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Rejuvenating potentials of *Curcuma longa* on Sudan IV dye induced hepato-renal injury

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ABSTRACT

Objectives: Sudan IV dye (S4D), has been reported to enhance the color of food products example palm oil, despite the health issues associated with these colorants. The potent antioxidant attribute of *Curcuma longa* facilitates its capacity to protect the kidney against deleterious attacks by free radicals. This study investigated the rejuvenating potentials of the extract of *C. longa* by S4D induced hepato-renal injury in rats.

Material and Methods: Twenty-five female albino rats were distributed into five groups, as follows: Group 1 received distilled water and diet only, Group 2 received 10% S4D+100 mg C.L, Group 3 received 15% S4D +100 mg C.L, while Groups 4 and 5 received 200 mg and 250 mg C.L, respectively. After 3 weeks of administration of *Curcuma* treatment, animals were sacrificed; liver, kidney, and small intestine were collected for analyses. Aspartate and alanine aminotransferases, gamma-glutamyl transferase, glutathione-s-transferase, alkaline phosphatase, and lactate dehydrogenase, as well as some selected electrolytes were measured.

Results: Results show that treatment with 200 mg and 250 mg/kg significantly ($P < 0.05$) rejuvenated these enzyme biomarkers while modulating the physiological levels of the serum electrolytes and kidney markers.

Conclusion: *C. longa* exhibited a significant antioxidant potential that sustain the cells from the effect of S4D toxicity. Hence, the results indicate a possible ameliorative mechanism of the plant extract against S4D- induced hepato-renal injury. Therefore, the inclusion of *C. longa* into our daily diet might limit the effect of the hepato-renal injury.

Keywords: *Curcuma longa*, Hepato-renal, Sudan IV dye, Toxicity, Antioxidant.

INTRODUCTION

Sudan IV dye (S4D) ($C_{24}H_{20}N_4O$) is an azo dye found used as a colorant in industrial products, such as shoe polish, petrol, soaps, plastics, oil, and printing inks.[1] It has been ruled unsafe under the 1995 Colors in Food Regulations and is an illegal food dye. However, due to the intense red-orange color and inexpensiveness of S4D, it has been deliberately added to foods products (like chili powder, palm oil, egg products, and poultry meat) as coloring agents.[1,2] The increased usage of palm oil, particularly in homemade and processed meals, coupled with its bright reddish color makes it a prime target for adulteration with S4D; an incident that has, unfortunately, continued to escalate.[3] In Washington DC, the United States, a survey by Andoh *et al.*, Ireson *et al.*[3,4] observed S4D as the principal azo-dye in food, with concentrations reaching 24 µg/ml, as against the EU detection limits of <1.2 µg/ml. Another study, this time in Western Africa, confirmed the

adulteration of food samples with S4D, previously reported by the Food and Drug Authorities of Ghana.[5] Even in Nigeria, the adulteration of food with S4D is rampant and may give an impression that it's a normal event.[5,6]

Several studies, using different animal species, have shown the safety of concoctions from turmeric (*Curcuma longa*),[4,6] although other studies have reported incidences of hepatotoxicity, following the intake of excessive amounts of *C. longa* extracts[5,6] This discrepancy has been attributed to the differences in metabolism between different species. For instance, a recent study Eteng *et al*.[7] demonstrated species-dependent susceptibility to the high dose of *C. longa*-induced hepatotoxicity, due to differences in the metabolism of curcumin between humans and rats. Humans seem capable of tolerating high doses of curcumin without displaying noticeable adverse effects. No side effect was observed in a phase 1 study by Amin *et al*.,[8] following 90 days of curcumin injection with a dose as high as 8 g/day. The findings were that patients receiving *Curcumin* and phenylbutazone each for 2 weeks shows some significant levels of improvement seen at the end of both treatment regimens compared to the baseline studies. However, the magnitude of improvement was greater in the phenylbutazone group as a result of not providing a placebo group to show the efficacy of *Curcumin*.

