (LC_{50}/10, 7.89mg/l) after all exposed periods. While the reduced frequency of micronuclei induction was observed in simultaneous exposed with curcumin alongwith chromium (LC_{50}/10, 7.89 mg/l) in group IV, V and VI (1mg/l, 2mg/l and 3mg/l) for 24hr, 48hr, 72hr and 96hr are presented in Table 1. However, fishes of group II exposed with highest selected dose of curcumin, does not induced any genotoxic effect itself. Thus, the selected dose of curcumin is safe against chromium trioxide for fish *C. punctatus*. The increased frequency of micronuclei induction, induced by chromium trioxide was noted as 1.15±0.09, 1.29±0.05, 1.43±0.12, and 1.66±0.17 after 24hr, 48hr, 72hr and 96hr, respectively. In comparison to control, in group II (Cur, 3mg/l), observed frequency of micronuclei was found 0.25±0.20, 0.28±0.25, 0.29±0.20 and 0.29±0.1 after 24hr, 48hr, 72hr, and 96hr and respectively. However, in comparison to alone exposed group III (LC_{50}/10 of Cr,

7.89mg/l), frequency of micronuclei was found reduced in simultaneously exposed with curcumin (1mg/l, 2mg/l and 3mg/l) in group IV, V and VI after 24hr-96hr. The reduced micronuclei profile in group IV, V and VI (1mg/l, 2mg/l and 3mg/l) with chromium trioxide were recorded as 0.83±0.11, 0.76±0.05, 0.73±0.05 (24hr), 1.02±0.22, 0.96±0.19, 0.93±0.05, (48hr), 1.26±0.26, 1.12±0.14, 1.06±0.10 (72hr) and 0.83±0.11, 1.32±0.06, 1.21±0.11 at (96hr) respectively. In our result the maximum frequency of MN induction was observed after 96hr (1.66±0.17) in group III and minimum frequency after 24hr (1.15+0.09). Thus, the result of present finding showed that the main polyphenolic compound of *C. longa*, curcumin is an excellent antigenotoxic agent against chromium trioxide which effectively reduced the toxicity of chromium in fish *C. punctatus*.

Table 1: Induction of Micronuclei frequency in peripheral erythrocytes of fresh water fish *C. punctatus* after exposure of Chromium with Turmeric Powder Extract Curcumin.

Experiment-al Groups	Concentration (mg/l)	Exposure Days	Total No of Count Cells	Total No of Cells Count With Micronuclei	Frequency of MN Mean(%)+S.D
Group I	Control	24	3024	8	0.26±0.06
		48	3023	9	0.29±0.10
		72	3018	9	0.29±0.17
		96	3010	9	0.29±0.26
Group II	Curcumin (3mg/l)	24	3063	8	0.25±0.20
		48	3057	9	0.28±0.25
		72	3033	9	0.29±0.20
		96	3036	9	0.29±0.17
Group III	96 h-LC_{50}/10 of CTO (7.89mg/l)	24	3104	36	1.15±0.09
		48	3071	40	1.29±0.05
		72	3071	44	1.43±0.12
		96	3061	51	1.66±0.17
Group IV	96 h-LC_{50}/10 (7.89mg/l) of CTO +1mg/l Curcumin	24	3091	26	0.83±0.11
		48	3096	32	1.02±0.22
		72	3074	39	1.26±0.26
		96	3080	46	1.49±0.25
Group V	96 h-LC_{50}/10 (7.89mg/l) of CTO+2mg/l Curcumin	24	3116	24	0.76±0.05
		48	3093	30	0.96±0.19
		72	3097	36	1.12±0.14
		96	3092	41	1.32±0.06
Group VI	96 h-LC_{50}/10 (7.89mg/l) of CTO+3mg/l of Curcumin	24	3108	23	0.73±0.05
		48	3110	28	0.93±0.05
		72	3102	35	1.06±0.10
		96	3115	38	1.21±0.11

