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# Repercussions of gallic acid loaded chitosan nanoparticles upon fluoride induced hepatotoxicity in Gold fish

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**ABSTRACT**

Nano delivery system is a rapidly developing science where materials in the nanoscale range are employed to serve as means to deliver therapeutic agents to specific targeted sites in a controlled manner. Gallic acid chytosan nanoparticle (GA-CSNPs) is found to be superior due to its high phenolic content and possesses antioxidant, immunomodulatory, anti-cancer, and antimicrobial effects. Fluoride, a naturally occurring element found in air, soil and water, has high affinity for calcium. It is abundantly used in semi-conductor and nanotechnology industries and hence can be found in industrial effluents. Exposure to excessive fluorides can be deleterious to both aquatic and terrestrial life. Fish have been recognized as bio-indicators for environmental contamination. The toxicants enter the fish mainly through the gills and accumulate in other tissues, such as the liver and kidneys, as they are the major organs of detoxification. Goldfish, a popular freshwater fish is employed in reducing mosquito populations in stagnant water bodies. They were chosen as a model system since they are small, inexpensive, colourful and very handy. GA-CSNPs will be prepared and characterized by scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR). Exposure of fish to sodium fluoride would induce stress, which in turn will reduce the protein and disturbance in the liver can be monitored by assaying marker enzyme (ALT, AST) and histopathological changes in the liver. Administration of GA-CSNPs is expected to de-escalate the effects of sodium fluoride.

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## 1. Introduction

Direct atmospheric deposition, volcanic eruptions, earthquakes, weathering of rocks and mountains, discharge of agricultural, municipal, domestic, and industrial waste and other anthropogenic activities upset the physiochemical parameters of the aquatic environment, perturbing its ecosystem. The contaminants gain entry either directly from the surrounding water through skin, muscles, and gills or indirectly by consumption of small fish, invertebrates and aquatic vegetation (Rajeshkumar and Li, 2018). Escalation of these contaminants beyond the threshold level will pose a serious threat to aquatic life due to sustainability, toxicity, bioaccumulation and biomagnification (Authman et al., 2015). Alterations in the characteristics of aquatic habitats were well documented using fish as bio-indicators (Lamas et al., 2007). *Carassius auratus* (Goldfish), a popular freshwater fish common in aquariums employed in reducing the mosquito population in stagnant water bodies (Gupta and Banerjee, 2009), was chosen as a model system since they are small, inexpensive, colourful and very handy. The liver is a metabolically active organ responsible for many vital life functions. Apart from being a vital detoxifying organ that brings about biotransformation and excretion of contaminants, it is also a crucial compartment that accumulates heavy metals (Jaric et al., 2011) that end up in organ injury. Measurement of biochemical indices in the liver helps us to study the impact of damage incurred and infer the response of fish to the degree and type of contaminant (Barhoumi et al., 2012).

Fluoride, a naturally occurring mineral, is distributed in water, soil, plants, rocks and air. The weathering of rocks forms the major doorway of fluoride into the aquatic system. Exposure to elevated levels of fluoride results in a condition called fluorosis. Among the aquatic species, fish are more vulnerable to the deleterious effects of fluoride toxicity because of its accumulation in bone, teeth and scales. Earlier studies with fluoride stress in experimental fish infers a reduction in the level of glucose and protein in blood and muscles (Gupta, 2003), elevation in the level of cholesterol and glycogen in liver (Aziz et al., 2013) and alteration in the activity of enzymes in the liver and muscles (Chitra et al., 1983) revealing the adverse effects of fluoride. This leads to an imbalance between oxidants and antioxidants, which results in metabolism and structural alterations (Yadav et al., 2014). Upon long-term exposure the effects may pose a challenge to their survival. The oxygen radical absorbing capacity and the bactericidal potential with respect to the inhibition of membrane function of the phenolic compounds have paved the way for its extensive use as a bioactive compound (Tripoli et al., 2007; Limwachiranon et al., 2019). Among the phenolic compounds, gallic acid is employed as a drug for pathological conditions such as diabetes (Fachriyah et al., 2017), cancer, inflammation (Faried et al., 2007), obesity (Hsu and Yen, 2007) also has the upper hand in controlling the microbial growth (Chanwitheesuk et al., 2007). Gallic acid retrieved both enzymatic and non-enzymatic antioxidants during oxidative stress induced by fluoride (Nabavi et al., 2012). The use of gallic acid in therapeutic interventions is unsatisfactory because of the alterations in its physiochemical properties and reduced bioavailability. The major reason for this could be its size (Fachriyah et al., 2017). Hence to increase the solubility, absorption, and bioavailability and decrease the risk of degradation of phenolic compounds it is necessary to encapsulate them onto a bio-polymer which can be polysaccharide, protein, or lipid in nature (Li et al., 2015). Studies by Ji et al. (2006), recorded that the natural compound GA is transformed

