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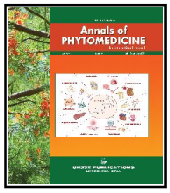

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Nanoquercetin abrogates fluoride induced cardiotoxicity in rats

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Abstract

We examined whether nano form of quercetin (QC) can reduce fluoride mediated cardiotoxicity by affecting oxidative stress pathway and myocardium structure and function in rats. The typical spherical nanoquercetin (NQC) particle size was 240.8 nm. Adult albino Wistar rats were randomly divided into four groups of six each. Group I was kept as the control. In Group II, rats were exposed to sodium fluoride (100 ppm) daily through drinking water for 42 days. Groups III and IV were treated with sodium fluoride as in Group II; however, they were administered QC (100 mg/kg BW) and NQC (50 mg/kg BW), respectively, by oral gavage during the last 14 days of sodium fluoride exposure. Elevated levels of cardiac troponin, heart specific markers, serum total cholesterol and triglycerides were seen in those exposed to sodium fluoride. Sodium fluoride increased lipid peroxidation, depleted reduced glutathione and decreased superoxide dismutase, catalase and glutathione peroxidase activities in cardiac tissue. Sodium fluoride was shown to significantly extend QT and QRS intervals when tested on electrocardiograms. QC and NQC treatment significantly attenuated these sodium fluoride-mediated effects and preserved the normal histological architecture of the heart tissue. However, the magnitude of the effects indicates that NQC has better ameliorative potential than normal QC even at the low dose level.

1. Introduction

Fluoride contamination of drinking water is a big issue for public health in many parts of the world. Fluoride has far-reaching effects on the body, affecting not only teeth and bones but also the heart and blood vessels after prolonged contact with water that is tainted with the mineral. Furthermore, the cardiovascular system is directly damaged by fluoride exposure. Several epidemiological studies have found a higher incidence of heart issues such as arteriosclerosis, hypertension, ischemia and cardiac failure when people are exposed to fluoride (Amini *et al*. 2011; Karademir *et al*. 2011; Tkachenko *et al*. 2021). Donmez and Cinar (2003), Cicek *et al*. (2005) and Shashi *et al*. (2001) discovered that experimental fluorosis in rats induced several pathological lesions in the heart and vascular tissues, as well as aberrant ECGs. Although, the exact mechanism by which fluoride exposure impacts the cardiovascular system is still unclear, it is known that it does so. Cummings and Mclvor (1988) found that fluoride decreases extracellular calcium and increases intracellular calcium by opening calcium dependent potassium channels. Fluoride also causes hypocalcemia and hyperkalaemia associated ventricular arrhythmias, which impair cardiac function and ultimately lead to cardiac arrest.

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Cardiovascular damage caused by fluoride has yet to be fully understood. Nevertheless, oxidative stress is a critical component that worsens organ damage caused by the majority of environmental toxicants. Producing free radicals is one of the best documented and most studied ways that fluoride causes toxicity. Fluoride also inhibits the respiratory chain and boosts superoxide, hydrogen peroxide and hydroxyl radicals; it quickly crosses cell membranes by simple diffusion; and it affects redox equilibrium *in vitro* and *in vivo* models. An increase in reactive oxygen species generation helps with the oxidation of lipids, proteins and nucleic acids, which in turn causes myocardial damage, cardiac arrhythmias and cardiomyopathy. Fluoride can also alter cellular intrinsic antioxidant defenses and cause oxidative stress by influencing the activity of enzymes that make up the cellular antioxidant system, including reduced glutathione, catalase, superoxide dismutase and glutathione peroxidase (Miltonprabu and Thangapandiyan, 2015). Myocardial stunning, infarction, arrhythmias, and cell death are theresults of an oxidative imbalance in the heart, which drastically changes the normal architecture of the heart.