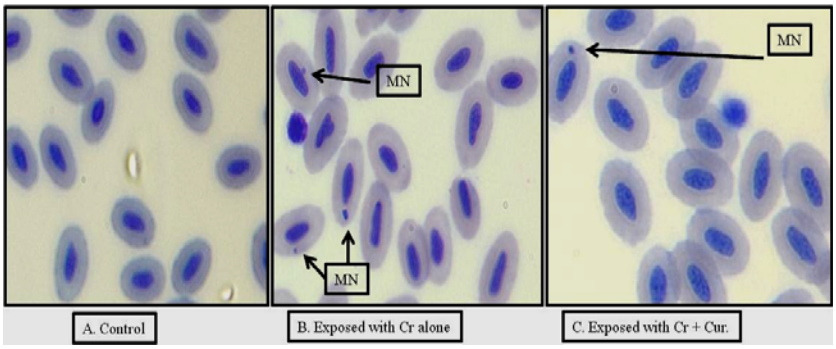

Fig 1: Shows genetic effect of chromium trioxide in erythrocytes of fish *C. punctatus*: A, Control, B, Increased frequency of micronucleus after exposure of highest alone dose of chromium trioxide C, Reduced frequency of micronucleus after exposure of alone dose of Turmeric Powder Extract Curcumin.

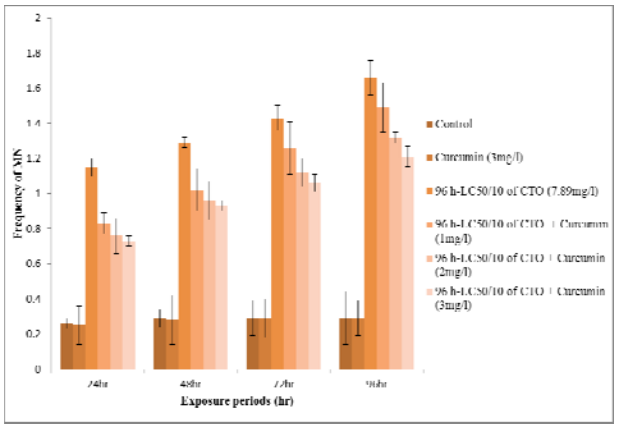

Fig 2: The frequency of Micronuclei induction induced by Chromium trioxide in erythrocytes of fresh water fish *C. punctatus* at different exposure period.

4. Discussion

In our environment, aquatic ecosystem is the major sink of industrial and agricultural pollutants, including those from vehicle traffic emissions and discharges of different heavy metals from different industrial areas is the major cause of aquatic pollution and their toxic role was reported a few hundred years ago. In respect to the genotoxicity mechanism of different heavy metals, there is some controversy [43]. In aquatic organisms, the role of these heavy metals may be related to the accumulation of free DNA-damaging radicals, aneugenic actions and clastogenic processor simultaneously to clastogenic [44, 45]. Among these heavy metals chromium is also responsible for inducing frequency of micronuclei induction in time and dose dependent manner in fishes and other vertebrates. In the present investigation fresh water fish, *C. punctatus* was exposed to alone sub lethal test concentration of chromium trioxide (LC_{50}/10; 7.89mg/l) for 24hr, 48hr, 72hr and 96hr. The highest frequencies of micronuclei induction were recorded in peripheral erythrocytes of fish after 96hr (1.66+0.17) in group III and lowest after 24hr (1.15+0.09). Thus, the present study shows that chromium is responsible for inducing micronuclei formation in fish erythrocyte of *C. punctatus*. In same experimental model Yadav and Trivedi [46] also reported that the number of micronuclei frequencies increased after exposure of heavy metal at 96 h. Similarly, work also reported by de Lemos *et al* [47] that the number of micronuclei decreased after exposure of chromium at 21 days. Therefore, in another study, the genotoxic activity of chromium was observed in cell lines of Medaka fin assessing chromosome damage and double strand of DNA breaks in a dose dependent manner [48]. This work is also supported by Ahmed *et al* [49] in term of micronuclei induction in freshwater fish stinging catfish, *H. fossilis* after exposure of different test concentrations of Cr (VI) at different time duration. Curcumin is a yellow, natural, insoluble in water, lipid-soluble, hydrophobic, polyphenol, phytochemical which is extracted from *C. longa* with no discernible toxicity for any organism [50]. Somparn *et al* [51] reported its antioxidant properties and its protective nature for tissues. Curcumin also act as a scavenger of oxygen free radicals and endogenous antioxidant enzymes such as glutathione transferase, superoxide dismutase, glutathione peroxidase and catalase [52]. For a longer time in Chinese medicine and Asian medicines it has been used as a therapeutic agent and dietary supplement by peoples in all over world [53]. In the present study when experimental group IV, V and VI were exposed with chromium trioxide (LC_{50}/10; 7.89mg/l) and simultaneous exposed with curcumin (1mg/l, 2mg/l, and 3mg/l) for 24hr, 48hr, 72hr and 96hr. The frequencies of micronuclei induction were recorded minimum in comparison to alone exposed group III with sub lethal test concentration of chromium trioxide (LC_{50}/10; 7.89mg/l) in time dependent manner. The antioxidant properties of curcumin such as hepato protection, cardiac protection, and anti-inflammatory protection has been also observed by Naik *et al* [54] and Prakobwong *et al* [55]**.** Same protective effect of curcumin in bone marrow cells of Albino Rates also reported by Bishnoi *et al* [56], Ali *et al* [57], in terms of chromosomal aberration and micronuclei assay after exposed with fried potatoes chips and roasted bread in dose and time dependent manner. In another study, Corona-Rivera *et al* [58] also reported the antioxidant properties of curcumin in dose dependent manner against copper induced genotoxicity in 4-6- week-old male Balb-C mice in term of micronuclei and comet. Curcumin is also reported in addition to anticancerous potential, anti-mutagenic agent, anti-inflammatory and as a good antioxidant agent. *In vivo* and *in vitro* condition, curcumin is act as anticlastogenic agent by Anto *et al* [59]**.** According to Polasa *et al* [60] curcumin shows protective effect in lymphocyte cells of human blood against B (a) P induced DNA damage. However, when bone marrow cells of mice acutely treated with curcumin shows weakly clastogenic [61]. So, in dose dependent manner curcumin shows both effects as genotoxicity and antigenotoxicity in HepG2 cells [62].