into a novel green antioxidant upon encapsulation with chitosan which is attributed to the high reducing property of its tri- hydroxyl group, hydrophilicity impacted due to the presence of hydroxyl and carbonyl groups and the obstruction posed on the hydrogen bonding network of chitosan due to its bulkier benzene ring.

Nano delivery system is a rapidly developing field that bridges biological and physical sciences employing materials in the nanoscale range. Due to their nano size (1-100 nm), they readily penetrate the tissue, ease the uptake of the drug by the cells and ensure its impact on the delivered site. This nanostructure not only helps in the delivery of sparingly water soluble compounds but also enhances the bioavailability by staying in the circulation for a longer duration and preventing the loss in the gastrointestinal region (Patra et al., 2018).

The primary component in the shell of cephalopods is chitin, a long-chain polymer of N-acetyl glucosamine. Deacetylation of this glucose derivative results in a non-toxic, biocompatible, biodegradable, non-antigenic polysaccharide chitosan (CS). CS finds major applications in biomedical science due to its anti-microbial properties (Ngo et al., 2015). This can act as a suitable vehicle for encapsulating diverse compounds that are employed in therapeutic interventions (Aljawish et al., 2014). Permutations in the reactive groups: the free amino groups on deacetylated units at C2 and the hydroxyl groups at C3 and C6 results in the conversion of CS into a nanoparticle. Ionic gelation is an encapsulation method that brings about the solid-gel transition of biopolymer in the presence of polyanions with a high density of negative charges (e.g., Sodium tripoly phosphate - TPP). The biocompatible cross-linked chitosan nanoparticles (CSNPs) are prepared as a result of the interaction of multivalent anion TPP with the positively charged amino group of CS (Jonassen et al., 2012). CSNPs help enhance the bioavailability and therapeutic efficacy of CS and other drugs like gallic acid that are poorly soluble (Lamarra et al., 2016), which could be attributed to the large surface area of the nanoparticle (Abdel-Wahhab et al., 2016).

This paper presents details of the stress imparted by toxicant (sodium fluoride) and the effect of drug-loaded nanoparticle (GA-CSNPs) by evaluating the bio-indicators such as total tissue protein, liver marker enzymes (ALT & AST), histopathological changes in the liver of goldfish.

## 2. Materials and Methods

### 2.1. Preparation of Gallic acid-loaded Chitosan Nanoparticles

Chitosan nanoparticles were synthesized by ionic gelation phenomenon following the method of Samrot et al., (2018), where the biopolymer in the presence of TPP is converted to a nanoparticle on which the gallic acid is loaded. The produced nanoparticles were lyophilized and stored at $4^0$ C.

### 2.2 Characterization of Nanoparticles

**2.2.1 Fourier-Transform Infrared Spectroscopy (FTIR):** The gallic acid loaded and unloaded chitosan nanoparticles were analysed by FTIR. The pellets were prepared on a KBr press. The spectra were scanned over the wave number range of 4000 to 400 $cm^{-1}$ using IR Affinity-1s (Shimadzu, Japan) instrument.

**2.2.2 Scanning Electron Microscopy (SEM):** The chitosan nanoparticles with and without gallic acid were loaded onto carbon strips and sputter coated with gold. The sputtered samples were examined under SEM.

### 2.3 Study animal

Disease-free fish *Carassius auratus* (goldfish), each weighing 50-70 grams were procured from ornamental fish breeders at Kolathur, Chennai. They were acclimatized to the laboratory conditions prior to the experiment in 100L glass tanks at room temperature. They were maintained at controlled conditions and fed with commercially available pellet feed.

