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NATURAL CURCUMIN PREVENTS CYPERMETHRIN INDUCED TOXICITY IN FISH VIA P53 PROTEIN MODULATION.

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ABSTRACT

Because of agricultural practices and disposal of industrial wastes, exposure to pesticides in the aquatic environment has become a serious health concern for non-target organisms especially fishes and fish-consuming human population. Phyto-based agents as possible protectors are preferred, mostly because of their otherwise non-toxic nature and easy availability. Curcumin (CUR), the test candidate, is one among them which is known for its anti-inflammatory, anti-oxidant, anti-genotoxic and anti-carcinogenic properties. Ethanolic extraction of CUR from turmeric rhizome by standard column chromatographic procedure and chemically confirmed by mass, NMR and FTIR spectral analyses, was pre-introduced in *Oreochromis mossambica* and in mammalian L6 muscle cell line, for evaluating its possible protective efficacy against toxicity of the commonly used pesticide, cypermethrin. Studies were conducted utilizing several suitable parameters like cellular-cytotoxicity, histopathology of liver, kidney, spleen and gill tissue, ROS generation and accumulation of lipid droplets in hepatic tissue, p53 protein expression and DNA damage. CUR was found to restrict ROS generation which inhibited deposition of lipid droplets in hepatic tissue and elevated pre-activation of p53 to combating the DNA damage, thereby preventing perturbation of morphology and physiology of cells and tissues. This result is direct evidence of prevention against toxicity imparted by pesticides, thereby implicating the possibility of use of CUR in designing protective drugs for both fish and human to combat against pesticide-induced toxicity.

INTRODUCTION

Agricultural practices and disposal of industrial wastes make major contributions to aquatic pollution. Chemical pesticides are often used in agricultural fields to control pests to increase the yield which are washed off from the fields and enter the ecosystem via water bodies, affecting almost all forms of organisms inhabiting different ecological niches. Further, some of the synthetic pyrethroids bring forth severe health hazards even in humans due to their lipophilic nature and lingering toxic impact in the environment [1]. Long-term exposure to pyrethroids is known to be associated with behavioural changes, impaired immunity, infertility and problems in proper brain functioning [2-3]. Pesticides are reported to

reach the human body through the food chain, which leads to serious health hazards [4-5]. Synthetic pyrethroids such as cypermethrin (CM) may easily get bio-accumulated in fish and other aquatic organisms that are exposed to them [6-7]. Thereafter, consumption of such fish can lead to an accumulation of the same in the human body, thereby causing life-threatening diseases in the human race [8-10]. Pesticide-induced toxic effects have therefore crossed their threshold limit in the ecosystem realm, which urges us to search for an immediate therapeutic protocol to combat against pesticide-induced toxic effects in fish and the human race [11].

Plant-derived bioactive components have the potential to combat a plethora of diseases. Phytochemicals are regularly used now-a-days in our lifestyle maintenance via vegetables, medicine, healthcare drinks, cosmetics, etc. because of substantial health benefits reportedly rendered by them. The phytochemicals obtained via food are also reported to protect against several medical conditions [12-15]. Although several phyto-chemicals (belonging to three main groups of natural products like terpenes, alkaloids and phenolic compounds) are known to act as fish poison which can cause fish death by interfering with fish biochemical respiratory pathways and suffocating them to death but there are certain plant-derived chemicals that are chosen over other synthetic compounds mainly because of their non-toxic/limited toxic nature, low cost and easy availability [16]. Likewise, several phyto-compounds have also been used by various researchers against pesticide toxicity in fish and other organisms [6,17-21]. However, the evaluation of chemical interactions/molecular mechanisms of many of these phytochemicals remain unexplored due to a lack of preliminary knowledge regarding the target of action of these phyto-chemicals in fish or human body [22-23]. Based on our preliminary literature survey, the phyto-chemical CUR, a yellow colour polyphenolic compound, appeared to be an ideal test candidate, because of its high antioxidant activity, anti-diabetic efficacy, anti-genotoxic property, anti-inflammatory response, antimicrobial activity, anticarcinogenic efficacy [24-30]. Further, CUR was also reported to have no toxic effect in the mammalian system as well as fish body and has usage against myriads of diseases since ancient times [31-32]. Therefore, extraction and isolation of CUR from the turmeric rhizome becomes an important part of our study to determine its protective efficacy in our concerned field. Although there are several advanced extraction and separation methods like ultrasonic extraction, Soxhlet extraction, microwave, dipping and zone-refining methods, solvent extraction method using polar and non-polar solvents like ethyl acetate, hexane, acetone etc. followed by column chromatography, remains the most common method for separating CUR from turmeric [33-37]. Further, CUR can be extracted in a cost-effective way from locally available turmeric roots. In the case of rearing fishes in farm, CUR has already been used as a supplementary diet which helps to improve their growth, immunity system, hepato-protective ability and enhancement of reproductive ability [38-41]. However, whether CUR could also protect fish from any specific pesticide-induced toxicity had not been studied earlier.

Therefore, in this study we primarily aimed to test the hypothesis if CUR, extracted from locally available turmeric, could demonstrate its ability to significantly prevent the extent of pesticide-induced toxicity in fish, and if it could, to determine what could be the possible chemico-biological mechanistic principle and major pathway behind the protective action. The pesticide chosen in the present study was CM, a commonly used pesticide, the toxicological effects of which had been elaborately reported earlier in fish [6,19].

Thus, the main objectives of our present work were: i) Chemical characterization of extracted active phytochemical from *Curcuma longa*, ii) to investigate the possible protective role of extract CUR against CM induced toxicity in tilapia fish and L6 mammalian muscle cell line.

Method and Materials

Chemicals

Chemicals and reagents were used to conduct the experiment at the analytical grade, ≤ 97% of purity.

Preparation of ethanolic extract of turmeric **rhizomes** *and its solvent extraction for isolation of active component*

Fresh turmeric rhizomes were purchased from the local market near Kalyani University. Ethanolic extract of turmeric rhizome was prepared in the following method: the rhizomes were macerated and soaked in ethanol (100%) overnight after they were filtered with Whatman filter paper (Manufacturer: Whatman – Cytiva cat no 1001125). The filtered ethanolic extract was evaporated in water bath, and one part of thick residue was stored at normal temperature for experimental use. The second part was used in TLC and column chromatography for separation of the active compound [30:70, Ethyl acetate (MERCK, 109623: Pet ether (MERCK, 101769), as eluent; silica gel 60-120 mesh size (MERCK 61806205001730). The different fractions of the isolated compound were sent for MS, NMR and FTIR analyses for the chemical characterization of possible active compound (CUR) anticipated to be present in turmeric extract.

Acclimatization of tilapia fish and experiment design

Around 22-27 g of disease-free and hormonal treatment-free 40 tilapia fishes (*Oreochromis mossambica*) were purchased from a fish farm near Kalyani University. They were kept in the fish vat/ small tank for acclimatization for 15 days and fed with normal fish pellets. Before the onset of the experiment, they were divided into three groups each containing nine fishes:

Group I: Control group-This group was fed with a normal diet.

Group II: Toxicant treated group (CM-treated).

Group III: Pre-treated drug group (CUR followed by CM exposure) – CUR (5mg/ g b.w. for 7 days) + CM.