Even though chronic fluoride poisoning is an important issue in public health, there is currently no treatment option for instances of chronic fluorideinduced cardiovascular toxicity. In the treatment of toxicity related to oxidative stress driven disorders, such as cardiovascular diseases, natural phytomolecules derived from herbs are now being employed. The capacity of herbal polyphenols to protect cells from oxidative damage has been the subject of much research (Nabavi *et al*., 2012). An herbal flavonoid with therapeutic benefits on different cardiovascular disorders is quercetin (QC), a polyphenolic molecule (Arts *et al*., 2005). Among quercetin's many

pharmacological and biological effects are its anti-inflammatory, antioxidant, antihyperglycemic, antiviral, anticancer, and analgesic effects (Nabavi *et al*., 2012; Yadav *et al*., 2021; Singh *et al*., 2023). Most studies have looked at its antioxidant properties and how it could help with cardiovascular diseases (De Boer *et al*., 2005). There are a few drawbacks to quercetin's therapeutic efficacy, such as its low absorption when taken orally and its quick removal from the body, even though it shows great promise as a useful and safe chemical with many potential medical uses. To lessen the dosage while increasing the compound's therapeutic efficiency, new quercetin formulation techniques such as microspheres, nanocapsules, and liposomes have been developed (Chakraborty *et al*., 2012; Ain *et al.,* 2022). The present state of the art in drug delivery is the transformation of phytomolecules into nano-size particles, which provide better pharmacological activities while being non-immunogenic, biodegradable, and non-toxic (Naik and Sundarrajan, 2023). Nevertheless, data regarding nanoquercetin (NQC) efficacy as a treatment for fluoride-induced cardiovascular damage is lacking. To determine whether NQC formulation has a more effective ameliorative effect than normal quercetin against fluoride induced cardiovascular damage in rats.

2. Materials and Methods

2.1 Preparation of NQC

The method for producing quercetin nanoparticles was slightly modified from the precipitation process described by Karan *et al*. (2012). For this study, quercetin nanoparticles were prepared by dissolving commercial quercetin in 99% ethanol at a concentration of 1 mg/ 20 ml. A syringe was used to hold the dissolved medicine solution and was then attached to a syringe pump. A drug solution was injected into the polyvinyl alcohol at a steady rate of 10 ml/min. 2 g of polyvinyl alcohol were mixed with 500 ml of distilled water and swirled with a magnetic stirrer overnight to create the solution. For 4 h the magnetic stirrer was used to agitate the whole liquid. The ethanol was completely removed by vacuum suction and the resultant mixture was air dried to get the purified NQC. Until they were needed again, the NQC was kept at 4^{o}C.

2.2 Characterization of NQC

We determined the shape and size range of the NQC using a field emission scanning electron microscope (JEOL-JSM 6701-F, Japan) at the Centre for Nanotechnology and Advanced Biomaterials, SASTRA University, Thanjavur, Tamil Nadu, India. During the 45-sec scanning period, the voltage was 30 kV and the current through the filament was 20 mA. The samples were dried by placing them on top of a glass plate. A thin coating of platinum was applied to the samples using an auto fine coater before imaging.

2.3 Animals and experimental design

Healthy male albino Wistar rats (80-100 g) were obtained from the Department of Laboratory Animal Medicine, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai-51. Before the start of the experiment, animals were keptin laboratory conditions for 7 days or more for acclimatization. They were properly managed under a 12h dark/light cycle with *ad libitum* feed and water. They were provided with pelleted rat feed procured from the M/s. Sai Enterprises, Chennai. Rats were randomly divided into four groups of six each. Group I was kept as the untreated control and Group II rats were considered sodium fluoride exposed experimental control. All the groups except Group I were exposed to sodium fluoride (100 ppm) daily through drinking water for 42 days. Groups III and IV were administered QC (100 mg/kg) and NQC (50 mg/kg) by oral gavage during the last 14 days, *i.e*., 29thto 42ndday of fluoride exposure. The concentration of fluoride (Atmaca *et al*., 2014) and the dose of quercetin (Oyagbemi *et al*., 2018) were selected based on earlier studies. Institutional Animal Ethics Committee (IAEC 10/VCRI-NKL 2021) approval was obtained for the study protocols by the Veterinary College and Research Institute, Namakkal. All the experimental animals were kept under constant observation during the entire period of the study.

2.4 Electrocardiographic examination

A 4-lead single-channel electrocardiogram (ECG) equipment (CARDIART GenX1 Electrocardiograph, BPL Medical Technologies, India) was used to capture electrocardiogram (ECG) data from rats for 42 days. The rats were anaesthetized with xylazine (10 mg/kg, b.wt) and ketamine (75 mg/kg, BW) administered intraperitoneally.The machine was calibrated at 50 mm/s paper speed and standard lead II ECG was recorded. From the ECG, parameters such as heart rate, p-wave duration, PR-interval, Q-T interval, S-T interval and QRS complex duration were calculated.