### 2.4 Experimental design

After two weeks of acclimatisation, the fish were divided into four groups each having six replicates and a completely randomized design (CRD) was followed to set up the experiment.

**Group I (control group)**: The fish were treated only with pellet food

**Group II (gallic acid-loaded chitosan nanoparticle)**: The fish were treated with 280mg/kg body weight of gallic acid-loaded chitosan nanoparticle (Abdel-Wahhab et al., 2016).

**Group III (Sodium fluoride-treated group)**: The fish were treated with 21 mg/ L of sodium fluoride (Aziz et al., 2013). Sodium fluoride was dissolved in the water.

**Group IV (Sodium fluoride/gallic acid-loaded chitosan nanoparticle co-administered)**: The fish were treated with sodium fluoride (21 mg/ L) and gallic acid-loaded chitosan nanoparticle (280mg/kg body weight) simultaneously.

At the end of the experimentation (96 hours), all animals were sacrificed under light anaesthesia, then rapidly dissected and subjected to the following biochemical assay and histopathological evaluation.

### 2.5 Preparation of homogenate

The liver was dissected out carefully, washed in ice-cold saline, blotted and weighed. It was then transferred into a centrifuge tube filled with PBS and homogenized. The resulting homogenate was centrifuged at 10,000 g for 20 min in a refrigerated centrifuge at $4^0$ C. The clear supernatants collected were used for protein estimation and assaying the activity of enzymes.

### 2.6 Estimation of protein

The levels of protein were estimated by the method described by Lowry et al. (1951), with alkaline copper reagent and Folins phenol reagent using BSA as standard. The protein levels were expressed as mg%.

### 2.7 Assay of amino transferase activity (ALT and AST)

The activity of ALT and AST was determined by Reitman and Frankel method (1957) with 2,4-dinitrophenyl hydrazine using sodium pyruvate as standard. The enzyme activity was expressed as µmoles of pyruvate liberated/ min/mg protein in tissue homogenate.

### 2.8 Histological evaluation

A part of the liver tissue was fixed in 10% formalin for

histopathological analysis. 5µm sections were cut from the paraffin-embedded blocks and were allowed to float on a hot water bath containing distilled water. The flattened sections were collected on clean poly-L-lysine coated glass slides and dried overnight. For optimal adhesion, the slides were placed in a $60^0$C oven for 1h.

### 2.9 Statistical Analysis

Results will be expressed as mean ± standard error (S.E.). The statistical significance of differences between the experimental groups will be calculated by student's t-test. Differences were considered significant at $p<0.05$, $p<0.01$ and $p<0.001$. Statistical analysis was carried out using the statistical software package version 7.0 (IBM SPSS statistics Advanced Analytical Solutions and Service provider in the UK and Ireland, Presidion London Office, Bracknell, UK).

## 3. Results and Discussion

Accumulation of contaminants in the water bodies for a longer period has led to a significant impact on aquatic species, especially the fish population. Fluoride, an essential trace element for the mineralisation of skeletal structure, becomes noxious when exceeding the threshold limits, increasing the generation of free radicals like superoxide anion ($O_2^-$), hydrogen peroxide and peroxynitrite (Puntoriero et al., 2018). GA a well-known antioxidant exhibits cytoprotection against liver fibrosis induced by carbon tetra chloride (Wang et al., 2014). The application of polyphenol extracts in the native form is restricted due to its unpleasant flavour, decreased solubility and reduced bioavailability. These hindrances can be overcome by encapsulation of gallic acid in a biopolymer such as chitosan resulting in the production of nanocomposite. This paves the way for the implication of nanotechnology in nutraceuticals and therapeutics (Pinheiro et al., 2015). The formed nanoparticle has to be characterised before ascertaining its use for therapeutic interventions.