The experimental aquariums were scientifically maintained at an ambient temperature of the water being 26-28 ˚C and pH= 6.7. At the end of the experiment, the fishes were anesthetized with benzocaine (200mg/1lit) [Sigma-Aldrich, Cas. No. 94-09-7] before sacrificing the above-mentioned groups.

Dose of CM administered in tilapia fish

The standardized dose of CM at 0.05 µl/ g b.w. determined through a range finding trial was taken from the 0.04 µg/µl stock solution, as per the report of Samadder et al., [6,19] and injected into the subcutaneous layer of each experimental fish irrespective of their sex.

Standardization of dose of CUR in tilapia

In a range-finding trial, the optimal dose of CUR showing maximum protective effect was determined to be 5% b.w. (Table 1). This dose was also reported to be optimum in a previous study [39]. Pre-acclimatized live tilapia fish were pre-fed with CUR at the optimum dose of 5% b.w. (50 µg/ g b.w.) daily for 7 days.

Histopathological evaluation of different tissues in fish

After dissecting out the hepatic, kidney, gill, spleen tissues these were immediately fixed in 10% neutral buffer for 24h. Then, the histological tissue sections were prepared and stained with haematoxylin and eosin stain as per standard protocol with slight modification [42]. Histological changes in tissue were photographed and analysed under a compound microscope (10X).

Measurement of reactive oxygen species (ROS) in hepatic tissue

Hepatic cells were isolated in PBS by perfusing and ROS was quantified as per standard protocol with slight modification [6,19].

Analysis of lipid droplets in hepatic tissue

After dissection of hepatic tissue, the tissue was immediately placed inside the cryostat microtome (Reichert, USA). Then frozen hepatic tissues sections were stained with Sudan III and IV [43] and observed under the compound microscope (10X). Morphometric study of lipid droplets were performed by using ImageJ 15.2 v software.

Assessment of DNA damage by fragmentation assay

DNA fragmentation assay was performed by kit-based extraction of the DNA (GSURE

FAST TISSUE KIT, Lot no- 19114K1169) from muscle tissue of fish and then DNA gel electrophoresis was performed in 1% agarose gel with 1% ethidium bromide. Intensity of DNA damage was determined by executing ImageJ 15.2 v software.

Analysis of ROS generation in histological section of hepatic tissue

Histological tissue sections of control and experimental set of fish were then stained with H_2DCF-DA stain in dark for 30mins after which they were rinsed in water and observed under confocal microscope (Carls Zeiss LSM 800) at 10X magnification. Density of ROS generation in same mean area of hepatic tissue was evaluated with help of Irfanview software.

Immunofluorescence study of p53 protein in hepatic tissue

To analyse p53 protein expression directly in hepatic tissue, anti p53 primary antibody (Santa Cruz Biotechnology, Inc., USA) and FITC conjugated secondary antibody (Sigma, USA) were added as per standard protocol and examined under a confocal microscope (Carls Zeiss LSM 800) at 10X [44]. Then, evaluation of the density of p53 expression level in same mean area of hepatic tissue was performed under Irfanview software.

Cell culture procedure

L6 Mammalian skeletal muscle cell line was purchased from NCCS (Pune, India) which were grown in an atmosphere maintained strictly at 5% carbon dioxide and temperature at 37^{O}C. The cells were nurtured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotic solution as per standard instruction.

Selection of dose of CM in L6 cells

L6 cells were exposed to the sub-lethal dose of 30 µM of CM, as standardized in our previous studies [6,19].

Selection of dose of CUR in L6 cell based on % cell viability

10 mg/ml of CUR was taken in autoclaved water and sonicated in sterile conditions to make a colloidal solution of CUR. 10, 20, 30, 40 and 50 µM of CUR was added to different wells of 96 well plate embedded with L6 cells and grown at desired density and incubated for 3h following the range-finding procedure adopted in a previous study [45]. After incubation, cellular cytotoxicity % was estimated by using MTT solution following the standard protocol [46] with slight modification [47]. The % of cell viability was calculated as:

$$\% \, Cellviability = \frac{OD \ of \ drug \ treated \ sample}{OD \ of \ control \ sample} X \ 100$$

Statistical analysis

The data presented in tables and figures were statistically analyzed by Student's t-test and one way ANOVA after performing three independent experiments and calculating the mean values as required.

Result

Characterization of the extracted compound analyzed from Mass, NMR and FTIR spectra

After performing the TLC and column chromatography (Figure 1) the ^{1}H NMR, Mass and IR spectra confirm that the extracted compound from the ethanolic extract of turmeric is CUR which might be in its keto form (a) or enol form (b) (Figure 2a-b).

^{1}H NMR: The ^{1}H NMR spectra were recorded in $CDCl_3$. The doublet peak at 6.41 ppm is attributed to the presence of alkene group. This doublet peak exhibits a coupling constant of *J* = 16 Hz which clearly indicates the presence of *(E)*-alkene in CUR moiety. In the aromatic region (>6 ppm), the presence of a total of ten protons accounts for the phenyl ring protons as well as *trans*-alkene protons of CUR. Two peaks at 5.81 and 5.73 ppm represent the flanked methylene (CH_2) protons. Besides, a sharp singlet peak at 3.89 ppm is ascribed to six protons for the pair of methyl groups of two methoxy (–OCH_3) functionalities (Figure 3a-c).

LCMS: In Mass spectra, the peak at 369.15 suggests the molecular ion peak (M + H) for CUR (Figure 4). The ionization of the peak at 369.15 confirms CUR structure.

FTIR: In FTIR spectra (Figure 5), peaks at 1627.99 cm^{-1} indicate predominantly to the overlapping stretching vibrations of alkenes (C=C) and carbonyl (C=O) character. Moreover, broadband at 3365.68 cm^{-1} appeared probably due to the presence of

phenolic –OH groups. The broadness appears due to intermolecular hydrogen bonding. The C=C aromatic stretching vibration appears at 1429 cm^{-1} and high intensity band at 1511.9 cm^{-1} is attributed to the mixed vibrations including stretching carbonyl bond vibrations (C=O), in plane bending vibrations around aliphatic CC-C, CC=O, aromatic CC-H of keto and enol configuration of CUR. Furthermore, significant intense band at 1281.18 cm^{-1} is attributed to the bending vibration of the C-O phenolic band.

Histopathological evaluation of different tissues

The histopathological parameters studies in different tissues of control and experimental fishes are given below (Figure 6):

Liver:

In the control group of tilapia fish, it was observed that the hepatic tissue has a perfect cytoplasmic compact architectural structure bounded by the intact cell membrane; the hexagonal hepatic cell displayed a centrally or sub centrally placed spherical nucleus. CM intoxicated tilapias showed that cytoplasmic compactness deteriorated because of vacuolation (marked with black colored arrow head) was formed in hepatocytes. CM exposed liver tissue exhibited that the centrally placed nucleus altered the position to the periphery of the hepatocytes, and pycnotic nuclei were observed in the hepatic cells. Blood ingestion (marked with yellow colored arrowhead) occurred in the central vein, and necrosis of hepatic tissue was also observed. However, in the CUR pre-treated group (group 3) of fish, there was a reduction in the pycnotic nuclei, restoration of the compactness of the hepatic tissues, almost centrally placed nuclei with restricted vacuolation and decline in necrosis was evident even after CM exposure (Figure 6.i a).

Kidney:

CM causes glomerulus detachment from the epithelial lining (marked with yellow colored arrowhead) through the loss of compactness of the cytoplasm. Peritubular edema (marked with black colored arrow head) and necrosis were also observed in the CM treated group as compared to control. On the contrary, in the CUR-pre-treated group reduction in the glomerulus detachment and peritubular edema was seen (Figure 6.i b).