Several *in vivo* and *in vitro* investigations have elucidated plausible mechanisms underlying the anti-inflammatory property of curcumin. It has been reported to inhibit a plethora of pro-inflammatory mediators, such as arachidonate pathway-related enzymes and molecules, nitric oxide synthase, hyaluronidase, collagenase, elastase, monocyte chemoattractant protein-1, tumor necrosis factor, interferon-inducible protein, and other proteins of the interleukin family[10] A double-blinded clinical study reported improvement in morning stiffness, joint swelling and walking time in eighteen rheumatoid-arthritic patients, who were administered curcumin and phenylbutazone each for 2 weeks, compared to baseline.[9,10] Nevertheless, very few studies have attempted to assess the *in vivo* effects of the adulteration of S4D, in food products, and, to the best of our knowledge, no study has investigated the rejuvenating tendencies of Curcuma in S4D induced hepato-renal injury in female rats. Therefore, this study will offer an improved discernment of the possible adverse health outcomes of ingesting S4D-containing food products. Further, the results of this study will propel the enactment of health intervention and management regulations, *vis-à-vis* the use of the chemical agent in food products. S4D is carcinogenic to human health as its harmful effect could be expressed for a long period of time. The active metabolites contained in S4D include ortho-toluidine and ortho-aminoazotoluene. There is a clear understanding that S4D has genetoxic effects thus, injecting it into food products could trigger oxidative stress thereby producing reactive oxygen species (ROS) and disabling the defenses of antioxidant cells.

MATERIAL AND METHODS

Plant materials

The plant[11] *C. longa* linn rhizomes were harvested from its natural habitat in Asasa Farms, Ile – Ise Awo, Abeokuta. The plant specimen was identified and authenticated by a botanist (Dr. Oleylika) in Pure and Applied Botany Department, College of Bi**o**sciences, Federal University of Agriculture, Abeokuta, Nigeria (Voucher number: FUNAAB – 0065).

Preparation of plant extract

The rhizomes were washed through and rinsed properly, cut into small sizes, and air-dried to remove moisture present after they were dried and blended (homogenized) using a mechanical blender. The powdery turmeric was weighed (1000 mg) and treated with 200 ml of ethyl acetate. The solution was filtered using Whatman No.1 filter paper. Subsequently, the solvent was evaporated using a rotary evaporator under reduced pressure at a controlled temperature. The extracts were stored for subsequent biochemical analysis.

Animals

The Department Animal Ethical Committee approved this study (FUNAAB – BCH 2014/1139). Thirty female albino rats (150–250 g), caged in the of Biochemistry Department, University of Ibadan, Nigeria, were acclimatized for 2 weeks before experimentation, housed in the plastic cage under ambient conditions, and fed with pellet and clean water *ad libitum*. The animals were handled in strict conformity with the guidelines approved by the ethical committee.

Experimental protocol

Experimentation was according to An *et al*., Jang *et al*.[11] Thirty female albino rats weighing 150–250 g were first separated into five groups of 5 animals[12] each shown in [Table 1]. The first group of animals was given distilled water only while the other four groups were given a composed diet of 65% carbohydrate, 20.3% protein, 5% tat, 5% fiber, 3.7% AIN – 93 mineral mixture, and 1% AIN -93 vitamin mature[13] The control group received diet alone. Group 2 received diet supplemented with S4D (100 mg) Group 3 (10%) containing SD4 + 100 mg, *C. longa* Group 4 received 200 mg *C. longa,* and Group 5 received 250 mg, respectively. The study lasted for 3 weeks, after which the rats were fasted overnight and sacrificed; blood collected through retro-orbital plexus into non-coagulated tubes was allowed to clot and centrifuged for 10 min at 3500 rpm to obtain the serum. The animals were dissected, after cervical dislocation and the liver was excised. *C. longa* was washed thoroughly with distilled water dried and blended (homogenized) using ROSEC 7.4 200 rpm electric

Table 1: Experimental design.

Groups	Dosage administered
1	Distilled water with diet alone
2	10% S4D + 10 mg C.L
3	15% S4D + 100 mg C.L
4	200 mg C.L only
5	250 mg C.L only

blender. The homogenized powder was stored in a frozen state (at −10°C) and lyophilized at University of Agriculture Abeokuta Ogun State, Nigeria using I.T.E Scientific Lyotrap ultra freeze dryer with ice capacity of 10 kg (5 kg in 24 h) at temperature −55°C (heat extraction rate of −40°C.