2.5 Blood and tissue collection

All the rats were fasted overnight (12-14 h) before the sacrifice. On day 43, blood was drawn from the retro-orbital plexus via a capillary tube and placed in clot activator vials to assess serum biochemical indicators. After blood collection, rats were sacrificed by cervical dislocation. The hearts were excised from each animal and weighed. Half of the excised heart tissues were rinsed with physiological saline and stored at -20°C for oxidative stress and antioxidant enzyme estimation.The remaining heart tissue from each group was fixed in a 10% neutral buffered formalin solution for the evaluation of histological changes.

2.6 Serum biochemical markers

Collected blood in the clot activator tubes were allotted to clot for about 20 min and then serum was separated by centrifuging at 1500 revolutions per minute (RPM) for 15 min. The biochemical parameters such as total cholesterol, triglycerides, aspartate amino transaminase (AST), alanine amino transaminase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), blood urea nitrogen (BUN) and creatinine were analyzed using M/s. Biosystems A50, India.

2.7 Cardiac troponin estimation

To estimate cardiac troponin, we used the method of Bhaskar and Rao (2002) and measured total salt soluble proteins, which are proteins associated with troponins and formed after acute myocardial injury.

2.8 Assessment of oxidative stress

We estimated many biochemical markers in the heart that are connected to oxidative stress. We dissolved 2 ml of ice-cold PBS in approximately 800 ml of purified water. After adjusting the pH to 7.4, the volume was increased to one litre by adding distilled water. We added the heart and aorta samples, which were weighed separately, to the mixture. For reduced glutathione determination, 200 mg of each organ's weight was mixed with 2 ml of 0.02 M ethylene diamine tetra acetic

acid (EDTA) in distilled water. Lipid peroxidation (LPO) in connection to malondialdehyde generation was evaluated using the method by Shafiq-Ur-Rehman (1984) using the amount of thiobarbituric acid-reactive molecules. To evaluate GSH, the method proposed by Sedlak and Lindsay (1968) was used. The superoxide dismutase (SOD) activity was assessed according to the method described by Madesh and Balasubramanian (1998). Aebi (1983) and Paglia and Valentine (1967) methodswere used to measure catalase (CAT) and glutathione peroxidase (GPx) activity, respectively.

2.9 Histopathology of heart tissue

The cardiac tissue and aorta were embedded in neutral buffer formalin at a concentration of 10% v/v. Haematoxylin and eosin stain were used to colour the paraffin blocks.