Spleen:

In the CM treated group, hemosiderin deposition (marked with yellow colored arrowhead) and aggregation of melano-macrophages (marked with black colored arrowhead) were observed along with a significant reduction in white pulp occurring in CM treated splenic tissue as compared to control group having ample evidence of the presence of white and red pulp present in them. On the other hand, the spleen tissue dissected out from CUR-pre-treated tilapia fish showed a total absence of melano-macrophages associated with an elevated number of white pulp in splenic tissue and reduction in hemosiderin deposition in them (Figure 6.i c).

Gill:

Gills of control group of fish showed the normal histological structure of gill tissue, which was composed of primary and secondary lamellae. In our study, CM exposed fish gill tissue showed a histological alteration in primary and secondary lamellae associated with formation of the large vacuole in the primary lamellae (marked with black colored arrowhead), which led to severe damage in gill tissue. Secondary lamellae were curled (marked with red colored arrowhead), interlamellar space reduced, which intended that degeneration of secondary lamellae occurred. However, in the CUR pre-exposed group, interestingly, the primary lamellae were not affected by the CUR-followed CM exposure, but the interlamellar space between the secondary lamellae remained the same as in group II. Further, a significant change occurred in the secondary lamellae of the CUR-treated tilapia group as compared to those exposed only to CM (Figure 6.i d).

Table 1: Range finding trial for dose selection of curcumin	
Group	**% Cytotoxicity in hepatic cells of fish**
Control	87±6
CM	52±3
CUR (I) + CM	74±4*
CUR (II) + CM	78±8*
CUR (III) + CM	69 ± 7^{NS}
CUR(I)= 100 µg/ g b.w., CUR(II)= 50 µg/ g b.w. and CUR(III)= 25 µg/ g b.w. *p<0.05 *vs* CM treatment, NS= non-significant analyzed through Student's t-test.	

Table 1: Range finding trial for dose selection of curcumin

a. TLC-Iodine blow plate b.Column Chromatography

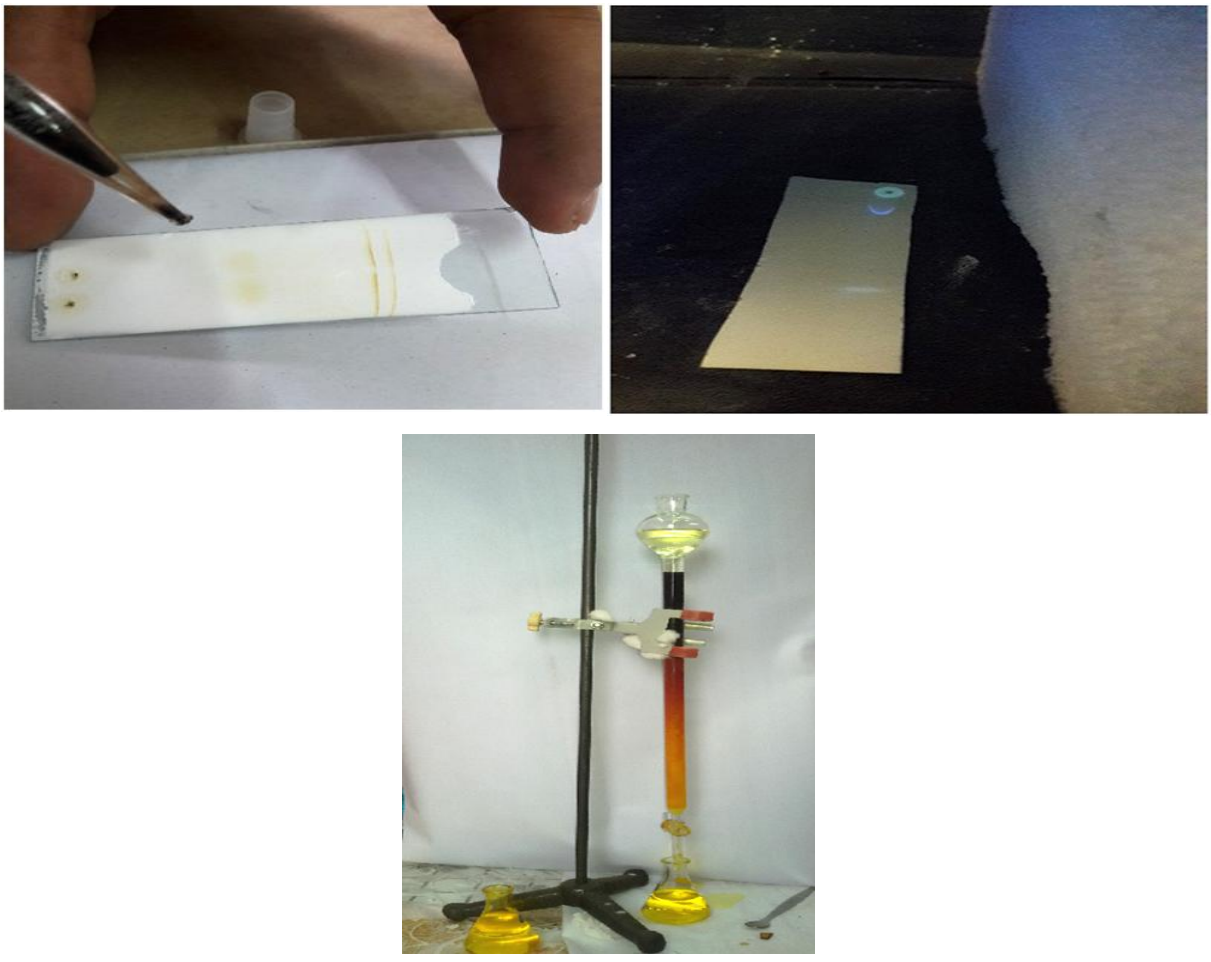


Figure 1: (a) Characterization of extract CUR through TLC- iodine plate, (b) & (c): Isolation of CUR through column chromatography