### 3.1 Characterization of Nanoparticle

#### 3.1.1 Fourier-Transform Infrared Spectroscopy (FTIR) analysis of nanoparticle

The interaction of TPP with CS brings about a spectral change, which is denoted by the frequencies signified by 1a-7a, which is represented in Fig. 1a. Characteristic bands at ~ 1250 $cm^{-1}$ and ~ 1150 $cm^{-1}$ attributed to the stretching vibration of P=O and the symmetric and anti-symmetric stretching vibrations in the O–P=O group, respectively. The ionic crosslinking of the amino group of CS by polyvalent anion of TPP is inferred from the presence of spectral bands at~ 1000 $cm^{-1}$ and ~ 850 $cm^{-1}$ which agrees with the data in the literature (Loutfy et al., 2016). A reduction in the absorbance at 1562 $cm^{-1}$ and a shift to at 1536 $cm^{-1}$ in the FTIR spectra of cross-linked chitosan, denoted the formation of ionic bonds (Lamarra et al., 2016). Consistently in the present study, the spectral band at ~ 1600 $cm^{-1}$ of CS was shifted to ~ 1550 $cm^{-1}$ with variation in absorbance which could be the result of the interaction of protonation of the amino group of CS by TPP. The report of Abdel-Wahhab et al. (2016) showed the presence of a new peak at 1256 $cm^{-1}$ and the decrease of intensity at 1628 $cm^{-1}$ inferring that anionic phosphate groups of sodium polyphosphate have interacted with the free amino groups of CS coincides with the present finding with CSNPs.

Analysis of the FTIR spectrum of GA-CSNPs as shown in Fig. 1b. (2a-2d) revealed a reduced absorption at ~ 3450 $cm^{-1}$ that denotes a fall in the O-H stretching of benzene ring. The disappearance of the band at ~ 1550 $cm^{-1}$ on comparison with the spectra of CSNPs and the presence of new peak at ~ 1450 $cm^{-1}$ reveals the encapsulation of GA by CSNPs which is supported by the findings of Abdel-Wahhab et al. (2016). This also ensures the change of the primary amine to the secondary amine resulting in the amide bond formation between the hydroxy groups of GA with the amino group of CS (Liu et al., 2013). The band at ~ 1750 $cm^{-1}$ is assigned for the C-O stretching in esters conferring the ester bond formation between the carboxyl group of GA and amino group of CS which coincides with the literature (Zheng et al., 2018). The report showing C=O band near 1690 to 1630 $cm^{-1}$and the peak around 3600$cm^{-1}$ denoting the OH stretching of benzene ring was identified in the FTIR spectrum of GA nanocomposites (Samrot et al., 2018) coincides with the present study. The successful interaction of GA with CS resulting in the formation of nanoparticle (GA-CSNPs) is evidencedby the spectral analysis of CS, CSNPs, GA and GA-CSNPs which can be further confirmed by the SEM studies that give information on the particle size.

