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Effect of nanoquercetin on sodium fluoride induced haemato-toxicity in rats

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Abstract

The present experiment pertains to the protective role of nanoquercetin against haematological toxicity induced by fluoride during 42 days exposure. Nanoquercetin (NQC) exhibited a spherical shape with the mean particle size of 240.8 nm. Rats were randomly divided into four groups of six each. Group I was kept as the control and other three groups (II, III and IV) were exposed to sodium fluoride (100 ppm) daily through drinking water for 42 days. Groups III and IV were administered quercetin (50 mg/kg bw) and NQC (25 mg/kg bw) respectively, by oral gavage for the period of 42 days. Exposure of rats to sodium fluoride caused significant changes of some haematological parameters in treated rats compared to controls. Co-administration of quercetin and nanoquercetin with fluoride restored some of the haematological parameters to near-normal values. Therefore, our investigation revealed that nanoquercetin given better protection against fluoride-induced toxicity.

Keywords: Quercetin, nanoquercetin, fluoride, cardiotoxicity, rats

Introduction

Blood is promptly affected by environmental pollutants and toxicants that can cause many metabolic disorders. Chronic exposure of fluoride acts as a potential pollutant with very high toxicity, associated with the haematological damage. Fluoride affects the formation of blood forming cells like hematopoietic cells in cavities of bone marrow and inhibits the transport of K^+/Cl^- ions (Choubisa *et al.* 1996) [1]. Susheela, (2001) [10] reported that fluoride intoxication of the human causes anemia and premature erythrocyte deaths i.e. life span of RBCs decreases due to membrane degeneration that turns them into echinocytes.

Rats exposed to 6 ppm of sodium fluoride for 30 days significantly reduced the hemoglobin level and total white blood count (Sharma *et al.* 2007) [9]. Kamble and Velhal, (2010) [5] shown that rats were exposed to 100, 200 and 300 ppm of sodium fluoride for 1 and 2 months caused significant alterations in blood corpuscles and the plasma protein. They reported that, sodium fluoride caused time dependent and dose dependent transient effect on red blood cells, white blood cell counts and hemoglobin level, which indicates immunological suppression. As dose and exposure period increases total red blood cell count and hemoglobin level were found to be decreased. On the other hand, initially total white blood cell count was increased, then after as the exposure period and concentration of fluoride increases total white blood count was found to be reduced.

Researchers are looking forward in search of protective agent in order to combat against fluoride induced haematological toxicity. Quercetin, a polyphenolic compound, which is known to exhibit a variety of pharmacological activities like antipyretic, anti-inflammatory, immunoprotected, hepatoprotective and bioenhancer (Nabavi *et al.* 2012) [8]. Therefore, the present study has been undertaken to evaluate the protective effect of quercetin and nanoquercetin on fluoride-induced haemato-toxicity in rats.

Materials and Methods

Apparently 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 kept in laboratory conditions for a period of 7 days or more for acclimatization. They were kept in proper managerial conditions under 12-h dark/light cycle with *ad libitum* feed and water. They were provided with pelleted rat feed procured from the M/s. Sai Enterprises, Chennai. All the animals 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 (50 mg/kg) and NQC (25 mg/kg) by oral gavage for 42 days, respectively. The experiments were carried out in accordance with the guidelines of the Institutional Animal Ethics Committee, Veterinary College and Research Institute, Namakkal (IAEC 10/VCRI-NKL 2021). All the experimental animals were kept under constant observation during the entire period of the study.

All the rats were fasted overnight (12-14 hours) before the sacrifice. Blood was collected in clot activator and K₃ EDTA coated vials on the 43rd day from retro-orbital plexus with the help of capillary tube for estimations of hematological parameters. The hematological parameters such as haemoglobin concentration (Hb), packed cell volume (PCV), total leucocyte count (TLC), differential leucocyte count (DLC), total erythrocyte count (TEC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), absolute granulocyte, lymphocyte and monocyte were estimated by using haematology analyzer at centralized clinical laboratory (Rayto-7600, Auto Haematology Analyzer, USA).

Results and Discussion

The mean values of various haematological parameters have been presented in Table.1 and 2. Circulatory system in any animal plays important role. The cellular constituents, plasma protein and chemical composition of blood play's vital role in the different metabolic activities. Any toxicants when entered in the body get circulated throughout the body by the circulatory system. When the concentration of these toxicant increases in the body it will cause qualitative and quantitative

abnormalities in the blood cell composition in exposed animals. The haemoglobin was decreased in the fluoride treated group suggesting anaemia which became pronounced with duration of fluoride exposure, which was also clinically evident as the rats body weight decreased. The associated hypoproteinaemia due to severe hepatotoxic degeneration and lack of erythropoietin secretion from severely damaged kidneys might be responsible for development of anaemia in rats of fluoride exposed groups.