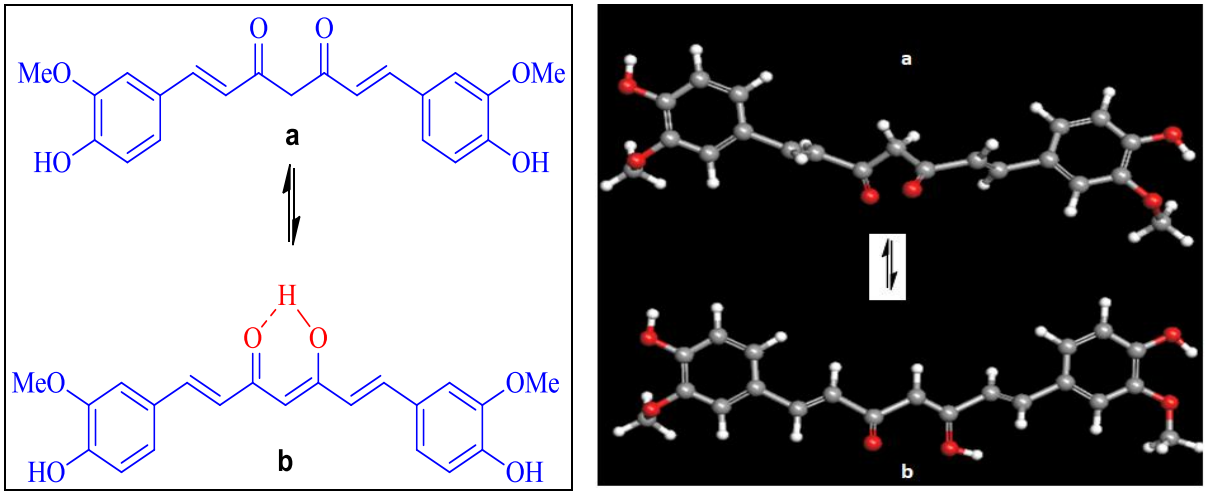
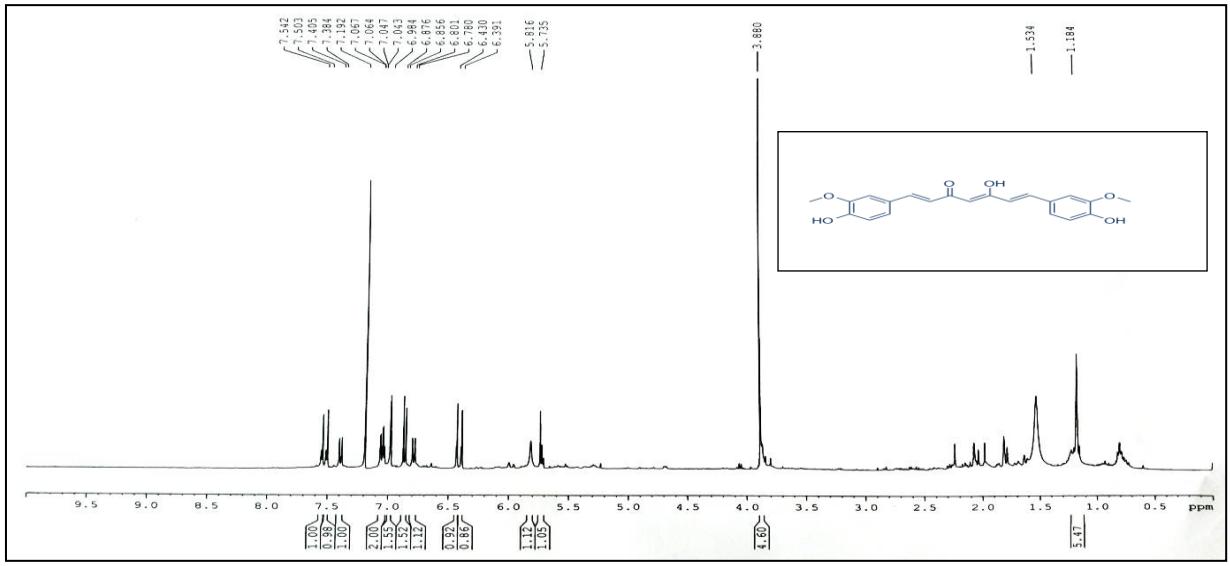
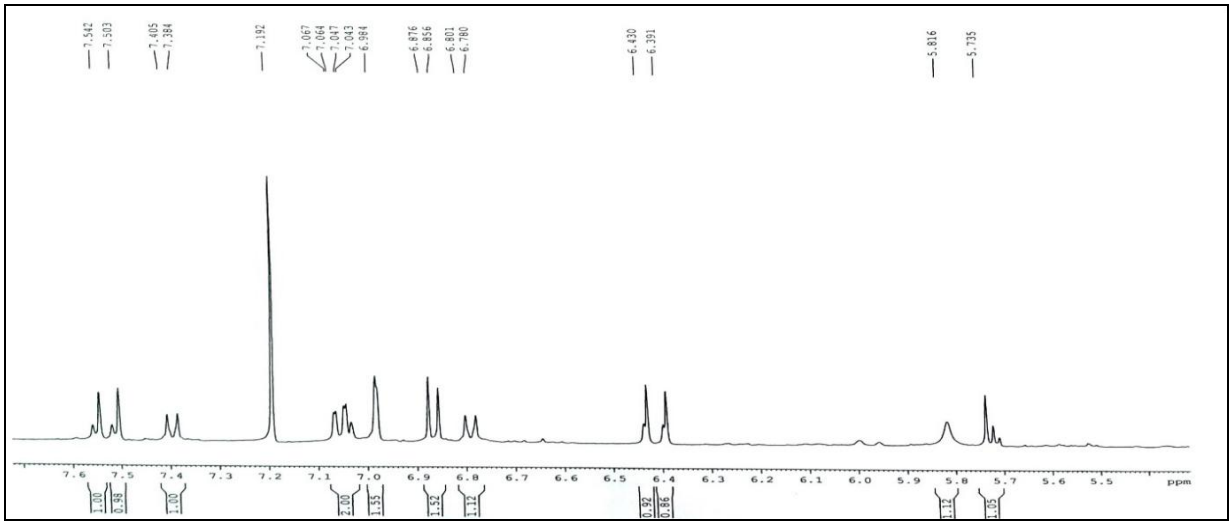


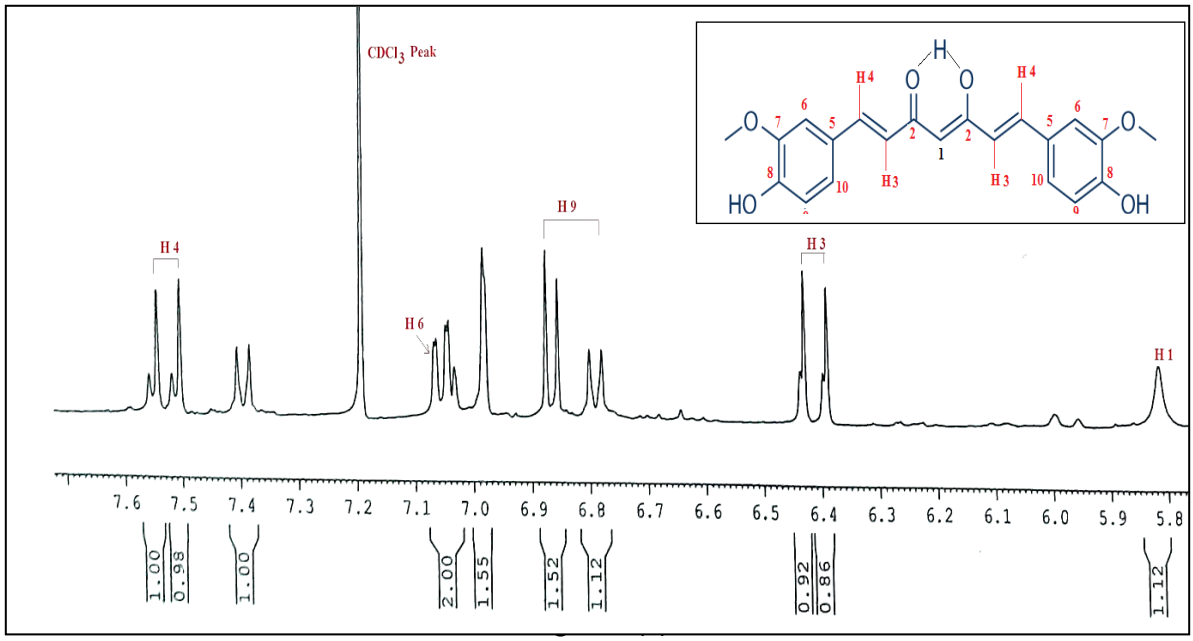
Figure 2: CUR in its bis-keto form (top a) and enol form (bottom b)



