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The effect of gold nanoparticles synthesized by *Curcuma longa* aqueous extract against liver toxicity induced by acetaminophen in *Oreochromis mossambicus*

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ABSTRACT

Liver-related illnesses are becoming a major problem on a global scale. Several harmful substances, including carbon tetrachloride (CCl_4), acetaminophen (APAP), antibiotics, and thioacetamide, primarily attack hepatic cells. Thanks to renowned pharmaceutical businesses, herbal medicines for the treatment of liver ailments have been used for a long time in India and have gained popularity across the world. Herbal medicines are still not allowed in certain places to treat liver problems due to their restrictive characteristics. In our research, we employed green synthesised gold nanoparticle as a hepatoprotective agent in our investigation, which might be useful as a treatment.

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1. Introduction

One of the greatest problems in aquaculture currently is liver damage. Many farms are experiencing the "liver and gall syndrome," which manifests as liver enlargement (up to a twofold increase in size) and colour changes. It is unclear what causes this illness; no known infectious bacteria or viruses have been found. One of the main reasons for the illness may be the xenobiotic challenge brought on by drug addiction and environmental contamination.

According to studies, acetaminophen (APAP, N-acetyl-4-aminophenol) is the medicine that causes liver damage the most frequently (Ostapowicz et al., 2002). Acetaminophen overdose results in "Acetaminophen hepatotoxicity," which damages the liver, and is one of the most typical forms of poisoning worldwide (Vidhya Malar and Bai, 2012). A safe therapeutic amount of acetaminophen can produce hepatotoxicity and abrupt liver failure, but an overdose can also be harmful (Michaut et al., 2014). In both human and animal models, a higher paracetamol dosage results in hepatotoxicity (Jaeschke et al., 2014). Hepatitis, liver cirrhosis, and other conditions can result from paracetamol hepatotoxicity events (Fontana, 2008).

A significant amount of antibiotics and chemicals have been introduced to the water environment and fish feeds in an effort to prevent and manage fish infections, yet these substances might harm the fish. However, no proven treatments for liver and gall syndrome have been identified. As a result, the use of medicinal plants and plant-based medications to prevent and control this condition has received a lot of interest (Yin et al., 2011).

A member of the *Zingiberaceae* family is *Curcuma longa* L.Antioxidant, hepatoprotective; anti-inflammatory, anticarcinogenic, and antibacterial capabilities are only a few of its qualities. As an anti-inflammatory medication, it treats flatulence, jaundice, menstruation problems, hematuria, haemorrhage, and colic (Yu et al., 2006). Curcumin, the primary ingredient that acts, was in responsible for the pharmacological functions. Curcumin has many properties such as antioxidant, anti-inflammatory, antiviral and antifungal actions.

Gold is a noble metal that has been used for ornamentation and medical applications since ancient times. Recently, the green production of gold nanoparticles has received more attention globally. These metallic nanoparticles might now be used for antibacterial activities (Sathish Kumar et al., 2009), medical imaging (Muthu & Wilson, 2010), and wound dressing (Ghosh et al., 2010). Animal studies have demonstrated the antioxidant and hepatoprotective benefits of various extracts of andrographolide (Roy et al., 2012), Phyllanthus amarus extract (Mishra et al. 2013), and green tea extract (Mukherjee et al., 2015). The current work investigated green synthesised gold nanoparticles mediated by Curcuma longa for their hepatoprotective and antioxidant activities in acetaminophen-induced hepatotoxicity in *O. mossambicus* fish.

2. Materials and Methods

Plant Material

Curcuma longa (*Zingiberaceae* family) were collected from the Botanical garden of Fatima Mata National College (Latitude: 8.8932 ^{0}North and Longitude: 76.6141^0 East), Kollam district of Kerala in December 2015. The Department of Botany Fatima Mata National College Kollam district, Kerala, has identified the Collected samples.

Preparation of *Curcuma longa* aqueous extract

A fresh and healthy *Curcuma longa* rhizome was cleaned with de-ionized water after being rinsed in flowing tap water with a clean brush to remove any dirt. The extraction was carried out based on Cooper and Gunn's methodology (2005). One gram of finely chopped *C. longa* was thoroughly pulverised in a mortar and pestle with 10 ml of distilled water.The aqueous extract of *C. longa* was filtered via the Whatman filter paper. The filtered extract was centrifuged at 1000 rpm for ten minutes.. The filter was cooled and stored in the refrigerator until it was needed.