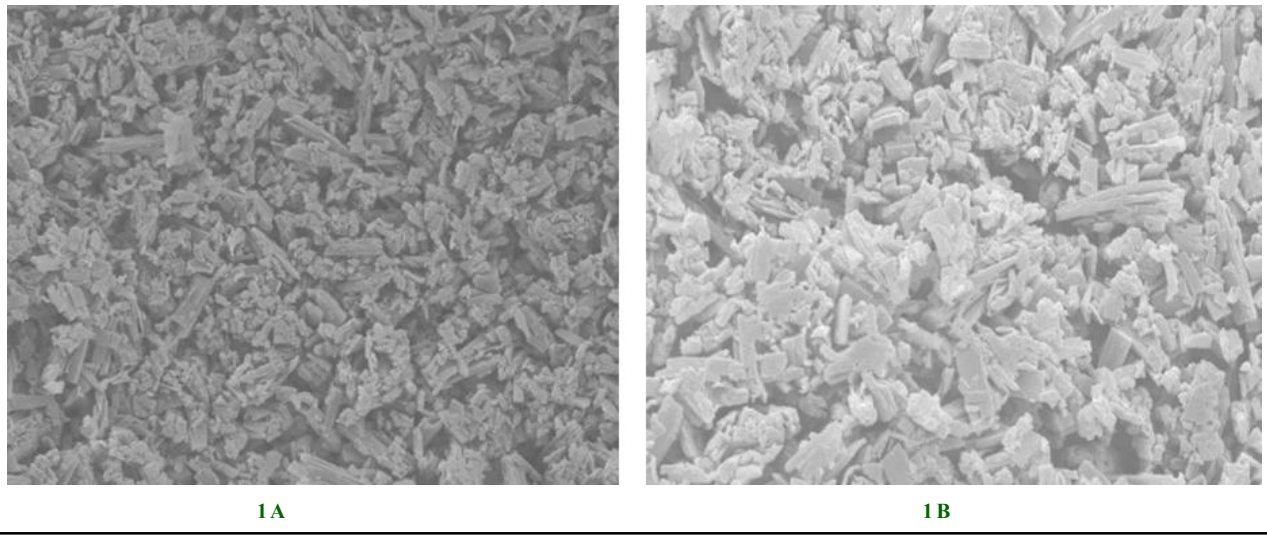
2.10 Statistical analysis

Data were analyzed by one-way ANOVA and means were compared with Duncan's post-hoc test using SPSS software. Data was represented as mean ± SE. A p-value below 0.05 was considered statistically significant.

3. Results

3.1 Preparation and characterization of NQC

QC was converted into nanoparticles with reduced particle size and it appeared in crystalline form by Scanning Electron Microscopy. The size distribution for the optimized batch of nanoquercetin ranged from 170 nm to 255 nm, with the mean particle size of 220± 20 nm (Figure 1A and 1B).



1 A 1 B

Figure 1: SEM images for nanoquercetin.

3.2 Electrocardiogram (ECG)

Table 1 shows that overall electrical activity, P-wave amplitudes, QRS complexes, P-R intervals, Q-T intervals and heart rates were all considerably higher in the fluoride-exposed group than in the control group. The effect of fluoride on ECG significantly restored the mean duration (s) of Q-T interval and heart rate by QC and NQC.

Table1: Effect of quercetin and nanoquercetin on fluoride induced altered electrocardiogram parameters of rats

Group	Treatment	Dose/ concentration	P wave		QRS complex	P-R interval	Q-T interval	S-T interval	Heart rate (beats/min)
			Amplitude (mv)	Duration (s)	Duration (s)	Duration (s)	Duration (s)	Duration (s)	
I	Control	Water	0.05 ± 0.02^{a}	0.010 ± 0.02^{a}	0.010 ± 0.01^{a}	0.05 ± 0.002^{a}	0.04 ± 0.00^{a}	0.02 ± 0.006	330.0 ± 3.65^{a}
II	Fluoride	100 ppm + MC	0.10 ± 0.01^{b}	0.020 ± 0.04^{b}	0.020 ± 0.01^{b}	0.06 ± 0.002^{b}	0.05 ± 0.00^{b}	0.02 ± 0.005	393.3 ± 19.09^{b}
III	Fluoride + QC	100 ppm + 100 mg/kg	0.10 ± 0.02^{b}	0.020 ± 0.03^{b}	0.020 ± 0.01^{b}	0.06 ± 0.003^{a}	0.06 ± 0.00^{b}	0.02 ± 0.004	340.0 ± 2.58^{c}
IV	Fluoride + NQC	100 ppm + 50 mg/kg	0.10 ± 0.02^{b}	0.020 ± 0.02^{b}	0.020 ± 0.01^{b}	0.05 ± 0.002^{b}	0.04 ± 0.00^{a}	0.02 ± 0.004	320.0 ± 2.58^{a}

Sodium fluoride was given through drinking water at 100 ppm for 42 days. QC or NQC were given by oral gavage to the fluoride-exposed rats during the last 14 days, *i.e.*, 29th to 42ndday of fluoride exposure. Values are expressed as mean ± SE (n=6). Values in the same column bearing common superscript did not vary significantly (p<0.05) in Duncan's multiple comparison test. QC: Quercetin, NQC: Nanoquercetin.

3.3 Serum cardiac functional markers

As shown in Table 2, the levels of cardiac indicators such as cardiac troponin, alkaline phosphatase, creatine kinase, AST and ALT were significantly higher in the fluorideexposed rats compared to the control group. Serum cardiac indicators were significantly reduced in rats administered QC or NQC, suggesting protection against heart injury, when compared to rats intoxicated with fluoride alone. However, there was no significant difference between QC and NQC treatment.