3.(a)

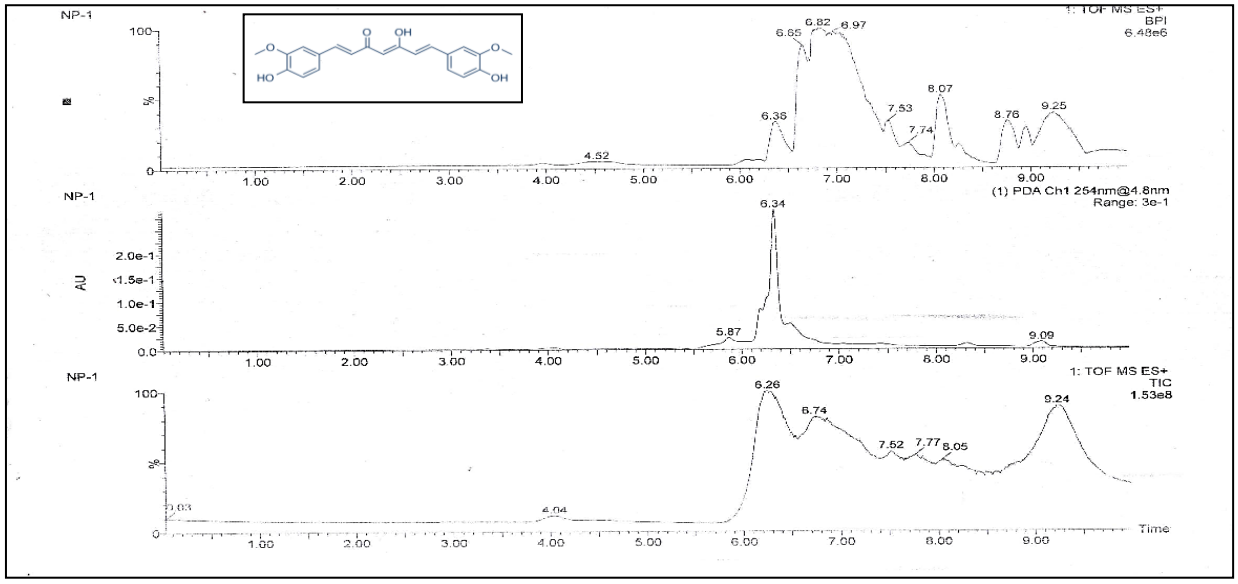


3.(b)

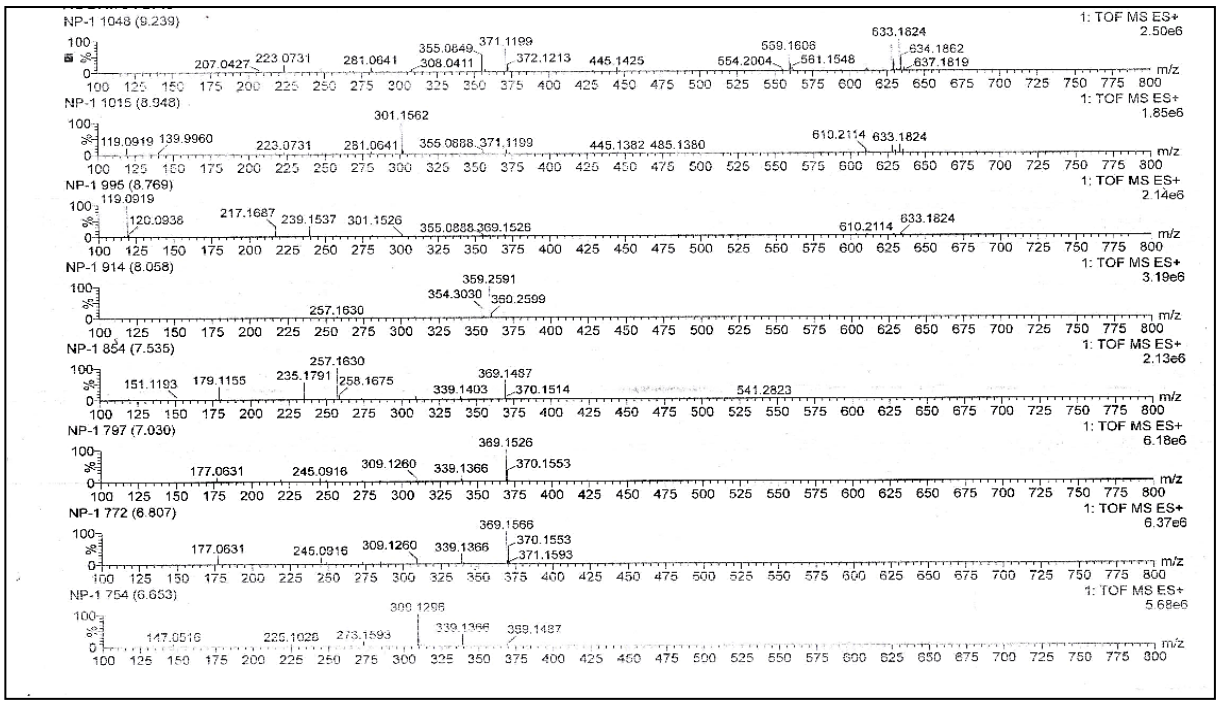


3.(c)

Figure 3. a) Characterization of CUR by analysis of NMR data, b) Expansion of aromatic region, c) Partial ^{1}H NMR spectra of extracted CUR in representing marked some aromatic and alkene protons.



4.(a)



4.(b)

Figure 4: (4a and 4b): Mass spectra showing peaks at 369.1 corresponding to CUR i.e. = M-1, i.e., 369.1-1= 368.1 M.W