Biosynthesis of gold nanoparticles

Gold nanoparticles were prepared from aqueous Chloro auric acid solution (0.3M) as a precursor and using prepared turmeric rhizome extract as a reducing agent in aqueous

medium. To synthesis gold nanoparticles from *C.longa,* 30 microliter of aqueous solution of 0.3M chloroauric acid ($HAuCl_4.3H_2O$) solution carefully added to 10 millilitre of *C.longa* extract from a conical flask at room temperature under static conditions Within 15 min of addition of extract, the colour of the solution changed from pale yellow to pale pink, indicating the formation of gold nanoparticles (Sree lekshmi *et al.,* 2013).

Collection and Maintenance of *Oreochromis Mossambicus*

O. mossambicus, a freshwater fish, is chosen as the experimental subject. Before the trial, O.mossambicus were obtained from the government hatchery of the Agency for Development of Aquaculture (ADAK) in Varkala, Kerala, and acclimated to the lab for two weeks. They were provided a control diet on ad lib during the acclimatisation period. Feed was rationed twice daily. *O. mossambicus* was raised in a lab environment with ideal salinity, pH, and temperature conditions. After rearing, fishes with an approximate size of (20±0.49g) were taken for the experiment.

Experimental Design

The fish were divided into four groups consisting of 6 fish in each. Fishes were treated as follows:

Group I: - Control fish (CON) were supplemented with commercial pellet feed

Group II:-Fishes were treated with (Acetaminophen) APAP for 7days

[APAP; 500 mg/kg was dissolved in 50 mL water at 70°C, cooled to room temperature, and added to the static tank].

Group III: - Fishes are treated with APAP for 7 days and simultaneously administered CL AuNPs [30µl($HAuCl_4.3H_2O$+10ml *C. longa* extract in 100gm basal feed]for 7 days.

Group IV: - Fishes were treated with CL AuNPs [30µl($HAuCl_4.3H_2O$+10ml *C.longa* extract in 100gmbasal feed] for 7 days

The experiments were set up in triplicate, and at the end of the experimental period, the selected fishes were sacrificed by decapitation. Blood was collected and allowed to clot at room temperature and then centrifuged at 3000 rpm at 4˚C for 20 min to obtain serum in which analysis of protein and enzymes (ALT, AST and ALP) were carried out. The liver was dissected immediately and homogenized in 0.01 M Tris-HCl buffer (pH 7.2) using Teflon homogenizer and centrifuged at 12,000 g at 4˚C for 20 min to obtain supernatant fractions for the determination of metabolic enzymes and hepatotoxic markers.

Biochemical Assessment of Hepatotoxicity

Alanine Transaminase (ALT, E.C.2.6.1.2), Asparate transaminase (GOT, E.C.2.6.1.1), Alkaline phosphatase (ALP, E.C.3.1.3.1) albumin, bilirubin, creatine were estimated by UV double spectrometry using the kits prepared by Span Diagnostic Ltd India.

Statistical Analysis

The values were expressed as mean ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by the Tukey multi comparison test. P values < 0.005 were considered as significant.

3. Results and Discussion

Assessment of serum marker enzymes in acetaminophen-induced hepatotoxicity in O. *mossambicus.*

The damage to the plasma membrane caused by APAP was assessed by monitoring the levels of ALP, ALT and AST in the serum. Intoxification of tilapia treated with APAP significantly altered the liver biochemical parameters when compared with the normal control fish ($p<0.005$). Table 1 depicts the effects of CL AuNPs on hepatic marker enzymes in the serum of control and experimental fishes. In APAP-intoxicated fishes, there was an elevation in the levels of AST, ALT and ALP in comparison with the control group. The elevated levels of ALT (152.40±5.48), AST (156.85±4.95) and ALP (115.30±4.25) in group II decreased respectively as (90.52±2.86) (62.30±3.15) and (78.55±3.10) in Group III (APAP+CLAuNPS*)* treated fishes. GNP-treated control fish did not show any alterations in these enzymes.

Acetaminophen-induced hepatic damage was accompanied by significant elevation of both blood and hepatic tissue GPT, GOT and ALP about 60%. ALT and AST are two mitochondrial enzymes (Gharaei *et al.,* 2011) and are also found in the cell cytoplasm in higher concentrations, particularly ALT. Higher levels of these enzymes in blood might result from cell leakage due to their high concentration and cell membrane damage. It has been reported that paracetamol metabolism triggers lipid peroxidation and causes liver injury in fish and mice (Gharaei *et al.,* 2011). These results are in agreement with those reported earlier by Senthilkumar *et al.,* 2008. Treatment with green synthesized gold nanoparticles, significantly restored elevated levels of both blood plasma and liver tissue GPT, GOT and ALP. *Amaranthus tricolor L,* aqueous extract has significant hepatoprotective effects against paracetamol-induced toxicity. This has been evidenced by biochemical investigations of SGOT, SGPT, ALP, total bilirubin, and histopathological studies (Aneja *et al*., 2013).