Table 2: Effect of quercetin and nanoquercetin on fluoride induced altered serum cardiotoxic marks of rats

Treatment	Dose/ concentration	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	CK (IU/L)	Troponin level (ng/ml)
Control	Water	54.17 ± 3.21[a]	106.17 ± 6.63[a]	198.33 ± 24.87[a]	1098.67 ± 51.00[a]	25.80 ± 2.14[a]
Fluoride	100 ppm + MC	206.83 ± 4.33[b]	283.33 ± 18.34[b]	387.17 ± 21.48[b]	2596.67 ± 133.19[b]	48.39 ± 2.08[b]
Fluoride + QC	100 ppm + 100 mg/kg	144.00 ± 12.49[c]	120.83 ± 10.13[c]	277.00 ± 31.07[a]	1560.33 ± 111.10[c]	29.09 ± 2.10[c]
Fluoride + NQC	100 ppm + 50 mg/kg	121.17 ± 7.52[c]	140.00 ± 15.28[c]	244.0 ± 23.70[a]	1415.67 ± 179.45a[c]	28.22 ± 1.66[c]

Sodium fluoride was given through drinking water at 100 ppm for 42 days. QC or NQC were given by oral gavage to the fluoride-exposed rats during the last 14 days, *i.e.*, 29th to 42nd day of fluoride exposure. Values are expressed as mean ± SE (n=6). Values in the same column bearing common superscript did not vary significantly (p<0.05) in Duncan's multiple comparison test. QC: Quercetin, NQC: Nanoquercetin.

Table 3: Effect of quercetin and nanoquercetin on the level of serum total cholesterol and total triglyceride of rats given continuous exposure to sodium fluoride

Treatment	Dose/concentration	Total cholesterol (mg/dl)	Total triglyceride (mg/dl)
Control	Water	169.17 ± 15.62[a]	94.67 ± 7.23[a]
Fluoride	100 ppm + MC	315.67 ± 6.75[b]	174.0 ± 13.18[b]
Fluoride + QC	100 ppm + 100 mg/kg	250.67 ± 31.84[c]	137.17 ± 8.30[c]
Fluoride + NQC	100 ppm + 50 mg/kg	225.17 ± 17.33[ac]	100.67 ± 5.14[a]

Sodium fluoride was given through drinking water at 100 ppm for 42 days. QC or NQC were given by oral gavage to the fluoride-exposed rats during the last 14 days, *i.e.*, 29th to 42nd day of fluoride exposure. Values are expressed as mean ± SE (n = 6). Values in the same column bearing common superscript did not vary significantly (p<0.05) in Duncan's multiple comparison test. QC: Quercetin, NQC: Nanoquercetin.

3.4 Serum lipid markers

The blood lipid levels of the rats in both the control and experimental groups are displayed in Table 3. The rats given fluoride had far greater levels of blood triglycerides and cholesterol than the rats given a control diet. QC and NQC treatment resulted in decreased serum cholesterol and triglyceride levels. While NQC treatment was statistically significant from QC treatment in triglyceride level.

3.5 Lipid peroxidation (LPO)

The effects of fluoride exposure on heart tissue LPO and the protective efficacy of QC and NQC in ratsare shown in Table 4. LPO level in heart tissue was significantly increased in rats exposed to fluoride compared to control. The levels of LPO in cardiac tissue were reduced by 66% following QC therapy and by 100% following NQC treatment, respectively. The NQC therapy had a high amelioration percentage, but it was not statistically significant compared to the QC treatment.

Table 4: Effect of quercetin and nanoquercetin on lipid peroxidation (nmole MDA formed/g tissue) in heart tissue of rats given repeated exposure to sodium fluoride

Treatment	Dose/concentration	LPO	Amelioration (%)
Control	Water	0.80 ± 0.38 [a]	-
Fluoride	100 ppm + MC	2.97 ± 0.20 [b]	-
Fluoride + QC	100 ppm + 100 mg/kg	1.50 ± 0.12[a]	66
Fluoride + NQC	100 ppm + 50 mg/kg	1.03 ± 0.12 [a]	100

Sodium fluoride was given through drinking water at 100 ppm for 42 days. QC or NQC were given by oral gavage to the fluoride-exposed rats during the last 14 days, *i.e.*, 29th to 42nd day of fluoride exposure. Values are expressed as mean ± SE (n = 6). Values in the same column bearing common superscript did not vary significantly (p<0.05) in Duncan's multiple comparison test. QC: Quercetin, NQC: Nanoquercetin.