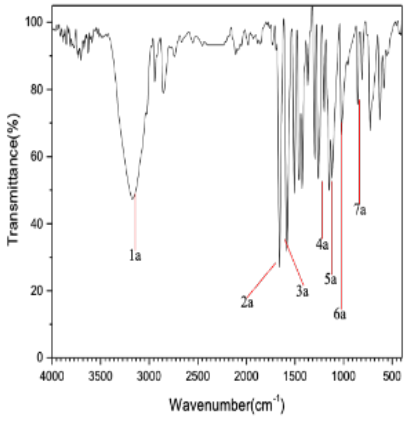


**Fig. 1a.** The FTIR spectrum of CSNPs

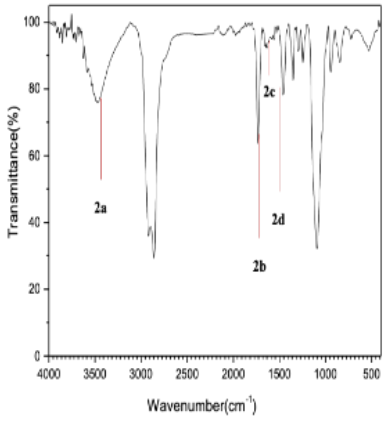


**Fig. 1b.** The FTIR spectrum of GA-CSNPs

### 3.1.2 Scanning Electron Microscopy (SEM)

Studies on drug encapsulation highlighted by Samrot et al. (2018) that the concentration of chelators and the pH of the reaction mixture will have an impact in determining the size of the nanocomposite. Encapsulation of GA on to CSNPs could be the reason for the variation in size between CSNPs and GA-CSNPs. In the present study ionic gelation of CS biopolymer into CSNPs using TPP is proved by the SEM images with the dimension range of CSNPs from 80 nm - 120 nm (Fig. 2B) against non-homogenous and non-smooth facade fluffy in nature with larger particle size with the diameter 5 µm (Fig. 2A) inferring the interaction of positively charged amino group of CS with the negatively charged phosphate ions of TPP. The blue arrows in plate B represent nanoparticles of size 0.08µm, the yellow arrows represent nanoparticles of size 0.1µm and the red arrows represent nanoparticles of size 0.12 µm. The gallic acid-loaded CSNPs appeared spherical in shape with non-uniform distribution in size ranging from 140 to 190 nm (Fig. 2D) in contrast to the crystalline macro structure of GA (Fig. 2C). The yellow dots in plate D represents GA-CSNPs of size 0.17 µm and the blue dots represent GA-CSNPs of size 0.19 µm.

### 3.2 *In-vitro* studies

The long term exposure to certain crucial elements poses them as toxicants that induce stress which is de-escalated by the antioxidant system in the organism thereby alters cellular homeostasis (Puntoriero et al., 2018). Studies on the toxic effect of sodium fluoride by Vishal and Singh, (2012) reported a decrease in the protein content which is directly proportional to the concentration and duration of exposure. A fall in the level total protein, albumin, globulin, and glucose (Chen et al., 2013) was highlighted during stress induced by sodium fluoride in *C.carpio*. A study indicating such depletion in fish models during pollutants-induced hypoxic condition (Padmini et al., 2015) support to the decreasing levels of proteins in the present study when exposed to sodium fluoride. GA-CSNPs function as a potential chain breaking anti-oxidant which is depicted by an increase in the radical scavenging activity ($ABTS^{+}$). The synergistic action of CS as a deactivator of metal ions and GA as a donor of hydrogen atoms has improved the usage of GA-CSNPs in combating stress (Xie et al., 2014). The toxic effect of ochratoxin A (OTA) was curtailed by blocking its active site, which was enhanced by the grafting GA onto CSNPs. Co-administration of OTA and GA-CSNPs brought back the level of total protein, albumin, globulin and A/G ratio to near normal (Abdel-Wahhab et al., 2016). Similarly, the present research work shows a significant decrease in the level of liver proteins in group III when compared with group I, which could be due to the OS imposed on proteins and the cell's response to defend against the stress. From the significant increase in the hepatic protein level in group IV in comparison with group III we can infer the protection rendered by GA-

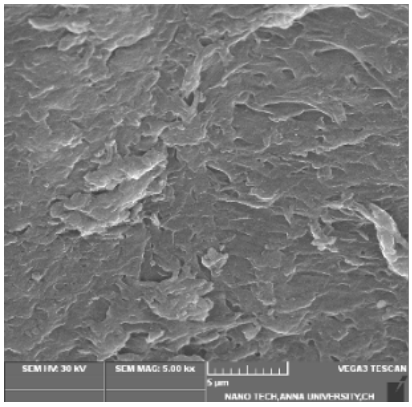

Fig. 2A.

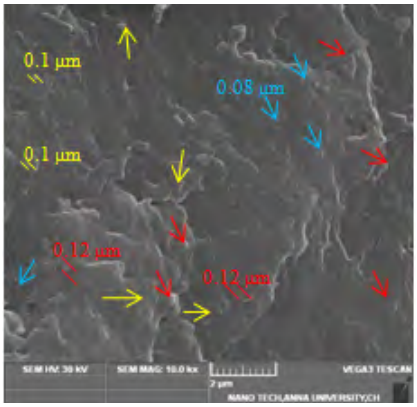


Fig. 2B.

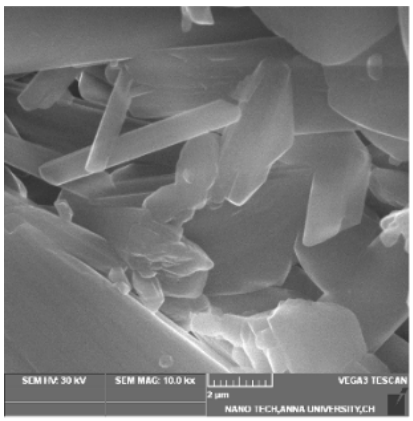

Fig. 2C.