5. Conclusion

The present investigation indicates that curcumin is highly antioxidant compound of *C. longa*. It reduced the increased frequency of micronuclei induction in peripheral erythrocytes of fish *C. punctatus* against chromium trioxide after different exposure period. Its ameliorative effect may be beneficial for aquaculture because it act as antigenotoxic agent against chromium discharge effluents in water.

6. Acknowledgements

The present research work was financially supported by UGC-BSR (Award no. F- 25-1/2013-14 (BSR)/7-109/2007), authors gratefully acknowledge the UGC, New Delhi for financial assistance. The authors are also thankful to, Department of Zoology for providing central instrumentation facility developed under UGC-SAP, DST-FIST, and DST-PURSE.

7. References

1. World Health Organization. Background document for preparation of WHO Guidelines for drinking water quality, 2003.
2. Farag AM, May T, Marty GD, Easton M, Harper DD, Little EE *et al*. The effect of chronic chromium exposure on the health of Chinook salmon (*Oncorhynchus tshawytscha*). Aquat Toxicol. 2006; 76(3-4):57-246.
3. Li ZH, Zlabek V, Grabic R, Li P, Machova J, Velisek J *et al*. Effects of exposure to sublethal propiconazole on the antioxidant defense system and Na+–K+-ATPase activity in brain of rainbow trout, *Oncorhynchus mykiss*. Aquat Toxicol. 2010; 98(3):297-303.
4. Velma V, Tchounwou PB. Hexavalent chromium-induced multiple biomarker responses in liver and kidney of goldfish, *Carassius auratus*. Environ Toxicol. 2011; 26(6):56-649.
5. Abdel-Baki, Dkhil MA, Quraishy A. Bioaccumulation of some heavy metals in tilapia fish relevant to their concentration in water and sediment of Wadi Hanifah, Saudi Arabia. African J Biotechnol. 2011; 10(13):2541-2547.
6. Nagarani Nagarajan, Devi Velmurugan Janaki, Devi Chandrasekaran Archana, Kuppusamy Kumaraguru A. Genotoxiciy Assessment of Mercuric chloride in the Marine Fish *Therapon jarbua*. Environ Asia. 2009; 2(2):50-54.
7. Yadav KK, Trivedi SP. Evaluation of genotoxic potential of chromium (VI) in *Channa punctatus* fish in terms of chromosomal aberrations. Asian Pacific J Cancer Prev. 2006; 7(3):472-476.
8. Yadav KK, Trivedi SP. Sublethal exposure of heavy metals induces micronuclei in fish, *Channa punctatus*. Chemospher. 2009; 77(11):500-1495.
9. Kumar R, Nagpure NS, Kushwaha B, Srivastava SK, Lakra WS. Investigation of the genotoxicity of Malathion