Fluoride can cause anemia in two ways. Fluoride damaged but unlysed cells are removed from circulation by the reticuloendothelial system lowering their lifespan. Fluoride increases the phagocytic activity of splenic macrophages that engulf more red blood cells leading to their removal from circulation (Kumari and Kumar, 2011) [6]. Fluoride is also known to inhibit erythropoiesis thereby lowering red blood cells count and haemoglobin content. Fluoride accumulates not only in bones but also in bone marrow cavities where hematopoiesis occurs. Machalinski *et al.* (2000) [7] reported that fluoride toxicity causes injury to human hematopoietic (blood forming cells) progenitor cells which will inhibit erythropoiesis. Kahl *et al.* (1973) [4] explained that fluoride interacts with the iron of haemoglobin, thereby inhibiting globin synthesis and retarding the normal process of erythropoiesis leading to anemia. Kamble and Velhal, (2010) [5] demonstrated that rats were exposed to 100, 200 and 300 ppm of sodium fluoride for 1 and 2 months caused significant alterations in blood corpuscles and the plasma protein. Ingestion of fluoride through drinking water (10, 50, 100 ppm) for 12 weeks was found to affect haemoglobin synthesis in rats (Chouhan *et al.* 2010) [3]. In this study, fluoride was accumulated in the erythrocyte membrane and caused the formation of echinocytes. Since the life span of echinocytes is less than that of red blood cells, the early destruction of these red blood cells is likely to cause anemia. Treatment with quercetin and nanoquercetin improved the reduced levels of haemoglobin of fluoride exposed rats. This might be due to the ability of these quercetin to improve defense mechanism of the body, which is also supported by the enhanced level of cardiac tissue glutathione enzyme following the administration of this quercetin.

Table 1: Effect of quercetin and nanoquercetin on haematological parameters of rats given continuous exposure of sodium fluoride

Treatment	Dose/ Concentration	Hb (g/dl)	PCV (%)	TEC (x10 ⁶ /μL)	MCV (fL)	MCHC (g/dl)	TLC (x10 ⁶ /mm ³)
Control	Water	12.95±0.58 ^a	34.066±0.63	6.18±0.18	56.51±0.59	34.93±0.54	12.59±0.70
Fluoride	100 ppm + MC	11.18±0.35 ^b	34.73±0.569	6.15±0.10	56.48±0.65	34.61±0.56	11.96±0.93
Fluoride + Q	100 ppm + 50 mg/kg	12.55±0.27 ^{ab}	35.25±0.902	6.22±0.12	56.63±0.61	35.63±0.85	14.54±0.97
Fluoride+ NQ	100 ppm + 25 mg/kg	12.50±0.13 ^{ab}	37.06±0.619	6.45±0.13	57.46±0.35	33.78±0.34	14.08±0.80

Sodium fluoride was given through drinking water at 100 ppm for 42 days.

Groups III and IV: Q or NQ were given by oral gavage to the fluoride-exposed rats from day 1 to 42 days (Prophylactically).

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.

Q: Quercetin, NQ: Nanoquercetin, Hb: Hemoglobin, PCV: Packed Cell Volume, TEC: Total Erythrocyte Count, MCV: Mean Corpuscular Volume, MCHC: Mean Corpuscular Hemoglobin Concentration, TLC: Total Leucocyte Count,

Table 2: Effect of quercetin and nanoquercetin on haematological parameters of rats given continuous exposure of sodium fluoride

Treatment	Dose/ Concentration	GRA (%)	LYM (%)	MON (%)	Platelet (x10 ³ /μL)	RDWCV (%)	RDWSD (%)
Control	Water	33.66±2.90	60.33±2.88	5.50±0.22	892.33±24.15	12.93±0.06	34.01±0.38
Fluoride	100 ppm + MC	33.16±2.84	61.83±3.17	5.00±0.36	889.16±28.98	12.71±0.09	33.58±0.45
Fluoride + Q	100 ppm + 50 mg/kg	31.33±1.64	63.66±1.80	5.00±0.25	927.50±30.11	13.03±0.11	34.31±0.61
Fluoride+ NQ	100 ppm + 25 mg/kg	30.83±1.74	64.00±2.04	5.16±0.40	915.00±15.28	12.68±0.08	34.10±0.43

Sodium fluoride was given through drinking water at 100 ppm for 42 days.

Groups III and IV: Q or NQ were given by oral gavage to the fluoride-exposed rats from day 1 to 42 days (Prophylactically).

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.

Q: Quercetin, NQ: Nanoquercetin, GRA: Granulocyte, LYM: Lymphocyte, MON: Monocyte, RDWCV: Red Blood Cell Distribution Width (CV), RDWSD: Red Blood Cell Distribution Width (SD)

Conclusion

The results of present study demonstrated that oral administration of quercetin and nanoquercetin along with sodium fluoride intoxication prevents haemato-toxicity in rat, probably because of the antioxidant effect of this compound.

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