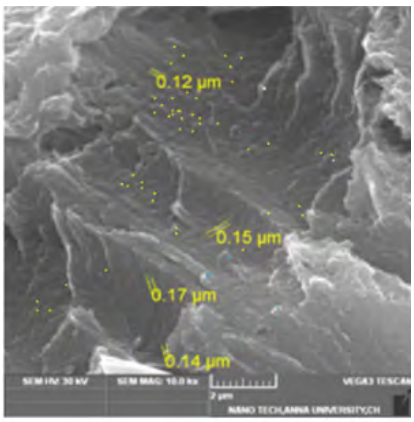


Fig. 2D.

**Fig. 2.** Result of scanning electron microscopic study of Chitosan (CS), CSNPs, Gallic acid (GA) and GA-CSNPs. 2A represents the biopolymer CS. The size and distribution of CSNPs are represented in 2B. 2C represents the crystalline structure of GA. The size and distribution of GA-CSNPs are represented in 2D

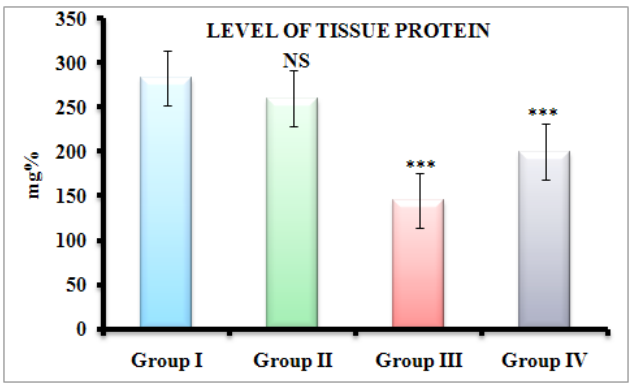


**Fig. 3.** Levels of protein in liver tissue of control and other treated groups

Comparisons are done between Group II vs Group I, Group III vs Group I, and Group IV vs Group III. Results are expressed as mean ± SEM (n = 6). NS= Not significant, *p<0.05, **p<0.01, ***p<0.001 statistical significance difference between control and treated groups

CSNPs to restore the energy homeostasis in the cell. No significant variation in the tissue protein level in group II when compared with group I portrays that GA-CSNPs treated group mimics the control group, emphasising the undisturbed metabolic status of the cell.

Toxicants disturb the energy balance by exploiting the food reserve of the cell. Increased catabolism of proteins to yield amino acids (Muley et al., 2007), which are fed to the central pathway that forms a major link between the carbohydrates-protein metabolisms via transaminases through anaplerotic reaction to satisfy the energy demands. The rise of marker enzymes in the extracellular fluid is due to its exudation from damaged tissue or accumulation within the cell, which is an index of altered membrane permeability (Yousafzai and Shakoor, 2011). Thus, the measurement of marker enzymes ALT and AST throws light on the functional capabilities of the liver, the major detoxifying organ. The intracellular localization of ALT and AST establishes that ALT is a more specific indicator of liver inflammation than AST. The threat induced by elevated levels of fluoride in *C. Carpio* was inferred by a marked rise in the level of ALT and AST (Chen et al., 2013). Exposure of freshwater fish *Oreochromis niloticus* to metals like cadmium and zinc showed increased activities of both ALT and AST of which ALT elevation was prominent during cadmium insult (Firat and Kargin, 2010). Consistent with these, the present study has shown a significant increase in the activity of ALT ($p<0.001$) and AST ($p<0.05$) in group III compared to group I revealing the damage imparted to liver by fluoride. The result is supported by the findings of Azis et al. (2013) in gills of fresh water fish *Tilapia mossambica* upon fluoride exposure. Studies of Latief et al. (2016) demonstrated that gallic acid de-escalates the hepatic injury due to N'-Nitrosodiethylamine (NDEA), a prominent hepato-toxin, carcinogen and a mutagen. The inhibitory effect of GA on fluoxetine-induced liver damage is represented by the changes in AST and ALT levels (Karimi-Khouzani et al., 2017). Treatment of catfish with OTA and GA-CSNPs succeeded in normalizing the ALT and AST levels (Abdel-Wahhab et al., 2016). These reports revealed support for the present finding, which recorded a significant decrease in the activity of ALT ($p<0.001$) and AST ($p<0.05$) in group IV compared to group III, revealing the promising hepatoprotective effects of GA-CSNPs. The insignificant variation of these marker enzymes in group II compared to group I depicts that the GA-CSNPs treated group mimics the control group, highlighting intact hepatic tissue. The above finding is reinforced by the report which states that treatment of cat fish with GA-CSNPs alone did not induce the biochemical parameter ALT and AST (Abdel-Wahhab et al., 2016).

