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Ameliorative potential of quercetin nanoparticles against tolfenpyrad-induced subchronic toxicity in male Wistar rats

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Abstract

Tolfenpyrad, a pyrazole-derived pesticide, is widely used in agriculture. This study evaluated the median lethal dose (LD₅₀) of tolfenpyrad and protective efficacy of quercetin-loaded chitosan nanoparticles against tolfenpyrad-induced subchronic toxicity in male Wistar rats. The LD₅₀ was determined using ten rats. Subsequently, for the subchronic study, 42 animals were assigned to seven groups (six animals/group): Group I: distilled water control, Group II: vehicle control (Olive oil, 4 mL/kg), Group III: quercetin control (20 mg/kg), Group IV: quercetin-loaded chitosan nanoparticle control (20 mg/kg), Group V: tolfenpyrad (8.6 mg/kg), Group VI: tolfenpyrad (8.6 mg/kg) + quercetin (20 mg/kg), and Group VII: tolfenpyrad (8.6 mg/kg) + quercetin-loaded chitosan nanoparticles (20 mg/kg). Treatments were administered orally for 60 days. Clinical signs, biweekly feed and water intake, and body weight were recorded. Tolfenpyrad caused perioral inflammation, a coarse hair coat, and significant reductions in feed intake, water intake, and body weight. Co-administration of quercetin ameliorated these effects, while co-administration of quercetin-loaded nanoparticles significantly reduced inflammation and restored physiological parameters closer to normal levels. These findings highlight the therapeutic potential of quercetin-loaded chitosan nanoparticles against tolfenpyrad-induced physiological disruptions.

Keywords: Tolfenpyrad, quercetin, nanoparticle, rat, pesticide

1. Introduction

Tolfenpyrad (TFP) is a modern pyrazole carboxamide insecticide and acaricide that has been increasingly recognized for its broad-spectrum efficacy against a wide array of agricultural pests like Hemiptera, Coleoptera, Diptera, Lepidoptera, Thysanoptera, and Acari (Chi *et al.*, 2021; Zhang *et al.*, 2022) [5, 25]. TFP exhibits potent efficacy across all developmental stages of the target pest, including eggs, larvae, nymphs, and adults (Hikiji *et al.*, 2013) [10]. TFP is applied to a range of vegetables, fruits, nuts, vines, and row crops such as chilli, grapes, okra, cabbage and mango. Its molecular configuration is 4-chloro-3-ethyl-1-methyl-N-(4-(p-tolyloxy)benzyl)-1H-pyrazole-5-carboxamide. TFP's cytotoxic mechanism is primarily mediated through the inhibition of mitochondrial Complex I (NADH:ubiquinone oxidoreductase) within the electron transport chain, a process that disrupts cellular respiration and energy metabolism (Yamaguchi *et al.*, 2012) [23]. TFP demonstrates marked efficacy and increased usage against pest populations that exhibit resistance to conventional insecticidal classes such as organophosphates and carbamates due to its distinct biochemical target (Hikiji *et al.*, 2013) [10]. The persistent application of insecticides combined with inadequate washing procedures of vegetables leads to residual pesticide deposits, raising significant concerns regarding potential adverse effects on both animal and human health (Dong *et al.*, 2018) [7]. Pesticide residues, even at low levels in crops, can accumulate in the human body through ingestion, inhalation, or consumption of contaminated food and water, leading to unintentional exposure. Mitochondrial complex I facilitates the coupling of NADH oxidation to the reduction of ubiquinone. During this electron transfer process, some electrons may leak from complex I and interact with molecular oxygen, leading to the generation of superoxide and subsequent reactive oxygen

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species (ROS) (Koopman *et al.*, 2010) [12]. Since TFP impairs mitochondrial complex I function, the consequent disruption promotes electron leakage, leading to increased ROS generation. Therefore, identifying effective mitigation strategies like flavonoids to mitigate the ROS-mediated toxic effects induced by TFP is essential.

Quercetin (QCT) is a naturally occurring flavonoid found in fruits and vegetables like apple, onion, peas, mulberry and apricot. It acts as an antioxidant by scavenging ROS and reducing oxidative stress through donating electrons, thereby neutralizing free radicals (Zhang *et al.*, 2011) [26]. Its anti-inflammatory effect is mediated by inhibition of NF- κ B activation and downregulation of inflammatory markers such as inducible nitric oxide synthase, cyclooxygenase-2, and C-reactive protein (García-Mediavilla *et al.*, 2007) [9]. Several studies have demonstrated that QCT mitigates the toxic effects induced by pesticides such as chlorpyrifos, acetamiprid, and fipronil (Uzun *et al.*, 2010; El-Gendy *et al.*, 2022; Uzunhisarcikli *et al.*, 2023) [21, 8, 22]. Bioavailability studies of QCT have shown that following absorption, over 96% of the ingested compound is excreted within 72 hours (Mullen *et al.*, 2008) [14]. This indicates rapid metabolism and elimination of QCT after oral administration, which is critical to consider when evaluating its potential biological effects.