3.6 Antioxidative enzymes

Table 5 reveals that the enzymatic antioxidant activities (SOD and CAT) were significantly decreased in cardiac tissue of rats exposed to fluoride. QC treatment significantly increased the activities of SOD (21%) and CAT (32%). NQC treatment significantly increased the activity of SOD by 50% and that of CAT by 47% in the heart. However, the effect of NQC treatment did not differ significantly from QC treatment but amelioration percentage was higher in NQC treatment. Table 6 shows the effects on the antioxidative glutathione system. Fluoride exposure resulted in significant depletion in GSH and GPx content in the heart. QC treatment increased GSH and GPx content by 34% and 29%, respectively, whereas NQC treatment significantly increased the GSH and GPx in the heart by 54%and 38%. However, the percent amelioration was high in NQC treatment in fluoride exposed rats.

Table 5: Effect of quercetin and nanoquercetin on the activity of SOD (units/mg protein) and CAT (mmole H_2O_2 utilized/min/mg protein) in heart tissue of rats given continuous exposure to sodium fluoride

Group	Treatment	Dose/concentration	SOD	Amelioration (%)	CAT	Amelioration (%)
I	Control	Water	26.22 ± 1.87[a]	-	1.61 ± 0.13[a]	-
II	Fluoride	100 ppm + MC	10.68 ± 0.79[b]	-	0.50 ± 0.08[b]	-
III	Fluoride + QC	100 ppm + 100 mg/kg	15.96 ± 0.90[b]	21	0.92 ± 0.10[b]	32
IV	Fluoride + NQC	100 ppm + 50 mg/kg	19.58 ± 0.72[c]	50	1.07 ± 0.12[c]	47

Sodium fluoride was given through drinking water at 100 ppm for 42 days. QC or NQC were given by oral gavage to the fluoride-exposed rats during the last 14 days, *i.e.*, 29th to 42nd day of fluoride exposure. Values are expressed as mean ± SE (n=6). Values in the same column bearing common superscript did not vary significantly ($p<0.05$) in Duncan's multiple comparison test. QC: Quercetin, NQC: Nanoquercetin.

Table 6: Effect of quercetin and nanoquercetin on the activity of glutathione peroxidase (µmole of NADPH oxidized to NADP/mg of protein/min) and reduced glutathione (mM of GSH per g of wet tissue) in heart tissue of rats given continuous exposure to sodium fluoride

Group	Treatment	Dose/ concentration	Glutathione peroxidase	Amelioration (%)	Reduced glutathione	Amelioration (%)
I	Control	Water	33.98 ± 5.17[a]	-	57.98 ± 3.03[a]	-
II	Fluoride	100 ppm + MC	12.98 ± 0.71[b]	-	24.82 ± 2.58[b]	-
III	Fluoride + QC	100 ppm + 100 mg/kg	19.24 ± 1.73[b]	29	36.15 ± 2.26[c]	34
IV	Fluoride + NQC	100 ppm + 50 mg/kg	22.47 ± 1.64[b]	38	42.73 ± 2.43[c]	54

Sodium fluoride was given through drinking water at 100 ppm for 42 days. QC or NQC were given by oral gavage to the fluoride-exposed rats during the last 14 days, *i.e.*, 29th to 42nd day of fluoride exposure. Values are expressed as mean ± SE (n = 6). Values in the same column bearing common superscript did not vary significantly ($p<0.05$) in Duncan's multiple comparison test. QC: Quercetin, NQC: Nanoquercetin.

3.7 Histopathology of heart

The heart tissue of the control group did not show any significant microscopic alterations and revealed normal histologic details (Figure 2A). Heart tissue of the fluoride exposed group showed mononuclear cell infiltration at epicardium and myocardial fibers degeneration (Figure 2B). Treatment with QC in fluoride exposed rats of heart tissue revealed moderate degeneration and necrosis with mononuclear cell infiltration at the left ventricle epicardium (Figure 2C). However, treatment with NQC in fluorideexposed rats of heart tissue showed mild degeneration and congestion of blood vessels in the myocardium (Figure 2D).