Biochemical assays

The assay for glutathione-S-transferase (GST) followed the procedure of Habig *et al*.,[9] while Randox Diagnostic kits (Randox Laboratory Limited, Crumlin, United Kingdom) were used for the determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), and lactate dehydrogenase (LDH). The concentrations of creatinine, uric acid, urea, albumin, sodium, magnesium, calcium, potassium, and chloride were also determined with Randox kits

Statistical analysis

The results were expressed as the mean ± SEM (5), the level of homogeneity among the groups was assessed using one-way analysis of variance (ANOVA). Was run and supported by the least significant difference multiple comparisons test. All analyses were done using the Statistical Package for Social Science 20.0 and $P < 0.05$ was considered statistically significant.

RESULTS

[Figure 1] the effect of *C. longa* Linn extract on the tissue damage enzymatic biomarkers in the serum of 10% S4D in female albino rats. The dosage of 10% of S4D significantly ($P < 0.05$) increased the specific activities of serum ALT, AST, ALP, GGT, and LDH compared to the normal group. However, co-treatment with 200 mg and 250 mg kg^{-1} body weight significantly lowered the elevated specific activity of ALT with a slight reduction in AST, ALT, and GST was observed in the 100 mg kg^{-1} of *C. longa* group. The administration of 250 mg kg^{-1} of *C. longa* actually showed a significant decrease in the specific activities of ALT, GGT, and LDH compared to the normal control. About 10% S4D shows a marked elevation in the activities of AST, LDH, and GST in Group 2 compared to Group 3 [Figure 2]. Furthermore, a significant decrease was observed in the specific activities of renal ALT, GST, and LDH in S4D treated group. Treatment with 200 mg and 250 mg k^{-1} body of *C. longa* in comparison with the control [Figure 3] shows significant increases in the serum bilirubin, urea, creatinine, and uric acid level in 10% S4D group, which was significantly ($P < 0.05$) reduced after treatment with 200 mg of *C. longa* extract. The effects of *C. longa* Linn extract on electrolyte concentration are presented in [Figure 4]. Compared to the control group, the 10% of S4D group showed a marked decrease in Na^+ and mg^{2+}. Treatment with 200 mg and 250 mg/kg *C. longa* extract showed a significant increase in the serum Mg^{2+}, while serum K^+ and Ca^{2+} concentrations were significantly ($P < 0.05$) reduced.

DISCUSSION

We report for the rejuvenating potential effect of S4D on the hepatic damage as a result of the cytotoxicity effect of S4D. On treatment with *Curcumin,* there was a reversal effect on the elevation of ALT, AST, ALP, LDH, and GGT in the serum of S4D exposed rats. The liver and kidney are very important organs which play a pivotal role in the physiological processes in biological systems any alteration may lead to oxidative damage. Thus, these biomarkers enzymes are localized in the cellular plasma membrane (ALP) cytosol (ALT and LDH), and mitochondria (AST) of the liver and kidneys among other tissues and their elevation in the blood are associated with tissue damage.[15] Thus, we attribute the elevation of serum activities of the enzymes to S4D induced tissue damage and destruction of cell membrane integrity resulting in the leakage of these enzymes, into the blood. The effect of S4D resulting in tissue damage may be meditated through the propagation of oxidative stress.[16] Lindamood reported a dose-dependent elevation in the generation of reactive species, following the exposure of $HepG^{2\ cells}$ to S4D. An *in vitro* study also showed exposure to S4D, even after just 60 min.[1,17]

The present study indicated that the treatment with different concentrations of *C. longa* extract (200 mg and 250 mg kg^{-1}) significantly augmented the specific activities of serum ALT, AST, ALP, LDH, and GGT, compared to 10% S4D of 100 mg extract of *C. longa* in Groups 2 and 3 compared with the normal control.

These rejuvenating tendencies shown by *C. longa* on enzyme markers of tissue damage may be eluded to its hepato-protective tendencies reported by Mizutani *et al*.[18] which protects the kidney against any biochemical alteration. The significant decrease in the level of ALT and ALP noticed in this study further supports the claims of Jang *et al*. Jaeschke and Wendel.[13,19] These authors demonstrated that the injection of synthetic food colorants impaired hepato-rental function in male rats. AST, ALT, and LDH are cytosolic enzymes involved in the metabolism of carbohydrate and