to freshwater teleost fish *Channa punctatus* (Bloch) using the micronucleus test and comet assay. Arch Environ Contam Toxicol. 2010; 58(1):123-130.
10. Zhitkovich A, Voitkun V, Costa M. Formation of the Amino Acid−DNA Complexes by Hexavalent and Trivalent Chromium *in Vitro* Importance of Trivalent Chromium and the Phosphate Group. Biochemist. 1996; 35(22):7275-7282.
11. Matsumoto ST, Marin Morales MA. Mutagenic Potential Evaluation of the Water of a River That Receives Tannery Effluent Using the *Allium cepa* Test System. Cytologia (Tokyo). 2004; 69(4):399-408.
12. Rathee JS, Patro BS, Mula S, Gamre S, Chattopadhyay S. Antioxidant activity of *piper betel* leaf extract and its constituents. J Agric Food Chem. 2006; 54(24):54-9046.
13. Santhanam G, Nagarajan S. Wound healing activity of *Curcuma aromatica* and *Piper betle*. Fitoter. 1990; 61(5):458-459.
14. FAO. Chemical and Technical Assessment. 61st JECFA, Food and Agriculture Organization. 2004. http://www.fao.org/fileadmin/templates/agns/pdf/jecfa/cta/61/Curcumin. Pdf.
15. Basnet P, Skalko Basnet N. Curcumin An Anti-Inflammatory Molecule from a Curry Spice on the Path to Cancer Treatment. Molecul. 2011; 16(6):4567-4598.
16. Sandur SK, Pandey MK, Sung B, Ahn KS, Murakami A, Sethi G *et al*. Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-independent mechanism. Carcinogenesis. 2007; 28(8):1765-1773.
17. Iqbal M, Sharma SD, Okazaki Y, Fujisawa M, Okada S. Dietary Supplementation of Curcumin Enhances Antioxidant and Phase II Metabolizing Enzymes in ddY Male Mice: Possible Role in Protection against Chemical Carcinogenesis and Toxicity. Pharmacol Toxicol. 2003; 92(1):33-38.
18. Surh YJ. Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer. 2003; 3(10):768-780.
19. Dairam A, Fogel R, Daya S, Limson JL. Antioxidant and Iron-Binding Properties of Curcumin, Capsaicin, and *S* -Allylcysteine Reduce Oxidative Stress in Rat Brain Homogenate. J Agric Food Chem. 2008; 56(9):3350-3356.
20. Al Sabti K, Metcalfe CD. Fish micronuclei for assessing genotoxicity in water. Mutat Res Toxicol. 1995; 343(2-3):121-135.
21. Çıkrıkçı S, Mozioğlu E, Yılmaz H. Biological Activity of Curcuminoids Isolated from *Curcuma longa*. Rec Nat Prod. 2008; 2(1):19-24.
22. Tajbakhsh S, Mohammadi K, Deilami I, Zandi K, Fouladvand M, Ramedani E *et al*. Antibacterial activity of indium curcumin and indium diacetylcurcumin. African J Biotechnol. 2008; 7(21):3832-3835.
23. Jurenka JS. Anti-inflammatory properties of Curcumin, a majorconstituent of *Curcuma longa*: a review of preclinical and clinicalresearch. Altern Med Rev. 2009; 14(2):141-153.
24. Bereswill S, Munoz M, Fischer A, Plickert R, Haag LM, Otto B *et al*. Anti-inflammatory effects of resveratrol, curcumin and simvastatin in acute small intestinal inflammation. PLoS One. 2010; 5(12):15099.
25. Barthelemy S, Vergnes L, Moynier M, Guyot D, Labidalle S, Bahraoui E. Curcumin and curcumin derivatives inhibit Tat-mediated transactivation of type 1 human immunodeficiency virus long terminal repeat. Res Virol. 1998; 149(1):43-52.