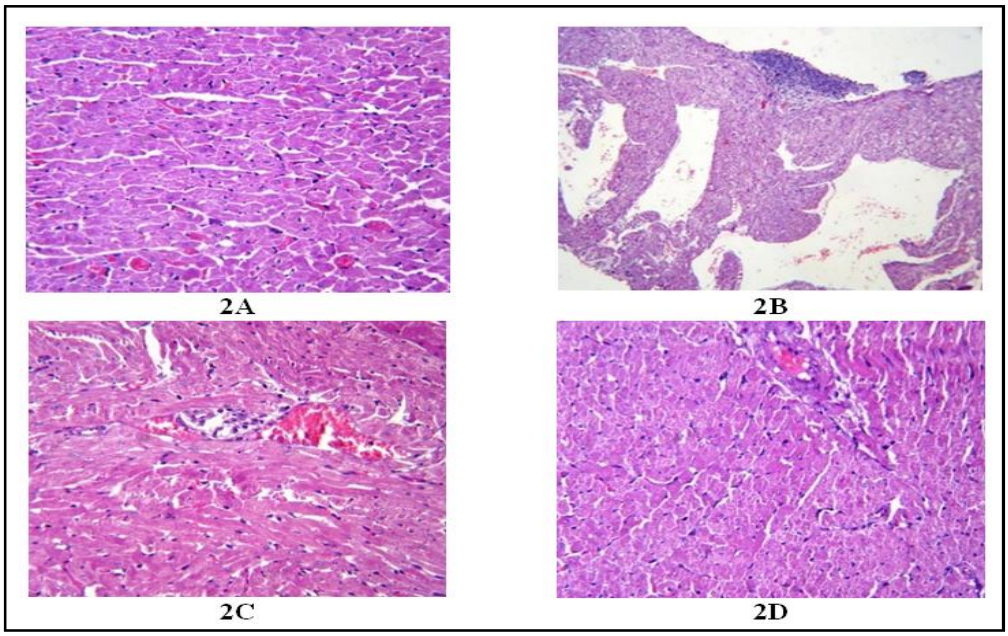


Figure 2: Effect of quercetin and nanoquercetin on histopathological changes in heart tissue of rats given repeated exposure of fluoride.

4. Discussion

The nanoprecipitation method was used to produce NQC, which yielded particles ranging in size from 170 to 255 nm. This size range verifies that the quercetin is convertedto nanoform. It was suitable for drug delivery to target organs, most importantly into the intracellular, which is the ultimate challenge in drug delivery. The small difference in size between the particles as prepared by Kakram *et al*. (2012) and our preparation may be attributed to the flow rate, solvent/ stabilizer volume ratio, stirring speed and drug solvent concentration.

Fluoride-mediated elevations in serum ALT, AST, alkaline phosphatase and creatine kinase levels may be linked to cardiotoxic changes that impair cardiomyocyte membrane integrity (Panneerselvam *et al*., 2015; Emejulu*et al*., 2016). Cardiac troponin is the myocardial tissue specific proteinthat is a highly sensitive and specific marker of myocardial damage. Further, it is more efficacious than earlier markers because they aid not only in diagnosis, but also in risk assessment and therapeutic decision making (Peela *et al.,* 2010). Our findings demonstrate that elevated levels of this maker reflect the severity of cardiac membrane necrosis caused by fluoride (Abdel-Baky and Abdel-Rahman, 2020). The heart's histological changes may be associated with these enzymatic changes. Both QC and NQC improved the fluoride mediated alterations in these marker enzymes and heart histology and this could beattributed to the antioxidant and membrane stabilizing action of QC.

This study found that high levels of triglycerides and total cholesterol in the blood are the primary determinants of cardiac dysfunction. (Miltonprabu and Thangapandiyan, 2015; Abdel-Wahab, 2013). The present study suggests that both QC and NQC have a hypolipidemic effect and offer protection against fluoride induced cardiovascular toxicity by stabilizing the effect on myocardial phospholipids. However, the treatment with NQC had a more potent effect on lipid profile parameters than normal QC. The antioxidant and antilipo-peroxidative characteristics of NQC may also explain why it was able to significantly ameliorate the serum lipid profile impairment in rats that had been exposed to fluoride (Bader *et al*., 2015).