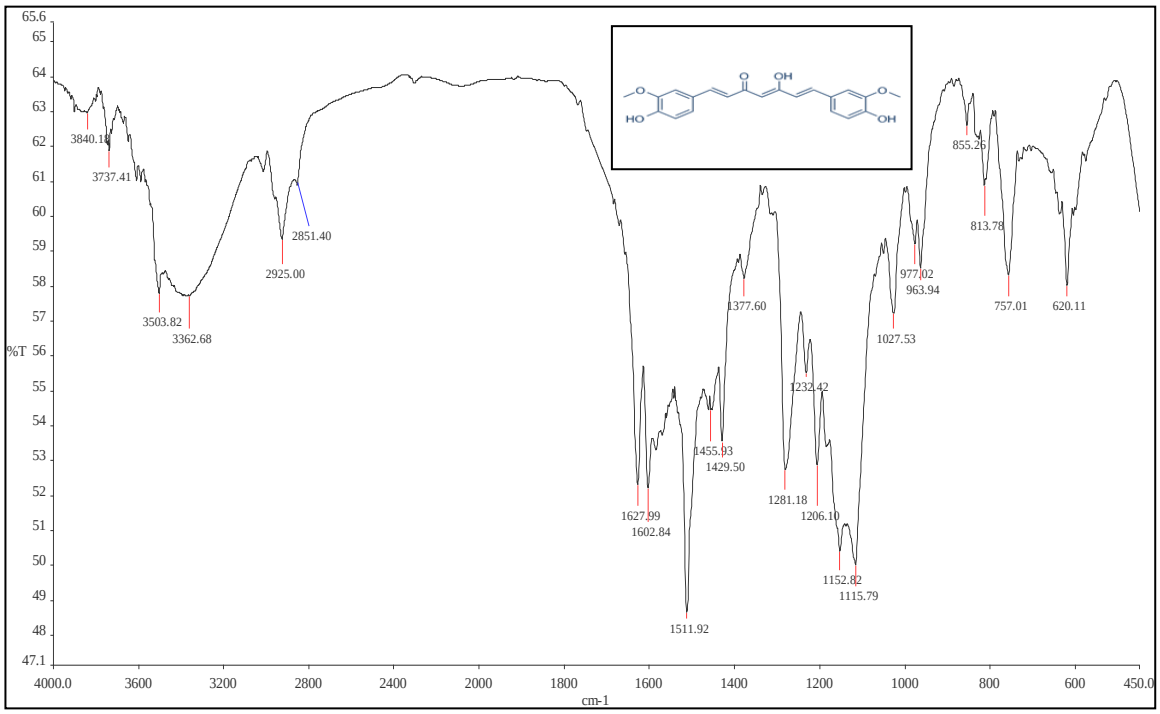
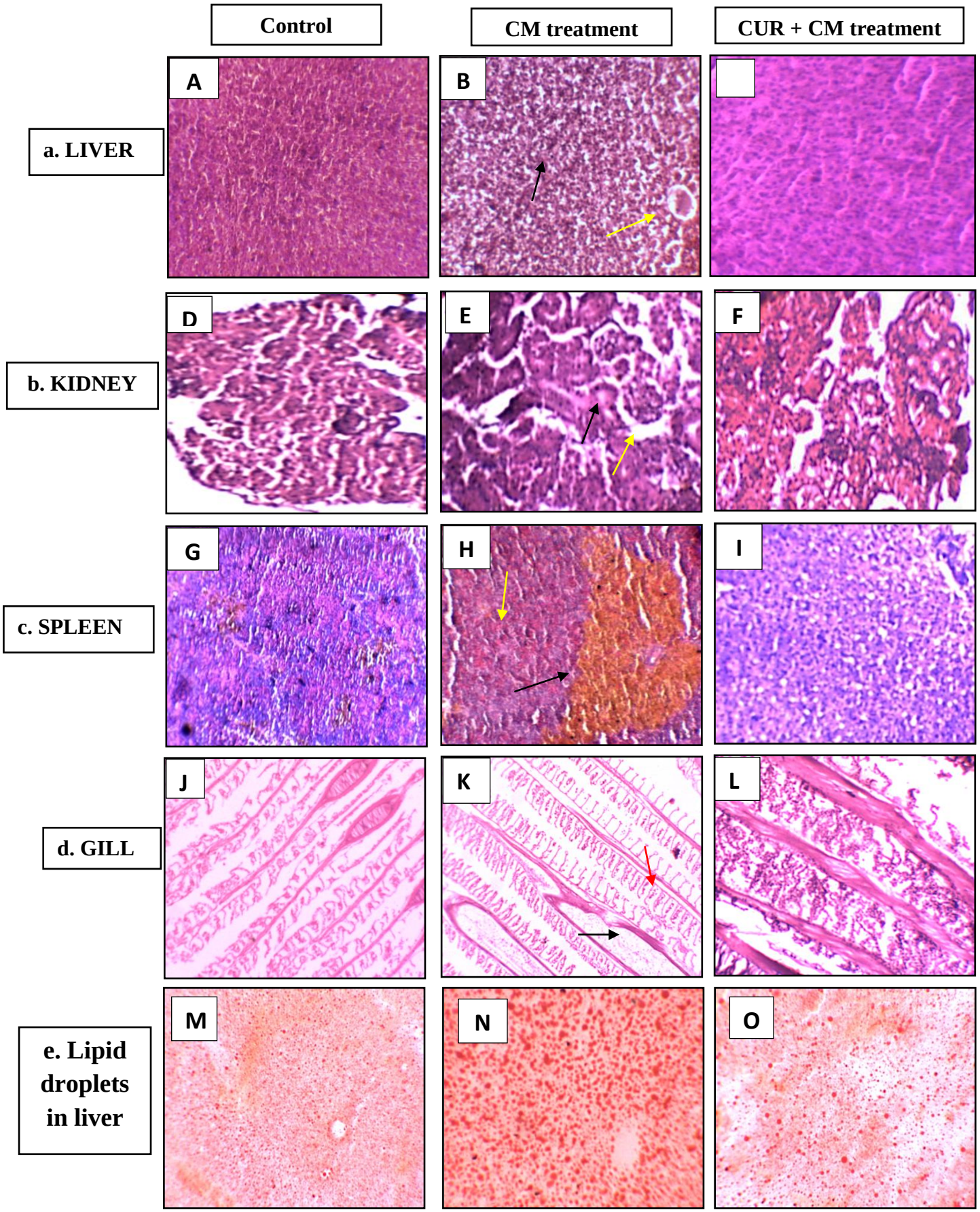
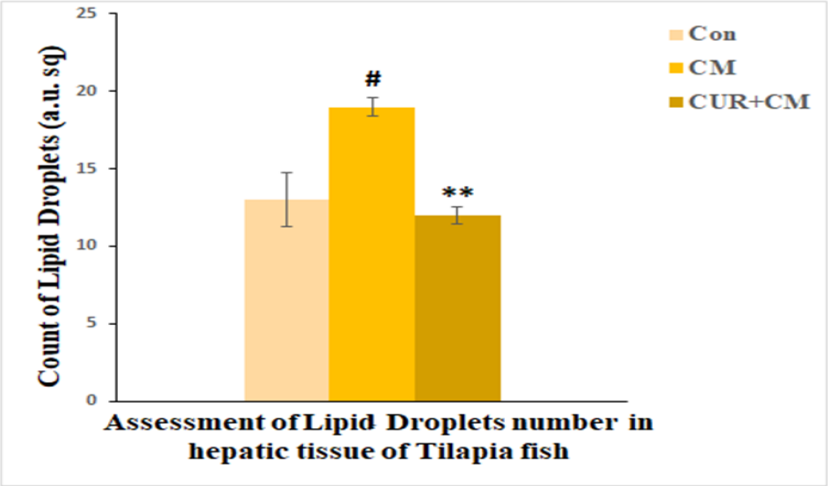


Figure 5: Characterization of CUR by analysis of FTIR spectra



6(i)



6 (ii)

Figure 6 :Histopathology and histo-chemistry based analysis of different tissue of Tilapia fish:
6.i (a) Liver tissue: (A-C) showing histopathological structure of hepatic tissue in tilapia fish, control group (A), vacuolation (black color headed arrow) and blood ingestion in central vein (yellow colored headed arrow) showed in CM treated group (B) and pre-treated CUR+ CM treated group (C).
6.i (b) Kidney tissue: (D-F) showing histopathological structure of kidney tissue, control group (D), peritubular edema (black color headed arrow) and glomerulus detachment (yellow color headed arrow) showed in CM treated group (E) and pre-treated CUR+CM treated group (F).
6.i (c): Spleen tissue: (G-I) showing histopathological structure of spleen tissue, control group (G), aggregation of melanomacrophages (black color headed arrow) and hemosiderin deposition (yellow color headed arrow) showed in CM treated group (H) and pre-treated CUR+CM treated group (I).
6.i (d) Gill tissue: (J-L) showing histopathological structure of gill tissue, control group (J), curled secondary lamella (red color headed arrow) and vacuolation in primary lamella (black color headed arrow) showed in CM treated group (K) and pre-treated CUR+CM treated group (L).
6.i (e) Histochemical analysis of Tilapia liver tissue (A- control, B- CM treated group, C- Pre-treated CUR+CM treated group) by Sudan staining of lipid droplets. An increased amount of lipid droplets were observed in CM treated group instead of pre-treated CUR+CM treated group.
Fig 6(ii): Quantitative analysis of lipid droplets number in hepatic tissue of tilapia fish. $^{\#}p< 0.05$ vs control, **p<0.01 vs CM treatment analysed through Student's t-test.