The histological changes in *Odontesthes bonariensis* from Chasico Lake revealed peliosis area, cellular degeneration, focal necrosis, nuclear pyknosis and dilation of blood sinusoids leading to hemosiderosis (Puntoriero et al., 2018) highlighting the impact of toxicants. Fluorotoxicosis in *Channa punctatus* recorded disorganised liver cord, damaged connective tissue, vacuolated hepatocytes, degenerated cytoplasm, enlargement and pyknosis of hepatic nucleus, thickening and inflammation of central vein capillary wall, dilation of central vein and sinusoids with haemorrhage and ultimately focal necrosis (Haque et

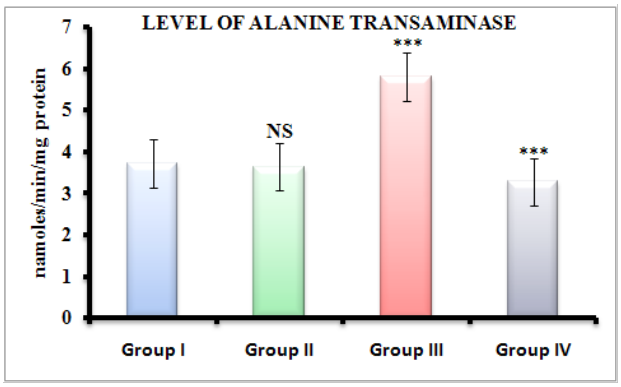


**Fig. 4.** Activity of ALT in liver tissue of control and other treated groups

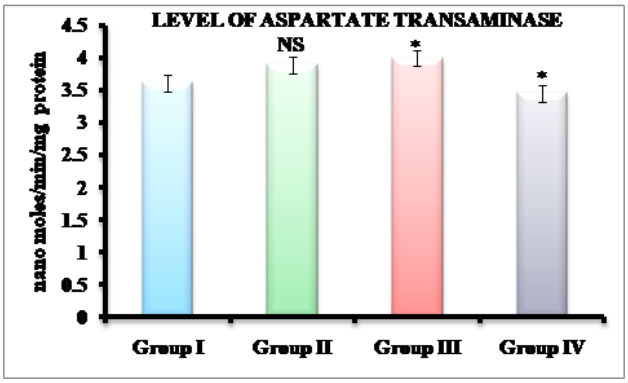


**Fig. 5.** Activity of AST in liver tissue of control and other treated groups

Comparisons are done between Group II vs Group I, Group III vs Group I, and Group IV vs Group III. Results are expressed as mean ± SEM (n = 6). NS= Not significant, *p<0.05, **p<0.01, ***p<0.001 statistical significance difference between control and treated groups.