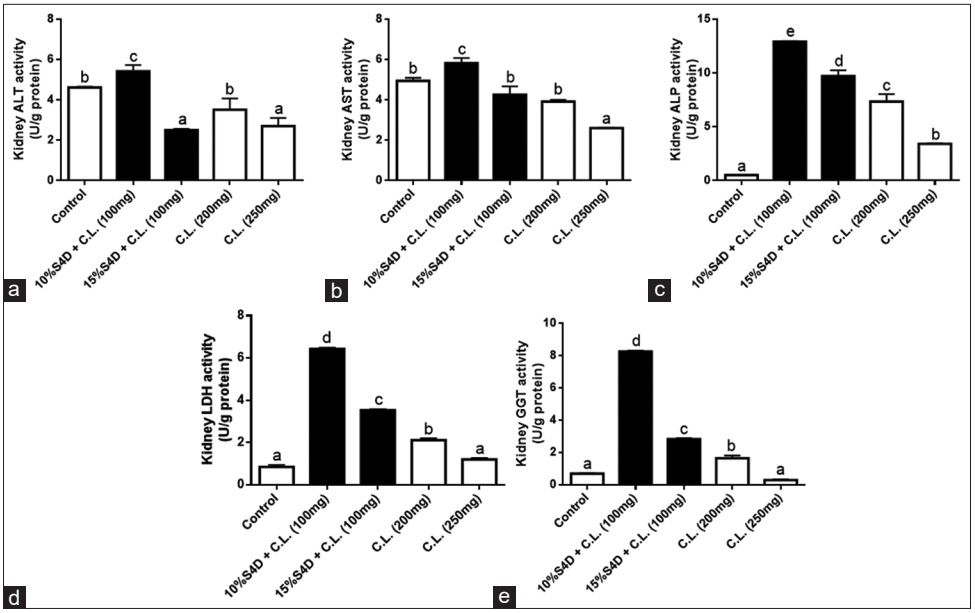

Figure 1: Effects of graded doses of Sudan (IV) dye and *Curcuma longa* (C.L.) extract on the activities of (a) alanine aminotransferase; (b) aspartate aminotransferase; (c) alkaline phosphatase; (d) lactate dehydrogenase; and (e) gamma glutamyl-transferase in the kidneys of experimental rats ($n = 6$). Bars are mean ± standard error. Bars with different letters are significantly different ($P < 0.05$).

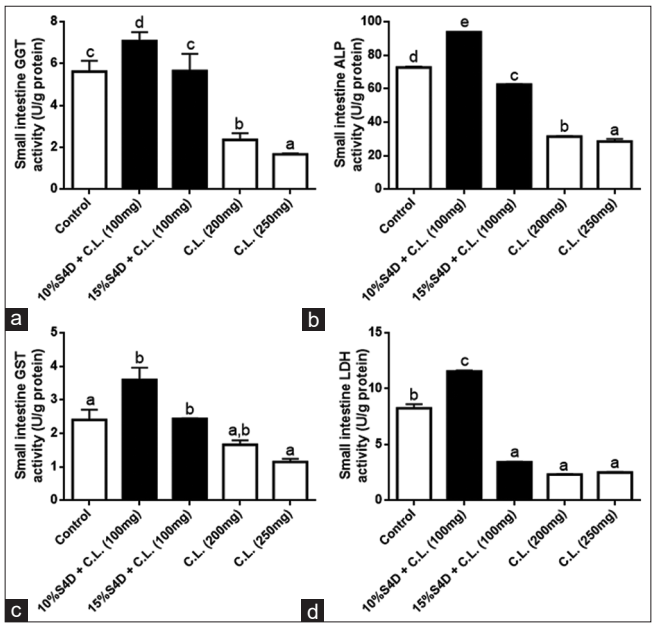

Figure 2: Effects of graded doses of Sudan (IV) dye and *Curcuma longa* (C.L.) extract on the activities of (a) gamma glutamyl-transferase; (b) alkaline phosphatase; (c) glutathione-S-transferase; and (d) lactate dehydrogenase in the small intestine of experimental rats ($n = 6$). Bars are mean ± standard error. Bars with different letters are significantly different ($P < 0.05$).

amino acids, which are highly expressed in the liver, muscle, and brain. In the course of liver damage, the functions of liver cells are compromised, while the levels of these enzymes increase in the serum, following the leakage across the hepatic plasma membrane into the blood. Therefore, increased serum activities of AST, ALT, and LDH, which we observed in all the groups exposed to S4D, indicate hepatic disease or necrosis.[20] This observed elevation in liver shows a dose-dependent trend.