26. Kutluay SB, Doroghazi J, Roemer ME, Triezenberg SJ. Curcumin inhibits herpes simplex virus immediate-early gene expression by a mechanism independent of p300/CBP histone acetyltransferase activity. Virology. 2008; 373(3):239-247.
27. Aggarwal B, Kumar A, Bharti A. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer res. 2003; 23(1A):98-363.
28. Aggarwal BB, Ichikawa H, Garodia P, Weerasinghe P, Sethi G, Bhatt ID *et al*. From traditional Ayurvedic medicine to modern medicine: identification of therapeutic targets for suppression of inflammation and cancer. Expert Opin Ther Targets. 2006; 10(1):87118.
29. Wang L, Shen Y, Song R, Sun Y, Xu J, Xu Q. An Anticancer Effect of Curcumin Mediated by Down Regulating Phosphatase of Regenerating Liver-3 Expression on Highly Metastatic Melanoma Cells. Mol Pharmacol. 2009; 76(6):1238-1245.
30. Youns M, Hoheisel JD, Efferth T. Traditional Chinese medicines (TCMs) for molecular targeted therapies of tumours. Curr Drug Discov Technol. 2010; 7(1):37-45.
31. Das L, Vinayak M. Anti-carcinogenic action of curcumin by activation of antioxidant defence system and inhibition of NF-κB signalling in lymphoma-bearing mice. Biosci Rep. 2012; 32(2):161-170.
32. Huang YC, Chao KSC, Liao HF, Chen, YJ. Targeting Sonic Hedgehog Signaling by Compounds and Derivatives from Natural Products. Evidence-Based Complement. Altern Med. 2013, 1-5.
33. Vijayalaxmi. Genetic effects of turmeric and curcumin in mice and rats. Mutat Res Toxicol. 1980; 79(2):125-132.
34. Mukhopadhyay MJ, Saha A, Mukherjee A. Studies on the anticlastogenic effect of turmeric and curcumin on cyclophosphamide and mitomycin C *in Vivo*. Food Chem Toxicol. 1998; 36(1):73-76.
35. Shukla Y, Arora A, Taneja P. Antigenotoxic potential of certain dietary constituents. Teratog Carcinog Mutagen. 2003, 35-323.
36. El Hamss R, Analla Campos M, Sanchez J, Alonso Moraga A, Munoz Serrano A, Idaomar MA. Dose dependent antigenotoxic effect of turmeric. Mutat Res. 1999; 446(1):9-135.
37. Corona Rivera A, Urbina Cano P, Bobadilla Morales L, Vargas Lares JDJ, Ramirez Herrera MA, Mendoza Magaua ML, *et al*. Protective *in vivo* effect of curcumin on copper genotoxicity evaluated by comet and micronucleus assays. J Appl Genet. 2007; 48(4):389-396.
38. Shafaghati N, Hedayati M, Hosseinimehr SJ. Protective effects of curcumin against genotoxicity induced by 131-iodine in human cultured lymphocyte cells. Pharmacogn Mag. 2014; 10(38):10-106.
39. American Public Health Association. Standard methods for the examination of water and waste water. 22th ed, American Public Health Association, New York, 2012.
40. Hamilton MA, Russo RC, Thurston RV. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol. 1977; 11(7):714-719
41. Fenech M, Kirsch Volders, Natarajan M, Surralles AT, Crott J, Parry JW *et al*. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis. 2011; 26(1):125-132.