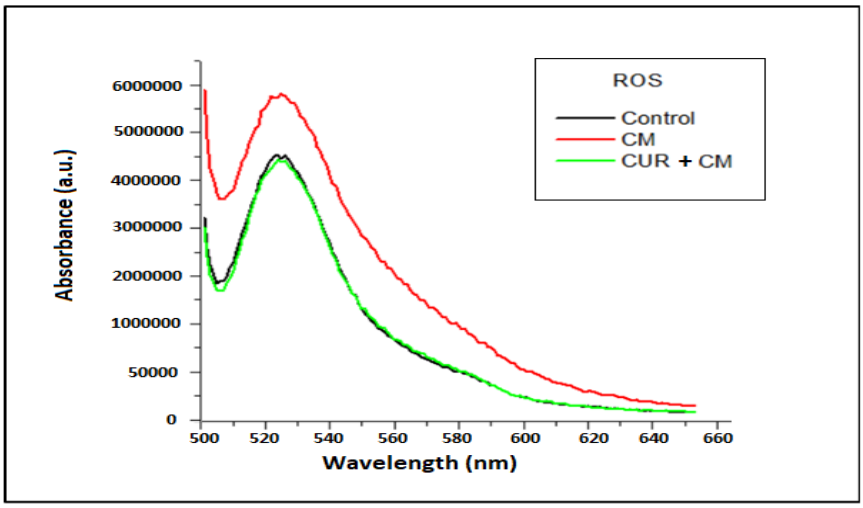
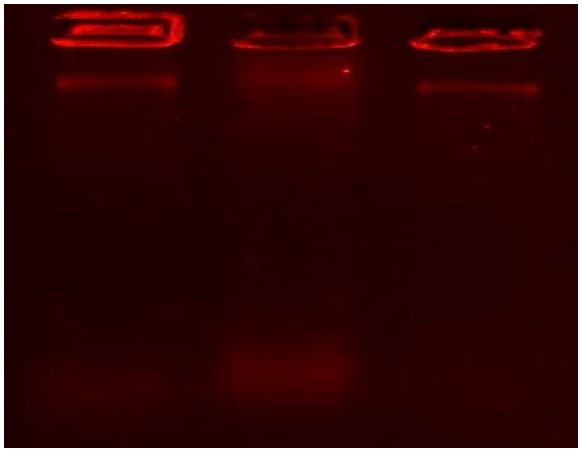


Figure 7: Measurement of ROS production in hepatic tissue by H2DCF-DA dye

8a



8b

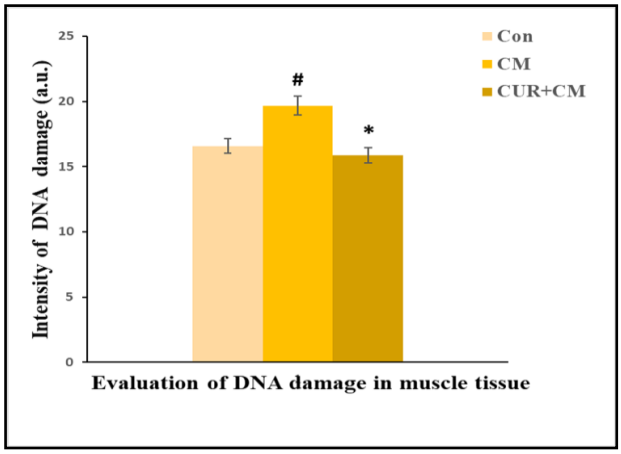
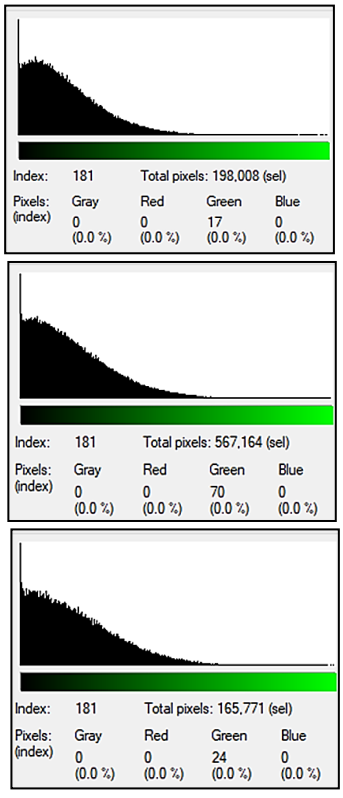


Fig 8(a-b): 8a: Analysis of DNA fragmentation induced by CM in muscle tissue of tilapia fish (Ln1- control, Ln2- CM treated group and Ln3- Pre-treated CUR+CM treated group); 8b: Estimation of the DNA damage intensity in muscle tissue. $^{\#}p< 0.05$ vs control, *p<0.05 vs CM treatment analyzed through Student's t-test.

Histogram of generation of ROS in hepatic tissues



Control

CM

CUR+CM

Figure 9: Image histogram of ROS generation shown by H2DCF-DA staining in hepatic tissue

Histochemistry study for assessment of lipid droplets in hepatic tissues

Histochemical studies of hepatic tissue of CUR-pre-fed and CM treated fishes showed a palpable reduction in the content of lipid droplets accumulated in hepatic tissues when compared to only CM exposed fish (group II) (Figure 6.i e).

Counting of lipid droplets in CM group showed higher frequency when it is compared with pre-treated CUR group as well as control group (Figure 6.ii).

Quantification of reactive oxygen species (ROS) generation

ROS production was significantly elevated after the fishes were exposed to CM. However, CUR-pre-fed CM intoxicated fishes showed an extreme reduction of ROS generation similar to the control set (Figure 7).

Estimation of DNA damage by fragmentation assay

Enlarged smearing of DNA was found in CM-treated group as compared to control sets. The pre-treated CUR followed by CM-exposed group showed a scanty amount of DNA smear when compared with CM-treated group as well as the control group (Figure 8a). Intensity of DNA damage decreased when experimental groups was pre-treated with CUR as compared to CM-exposed group (Figure 8b).

Evaluations of ROS production in histological section

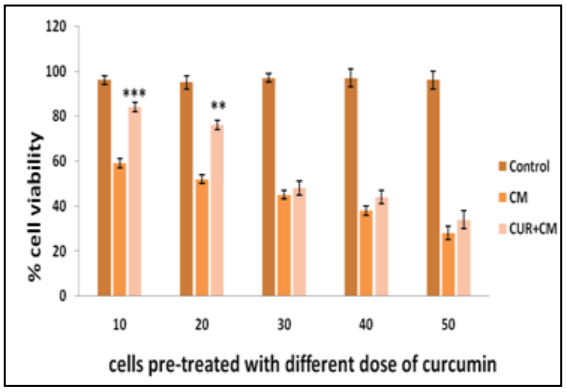
When compared with the CM-treated group of tilapia, it was observed that pre-treated

CUR followed by CM exposed fish reduced ROS generation (Figure 9).