GST is involved in the biotransformation and detoxification of harmful metabolites produced by S4D.[21] The liver and kidney are the major organs involved in the biotransformation of xenobiotic.[22] It is plausible to posit that these organs might have undergone oxidative stress instigated by S4D possibly leading to tissue damage and the observed elevation in serum enzymes and other markers. GST conjugates the electrophilic xenobiotics with the nucleophilic glutathione (GSH); thus, it is primarily involved in the detoxification of xenobiotics, including potential carcinogens.[23] Conservation of GST redox is, therefore, of inordinate relevance, to avert xenobiotic-induced hepatic damage.[24] The bio-transformed metabolite of S4D produced by the microsomal monooxygenase system is conjugated with GSH.[25] Meanwhile, in the liver, the induction threshold of the GSH conjugation system is greater than or equal to 100 mg/kg for S4D.[20] GST might be responsible for detoxifying the toxic metabolite produced by S4D. In addition, Glutathione-S transferase could also modulate the effect of *C. longa* has potent antioxidant properties that may be attributed to the bioactive components displayed by the extract to rejuvenate the tissue from toxic S4D. The liver produced the albumin which is present in the blood plasma protein play a key role in the transport of hormones, endogenous substances, and free tatty acid[26] also, bilirubin

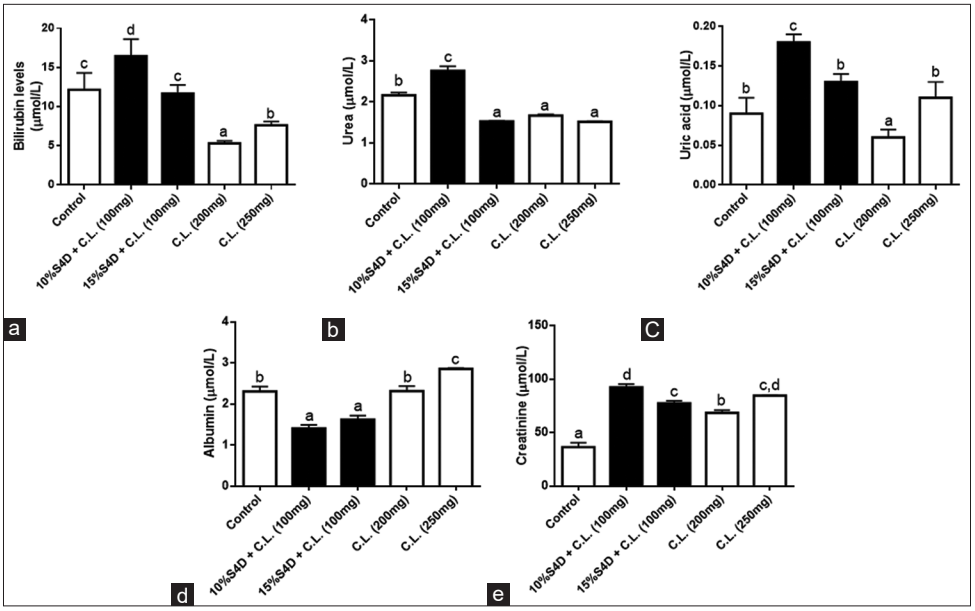

Figure 3: Effects of graded doses of Sudan (IV) dye and *Curcuma longa* (C.L.) extract on plasma levels of (a) bilirubin; (b) urea; (c) uric acid; (d) albumin; and (e) creatinine in experimental rats (n = 6). Bars are mean ± standard error. Bars with different letters are significantly different ($P < 0.05$).