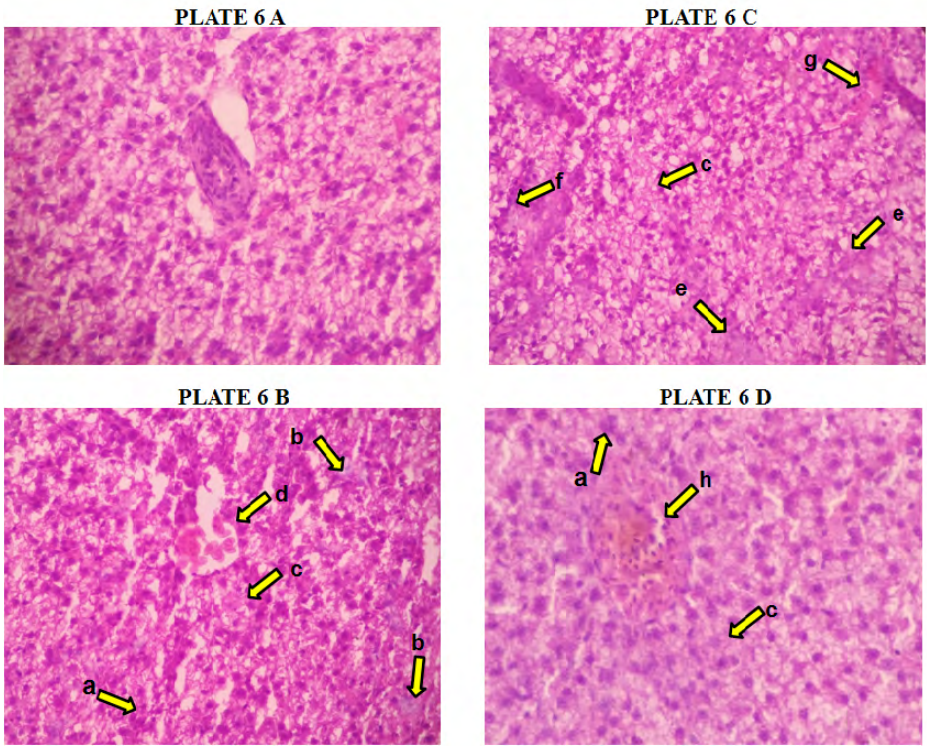


**Fig. 6.** Histological evaluation of fish liver tissue of control and treated groups

al., 2012). The main histopathological lesions inferred in the liver of fish upon exposure to fluoride were necrosis, hypertrophy, disorganization, vacuolization, fibrosis, congestion of blood vessels, dilation of sinusoids, nuclear atrophy, disruption and ruptured hepatocytes (Yadav et al., 2014). The NDEA treated group showed centrilobular sinusoidal dilation, inflammation, massive haemorrhage, and collagen accumulation along with disrupted hepatic cords in contrast to the perfect lobular architecture with intact liver parenchyma and absence of inflammation or any damage in the control group. GA supplementation reduced the histopathological disruptions observed in NDEA treated rats (Latief et al., 2016). The above results recommend gallic acid as a therapeutic supplement for protecting liver damage. But the bioavailability can be enhanced when converted to a nanocomposite. In accordance with the above findings, the present study revealed a typical compact characteristic distribution of central vein, portal tracts and hepatocytes with lobular architecture (Group I). Fish exposed to GA-CSNPs (Group II) reported marked congestion with crowding of hepatocytes (a), occasional inflammatory cell infiltrate (b), ballooning degeneration of cytoplasm (c) and central vein infiltered with RBC (d) are depicted in group II (Fig. 6 B) inferring the impact exerted by the nanocomposite. Histological examination of liver of fish treated with sodium fluoride (Group III) represented disrupted lobular architecture with dilated sinusoids along with hepatocyte ballooning degeneration (c), areas of necrosis and mild fibrosis (e) and pigment laden macrophages (f) are depicted in group III due to the impact of sodium fluoride (Fig. 6 C) conferring the hepato-toxic impact of sodium fluoride. Co-administration of sodium fluoride and GA-CSNPs to fish (Group IV) revealed marked congestion with crowding of hepatocytes (a), ballooning degeneration of cytoplasm (c) with haemorrhage and pigment laden macrophages (h) which almost resembled the findings of group II. The absence of necrotic and fibrotic changes observed in group IV (Fig. 6 D) highlights the ameliorative effect of GA-CSNPs upon fluoride induced toxicity. Hence the present study was successful in using GA-CSNPs to revert the distortions experienced by liver tissue on fluoride induced toxicity.

## 4. Conclusion

The deleterious effect of fluoride as a hepatotoxin is well documented by a decrease in the protein level, and increase in the activity of ALT and AST which is also supported by the histological changes in the liver. The efficacy of GA is enhanced by converting it into a nanoparticle. The characterisation using FTIR and SEM depicts the successful preparation of GA-CSNPs. Administration of these nanoparticles reversed the scenario, deciphering the impact of polyphenols to combat and quench the stress induced by fluoride. The non-toxic nature of the prepared nanoparticle is also highlighted by insignificant variation between group II and group I. From the above result, it can be concluded that the nanoparticle formulations could act as an effective candidate for the encapsulation of bio active compounds such as gallic acid and a promising alternative to improve the bio availability for therapeutic interventions.

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