42. Al Sabti K, Metcalfe CD. Fish micronuclei for assessing genotoxicity in water. Mutat Res Toxicol. 1995; 343(2-3):121-135.
43. Russo C, Rocco L, Morescalchi MA, Stingo V. Assessment of environmental stress by the micronucleus test and the Comet assay on the genome of teleost populations from two natural environments. Ecotoxicol Environ Saf. 2004; 57(2):168-174.
44. Ariza ME, Williams MV. Mutagenesis of AS52 cells by low concentrations of lead (II) and mercury (II). Environ Mol Mutagen. 1996; 27(1):30-33.
45. Nepomuceno JC, Ferrari I, Spanó MA, Centeno AJ. Detection of micronuclei in peripheral erythrocytes of Cyprinus carpio exposed to metallic mercury. Environ Mol Mutagen. 1997; 30(3):7-293.
46. Yadav KK, Trivedi SP. Sublethal exposure of heavy metals induces micronuclei in fish, *Channa punctatus*. Chemospher. 2009; 77(11):500-1495.
47. De Lemos CT, Rödel PM, Terra NR, Erdtmann B. Evaluation of basal micronucleus frequency and hexavalent chromium effects in fish erythrocytes. Environ Toxicol Chem. 2001; 20(6):1320-1324.
48. Goodale BC, Walter R, Pelsue SR, Thompson WD, Wise SS, Winn RN *et al*. The cytotoxicity and genotoxicity of hexavalent chromium in medaka (Oryzias latipes) cells. Aquat Toxicol. 2008; 87(1):60-67.
49. Ahmed MK, Kundu GK, Al Mamun MH, Sarkar SK, Akter MS, Khan MS *et al*. Chromium (VI) induced acute toxicity and genotoxicity in freshwater stinging catfish, *Heteropneustes fossilis*. Ecotoxicol Environ Saf. 2013; 92:64-70.
50. Aggarwal B, Bhatt l, lchikawa H, Ahn K, Sandur S, Natarajan C *et al*. Curcumin biological and medicinal properties. Turmeric genus Curcuma: Med Aromat plants industrial profiles CRC Press United States Arn. 2007, 297-368.
51. Somparn P, Phisalaphong C, Nakornchai S, Unchern S, Morales NP. Comparative antioxidant activities of curcumin and its demethoxy and hydrogenated derivatives. Biol Pharm Bull. 2007; 30(1):8-74.
52. Sivalingam N, Basivireddy J, Balasubramanian KA, Jacob M. Curcumin attenuates indomethacin induced oxidative stress and mitochondrial dysfunction. Arch Toxicol. 2008; 82(7):81-471.
53. Itokawa H, Shi Q, Akiyama T, Morris Natschke SL, Lee KH. Recent advances in the investigation of curcuminoids. Chin Med. 2008; 3(11):1-13.
54. Naik SR, Thakare VN, Patil SR. Protective effect of curcumin on experimentally induced inflammation, hepatotoxicity and cardiotoxicity in rats: Evidence of its antioxidant property. Exp Toxicol Pathol. 2011; 63(5):419-431.
55. Prakobwong S, Gupta SC, Kim JH, Sung B, Pinlaor P, Hiraku Y *et al*. Curcumin suppresses proliferation and induces apoptosis in human biliary cancer cells through modulation of multiple cell signaling pathways. Carcinogenesis. 2011; 32(9):80-1372.
56. Bishnoi M, Chopra K, Kulkarni SK. Protective effect of Curcumin, the active principle of turmeric (*Curcuma longa*) in haloperidol-induced orofacial dyskinesia and associated behavioural, biochemical and neurochemical changes in rat brain. Pharmacol Biochem Behav. 2008; 88(4):511-522.
57. Ali RA, Sayed NH, Abd El, Monem DD. Curcumin Reduced Potato Chips and Roasted Bread Induced Chromosomal Aberrations and Micronuclei Formation in Albino Rats. Life Sci J. 2012; 9(1):330-336.
58. Corona Rivera A, Urbina Cano P, Bobadilla Morales L, Vargas Lares JDJ, Ramirez Herrera MA, Mendoza Magaua ML *et al*. Protective *in vivo* effect of curcumin on copper genotoxicity evaluated by comet and micronucleus assays. J Appl Genet. 2007; 48(4):389-396.
59. Anto RJ, George Babu J, Rajasekharan KVD, Kuttan RKN. Antimutagenic and anticarcinogenic activity of natural and synthetic curcuminoids. Mutat Res Toxicol. 1996; 370(2):127-131.
60. Polasa K, Naidu AN, Ravindranath I, Krishnaswamy K. Inhibition of B (a) P induced strand breaks in presence of curcumin. Mutat Res Toxicol. Environ Mutagen. 2004; 557(2):203-213.
61. Mukhopadhyay MJ, Saha A, Mukherjee A. Studies on the anticlastogenic effect of turmeric and curcumin on cyclophosphamide and mitomycin C *in vivo*. Food Chem Toxicol. 1998; 36(1):73-76.
62. Cao J, Jiang LP, Liu Y, Yang G, Yao XF, Zhong LF. Curcumin-induced genotoxicity and antigenotoxicity in HepG2 cells. Toxicon. 2007; 49(8):1219-1222.