Electrocardiograms (ECGs) are a common tool for assessing heart health because they measure electrical activity in the heart with the use of sensors. As it is, it helps identify cases of xenobiotic-induced cardiotoxicity at an early stage. The P wave, which indicates atrial depolarisation, exhibited a marked increase in both length and amplitude after 42 days of fluorideexposure in rats. The P-R interval represents the period of atrial depolarization to initiation of ventricular depolarization was significantly longer in fluorided exposed rats. The significant increase in P-R interval is due to delayed impulse conduction from the SA node to the AV node. This may be due to a significant decrease in sodium ion concentration (data not shown) observed in the present study and may be responsible for the delayed atrial depolarization. ECG results revealed elevated heart rates and QRS and Q-T intervals in fluorideexposed rats compared to controls. Consistent with previous findings, changes in the QRS and Q-T intervals may be linked to hypocalcemia, which causes delayed repolarisation and ultimately ventricular fibrillation-mediated dysrhythmia (Panneerselvam *et al*., 2015; Oyagbemi *et al*., 2018). In addition, sinus bradycardia develops when the lengths of individual waves are so long that the activity of the heart's cells is drastically reduced. This work provides further evidence that NQC therapy mitigates the negative effects of fluoride intoxication on ECG parameters and heart rate in rats. QC can restore the ECG parameters due to its antioxidant, membrane stabilizingand electrolyte repair properties. Additional research is required to determine the precise process by which these antioxidants safeguard the fluoride induced ECG modification.

One possible mechanism by which fluoride cardiovascular damage develops is oxidative stress (Genge*t al*., 2014).We observed a significant increase in LPO levels in heart tissue in rats exposed to fluoride. The elevation in LPO levels suggests a rise in the formation of reactive oxygen species and reactive nitrogen, which may impede myocardial function in the long run (Thangapandiyan and Miltonprabu, 2013; Ameeramja *et al*., 2016). Both QC and NQC decreased the fluoride mediated increase in cardiac LPO. More interestingly, it is evident from the result that treatment with NQC caused a better reduction of LPO levels in heart tissue. This effect suggests that nanoconversion of QC may increase aqueous solubility and enhance bioavailability and bioactivity.

Antioxidants both enzymatic and non-enzymatic are the first line of defense against free radical induced toxicity. The proper operation of cells depends on a redox equilibrium between antioxidants and pro-oxidants (Nordberg and Arner, 2001). Fluoride reduced the activities of SOD, CAT, and GPx and depleted GSH levels in heart tissue (Oyagbemi *et al*., 2017). These reflect perturbations in normal oxidative mechanisms during fluoride toxicity. It has been reported by several workers that fluoride might reduce antioxidant levels. (Basha and Sujitha, 2011; Oyagbemi*et al*., 2017). In the present study, significant depletion of GSH, GPx, SOD and CAT activity was observed in the cardiac tissue of rats after fluoride exposure. According to Barbier *et al*. (2010), there was a marked rise in LPO and a dramatic drop in antioxidant levels, both of which could be detrimental to heart tissues. Both QC and NQC treatments raised cardiac tissue GSH, GPx, SOD, and CAT activity, although the NQC group showed a much higher percentage of improvement. Oyagbemi *et al.* (2017) found that quercetin improved cardiac GSH, GPx, SOD and CAT activity in fluoride treated rats. Furthermore, in the present study, we found that NQC treatment prevented the fluoride induced reduction in the activities of the antioxidant enzymes, non-enzymatic antioxidants and GSH levels in the cardiac tissue of the experimental rats via its extraordinary antioxidant and free radical scavenging properties.

5. Conclusion

When the ameliorative effects produced by QC as well as NQC were compared, it was found that mostly they caused statistically comparable effects. However, going by the percentage of amelioration, it was evident that the magnitude of amelioration caused by NQC was more pronounced than that caused by normal QC. Overall, the present study shows that QC in the nano form is more effective than normal QC against fluorideinduced cardiac toxicity in rats. Further, QC is a pleiotropic agent possessing anti-inflammatory and antioxidative properties and is taken daily as a food supplement, they would be effective in mitigating cardiovasculartoxicity owing to fluoride exposed humans and animals.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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