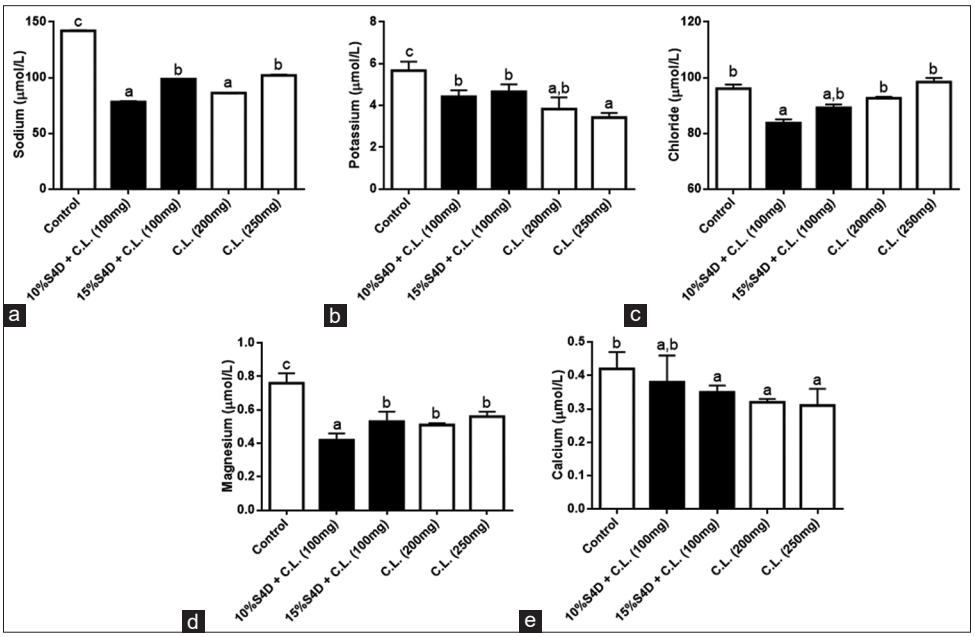

Figure 4: Effects of graded doses of Sudan (IV) dye and *Curcuma longa* (C.L.) extract on plasma levels of (a) sodium; (b) potassium; (c) chlorine; (d) magnesium; and (e) calcium in experimental rats (n = 6). Bars are mean ± standard error. Bars with different letters are significantly different ($P < 0.05$).

is a breakdown product of heme used in the assessment of hepatobiliary injury in preclinical and clinical studies of hepatic necrosis. Creatinine is a waste product of creatinine phosphate by muscle metabolism, while urea and uric acid are excreted in urine which is from the breakdown product of protein and purine metabolism, respectively. The observed concentration of serum bilirubin, creatinine, urea, and uric acid was consistent with previous reports Senthilkumar *et al*.,[26] Administration of S4D causes the accumulation of bilirubin, creatinine, urea, uric acid, and decrease albumin concentration in the serum. Co-administration with 200 mg and 250 mg kg body weight of *Curcumin,* however,

significantly increased the serum albumin concentration and decreased the serum levels of bilirubin, creatinine, urea, and uric acid compared with the normal control. On modulation of the serum bilirubin and albumin by *Curcumin* extract might be due to the enhancement of the activity of the microsomal enzymes UDP-glucuronyl transferase which is markably known for conjugation processes.[27] Electrolyte disturbance with xenobiotic could increase intracellular and extracellular fluid.[1,27] Increase magnesium excretion may occur in hypophosphatemia that results to reduced magnesium reabsorption at the loop of Henle and distal convoluted tubules.[28] Sodium (Na^+) level is always determined by the balance of the fluid in relation to the availability of sodium in the biological system.[28] Hyponatremia does not only constitute abnormality but also results in biochemical and physiological consequences.[28] Potassium (K^+) is another electrolyte in the kidney that plays an important role in the regulation of the physiological system exogenous substances have been found to reduce the amount of potassium excreted by the organ responsible for the metabolism of xenobiotic.[26] The reactive metabolite of S4D is eliminated by the kidney but may causes impairment in its ability to regulate the composition of the electrolyte balance[29] interestingly, the therapeutic potentials of this medicinal plant against S4D induce hepato-renal injury have become an area of scientific focus in this part of the country as most of our food products are not safe for human consumption as a results of intentional use of hazardous substances for attraction by vendors. From the results *Curcumin* exerts some pharmacological effect by maintaining the electrolytes balance and the metabolism of the liver enzymes by alleviating them from hepatic damage as well as antioxidant properties which can help to protect the cells against oxidation and several complications.

CONCLUSION

The recent studies exhibited significant antioxidant potentials that sustain the cells from the effect of S4D toxicity. Hence, the results indicate a possible ameliorative mechanism of the plant extract against carcinogenic effect of S4D induced hepato-renal injury. Therefore, we conclude that the extract of *C. longa* could be seen as a novel natural antioxidant that might have a protective effect against S4D induced hepato-renal injury by rejuvenating the activities of the enzymes and modulating the physiological activities of the serum electrolyte. Thus, the study indicates that the inclusion of *C. longa* into our daily diet might limit the effect of hepato-renal injury.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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