Determination of p53 expression by immunofluorescence study in hepatic tissue of tilapia

Our result revealed that the expression level of p53 was much higher in liver tissue of pre-treated CUR+CM group as compared to CM treated group and the control group of tilapia (Figure 10).

Evaluation of standardized dose of CUR based on % cell viability of L6 muscle cells



The % cell viability of L6 cells treated with CM was found to be decreased as compared to control set. However, there was a restriction in the % of cell mortality in L6 cells which were pre-treated with CUR followed by exposure with CM. The 20 µM dose of CUR was selected for our further study based on the results of the % cell viability test (Figure 11).

Fig 11: Assessment of % cell viability of L6 cells pre-exposed to curcumin (CUR) dose followed by treatment with cypermethrin (CM). ***p<0.001 *vs* CM, **p<0.01 *vs* CM treatment analysed through Student's t-test.

Discussion

In the present study, we isolated and chemically characterized CUR from the rhizomes of turmeric collected from local market as a very affordable source and found that it effectively reduced the ROS generation in the CM exposed fish. Similarly, CUR reduced the tissue damage in liver as there was a reduction in the pycnotic nuclei, restoration of the compactness of the hepatic tissues, brought forth centrally placed nuclei with restricted vacuolation and decline in necrosis. CUR also reduced lipid droplets produced in hepatic tissue of CM exposed fish. Similar protective changes in tissue damage were also observed in other tissues like kidney, gills and spleen of CUR fed CM intoxicated fish in respect of various sub-structures in them, as also encountered in our earlier study [19]. Thus, CM exposure caused histopathological changes while pre-feeding of CUR appeared to anticipate and antagonize these changes to render protection to different vital organs. Thus, pre-feeding of CUR could also bring about protective changes in other non-target

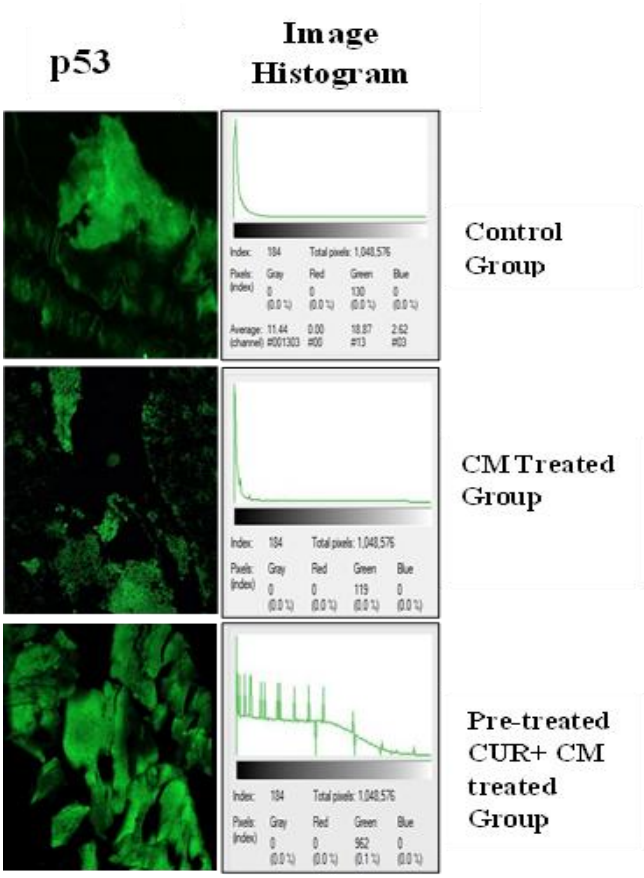


Fig 10: Evaluation of p53 expression density in hepatic tissue of tilapia fish.

organisms, bringing a favourable impact on maintaining biodiversity.

Over the years, researchers have been investigating different possible pharmaceutical interventions to combat the ill effects of conscious and unconscious exposure to such environmental toxicants

globally. Therefore, an ardent need for inhibiting such toxic effects of pesticides led us to search for a natural, phyto-based compound which would not only be environment friendly but also would be effective in living systems. Phyto-based flavonoid compounds with no/limited side-effects have earlier been used to combat toxic effects in humans [48]. However, reports on the evaluation of target-specific action of CUR against pesticide induced toxicity are scanty. In our present study tilapia fish receiving pre-treatment with CUR and then exposed to CM showed significantly reduced deleterious effects of the pesticide induced toxicity in respect of necrotic tissue, blood haemorrhage, and in the reduction of overall tissue mass, histological structure and morphology, when compared with that of the only CM treated fish.

CUR has been reported earlier to reduce DNA damage thereby having immense use against myriads of diseases [49]. In the present study, pre-treatment with CUR not only helped in restricting DNA fragmentation in the fish by CM, but also aided excessive production of p53. From the earlier studies, we observed that production of reactive oxygen species in tissue could destroy the cytoarchitecture by inducing DNA damage which would lead to the initiation of apoptosis process through modulating the mitochondrial membrane potential. ROS can induce oxidative stress to DNA which efficiently breaks DNA into the single strands. From our study, we found that fish pre-treated with CUR prior to CM exposure diminished the ROS generation in hepatic tissue which turns on different molecular signalling pathways to promote protection against CM induced toxicity.

Curcuma longa is a well-known medicinal plant containing active flavonoid phytochemical CUR. Ethanolic extraction and purification of CUR by using column chromatography have showed significant protection against pesticide-induced toxicity in both tilapia fish and L6 cell line. Turmeric rhizome extract contains several natural compounds which aid in increasing the functionality of CUR making it more permeable when administered in living cells and tissues [50]. Thus, the overall increase in the transport and absorption of CUR into cell or tissues is the key cause for effective protective potential of the natural CUR even against one of the common toxic pesticide CM when administered in fish. Since consumption of pesticide exposed fish causes several health hazards in human as well due to bio accumulative property of CM, therefore, the cyto-protective efficacy of CUR with no cellular toxicity in normal cells makes it a promising noble phytomedicine which may be intaken in our daily diet to bring about a kind of natural immunity or protection against pesticide toxicity in future.

CONCLUSION

Therefore, the bio-active component CUR, extracted from rhizomes of turmeric, serve as a possible natural preventive molecule playing effective role in inhibiting pesticide toxicity in fish and mammalian cell line which ensues a low cost environment friendly approach to combat against pesticide induced toxicity in every possible ecological and economical niche.

CONFLICT OF INTEREST

None to declare.

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Authorship credit statement

AS projected and conceptualized the study, Data curation-PS, RDas, SD, RDey; Formal analysis- PS, RDey; Funding acquisition-AS; Investigation- PS, RDey; Methodology and Project administration–PS, SD and RDas did this research under supervision of AS and MB (*in vivo*), chemical characterization: PS, SD, RDas and RDey did the research under supervision of DT; Resources-AS and SN; Software- AS provided *in vivo* and *in vitro* related analysis software; Supervision- AS and SN; Validation-RD under supervision of AS and SN ; Visualization: PS, RDey, RDas, SD, DT, MB, AS, SN; Roles/Writing - original draft- jointly by AS and SN; Writing -

review & editing: AS , Overall supervision and final editing: ARKB.

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