Table 1. Fluctuations of serum marker enzymes in acetaminophen induced hepatotoxicity in *O.mossambicus* supplemented with CLAuNPs

Group	Treatment	ALT U/L	AST U/L	ALP U/L
I	(Control)	86.78±4.12	49.42 ±2.40	67.65±2.23
II	(APAP)	152.40±5.48^{a*}	156.85±4.95^{a*}	115.30±4.25^{a*}
III	(APAP+CLAuNP)	90.52±2.86^{b*}	62.30±3.15 ^{b*}	78.55±3.10 ^{b*}
IV	(CON+CLAuNP)	89.30±2.62 ^{c*}	50.45±2.69 ^{c*}	70.35±3.66^{a*}

Values are listed as mean± SD (n=6 in each group) CON control, APAP acetaminophen, CLAuNP *C.longa* nanotized gold nanoparticles
a ($p < 0.05$) as compared to CON **b** ($p < 0.05$) as compared to APAP **c** ($p < 0.05$) as compared to CON and APAP+CLAuNP. *indicates significant level at 5% Data within the groups were analyzed using one-way ANOVA followed by Tukey multiple comparisons test.

Table 2. Fluctuations of serum biochemical levels in APAP induced hepatotoxicity in *O.mossambicus* supplemented with CLAuNPs

Treatment	Protein (g/dl)	Albumin (g/dl)	Bilirubin(g/dl)	Creatinine (mg/dl)
Control	9.50±0.336^{b*}	5.625±0.422^{c*}	2.55±0.191^{a*}	3.55±0.404^{a*}
APAP	6.325±0.298^{a*}	3.525±0.221^{a*}	4.725±0.170^{c*}	5.575±0.403^{C*}
APAP+ CLAuNP	8.375±0.262^{b*}	3.6±0.141^{ab*}	2.25±0.331^{a*}	3.425±0.309^{bc*}
CON+ CLAuNP	9.4±0.294^{b*}	4.25±0.25^{b*}	2.625±0.377^{b*}	3.675±0.9^{ab*}

a ($p < 0.05$) as compared to CON b ($p < 0.05$) as compared to APAP c ($p < 0.05$) as compared to CON and APAP+CLAuNP.

Assessment of serum biochemical constituents in acetaminophen-induced hepatotoxicity in *O. Mossambicus*

Table 2 shows the activities of the liver homogenate in protein, albumin, bilirubin, and creatinine of control and experimental fish. The levels of protein were significantly ($p<0.05$) reduced in the APAP-treated fish (6.325±0.298) compared to those of the control fish (9.50±0.336). Whereas the metabolic alterations were restored to near-control ($p<0.05$) in the APAP+GNaP treated fish (8.375±0.262). GNaP treated control fish did not show any alterations in the protein levels (9.4±0.294). The levels of albumin, were significantly ($p<0.05$) reduced and increased the value of bilirubin (4.725±0.170) and creatinine(5.575±0.403) in the APAP-treated fish compared to those of the control fish(2.55±0.191, 3.55±0.404) . Whereas, the metabolic alterations of these parameters were significantly restored to near-control ($p<0.05$) in the APAP+GNaP treated fish. GNP-treated control fish did not showed any alterations in the proteins and creatinine levels.

The present results showed that high dose of APAP deplete the hepatic levels of proteins and albumins and elevate the levels of bilirubin and creatinine. Since there is a close relationship between the rate of protein synthesis in the liver tissue and total protein concentrations in the plasma. The depleted level of total plasma protein (composed of albumin and globulin) reflects the decrease in protein synthesis in liver tissue. Administration of CL AuNPs showed a significant effect on both blood and liver tissue protein, and albumin levels. An increase in creatinine indicates pathological changes in hepatic biliary flow (Ravikumar *et al.,* 2005).

The herbal compounds present in *C.longa* (terpenoids, flavonoids and alkaloids) exerted their anti-inflammatory and antioxidant activities, which, combined with gold nanoparticle, helped ameliorate chemical/drug-induced hepatotoxicity in animal model.

4. Conclusion

To conclude nanotized gold nanoparticle exerted their hepatoprotective effects in experimental animals by their antioxidant property helped to reduce the pathogenesis of chemical/ drug-induced hepatotoxicity.

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