Recent advancements in nanotechnology have demonstrated that nanoformulation significantly enhances the bioavailability and therapeutic efficacy of various pharmaceutical agents. Among the different approaches, the ionotropic gelation method has gained considerable attention for the synthesis of chitosan nanoparticles. This technique is based on electrostatic interactions between the cationic amino groups present in chitosan, a modified natural polysaccharide from chitin and anionic crosslinking agents such as sodium tripolyphosphate (TPP). The resulting ionic bonds promote both inter- and intra-molecular cross-linking, thereby forming stable nanoparticles capable of efficient drug encapsulation (Calvo *et al.*, 1997) [4]. Quercetin-loaded chitosan nanoparticles (QNP) have proven effective in *in vitro* and *in vivo* studies (Nathiya *et al.*, 2014) [16]. *in vivo* studies have shown QNP successfully mitigates ethion and cypermethrin toxicity in experimental animals (Ranjith *et al.*, 2024) [2, 20].

Hence, this study was conducted to determine the LD₅₀ of TFP and ameliorative potential of QNP against TFP-induced subchronic toxicity over a 60-day period in male Wistar albino rats.

2. Materials and Methods

Tolfenpyrad (TFP; CAS No: 129558-76-5, 97% pure) was procured from Tokyo Chemical Industry (India) Pvt. Ltd., while HPLC-grade quercetin (QCT; CAS: 117-39-5), chitosan (CAS: 9012-76-4), and sodium tripolyphosphate (TPP; CAS: 7758-29-4) for nanoparticle preparation were obtained from Sigma-Aldrich. The study was conducted following approval from the Institutional Animal Ethics Committee (IAEC/COVAS/PKD/24/9/2025) using male Wistar rats sourced from the CCSEA approved Laboratory Animal Facility at the College of Veterinary and Animal Sciences, Pookode (registration no. 1271/GO/ReBiBt-S/ReRc-L/09/CPCSEA). Animals were housed under standard conditions (24±2 °C, 50-70% humidity, 12:12-hour light/dark cycle) with ad libitum access to feed and water, following a 7-day acclimatization period. A total of 6 male

Wistar rats were taken for acute toxicity study, the median lethal dose (LD₅₀) of TFP was determined in accordance with OECD TG 425 using the AOT 425 Stat PGM software. Subsequently, a repeated-dose subchronic study was performed using 42 rats randomly divided into seven groups (n = 6/group): Group I: distilled water, Group II: vehicle control (Olive oil, 4 mL/kg), Group III: quercetin (20 mg/kg[#]), Group IV: quercetin-loaded chitosan nanoparticles (20 mg/kg[#]), Group V: tolfenpyrad (8.6 mg/kg), Group VI: tolfenpyrad (8.6 mg/kg) + quercetin (20 mg/kg[#]), and Group VII: tolfenpyrad (8.6 mg/kg) + quercetin-loaded chitosan nanoparticles (20 mg/kg[#]). Treatments were administered orally for 60 days (#Mega *et al.*, 2022) [13]. Animals were fasted 6 hours before each daily oral administration; clinical signs were monitored at 30 minutes, 4, 6, and 24 hours post-dosing, and feed and water were restored after 1 hour. Body weight, feed intake, and water consumption were recorded biweekly throughout the study. Data were analyzed using GraphPad Prism 8.02 via repeated-measures two-way ANOVA followed by Tukey's test, with results expressed as mean±SEM.

3. Results

An acute oral toxicity test was performed as described above and LD₅₀ was determined to be 86 mg/kg body weight when administered in olive oil as vehicle (Fig.1).

Repeated-dose subchronic study in male Wistar rats treated with TFP at a dose of 8.6 mg/kg body weight did not display any apparent signs of severe toxicity or mortality during the 60-day study period. Mild inflammation was noted around the mouth (Fig.2), along with a slightly roughened body coat (Fig.3) and mild diarrhea in the TFP-treated group. Rats receiving TFP at 8.6 mg/kg and QNP at 20 mg/kg exhibited minor oral inflammation (Fig.4), while their coat condition remained comparable to that of the control group.

3.1 Mean biweekly change in body weight

The average biweekly body weight changes (g) resulting from oral administration across the seven treatment groups are illustrated in table 1. All rat groups exhibited weight gain, with a significant increase observed in the four control groups (Distilled Water, Olive Oil, QCT, and QNP). Among the treatment groups, rats receiving TFP at one-tenth of the LD₅₀ dose showed the least weight gain, where a 15.6% reduced body weight gain compared to the control. Conversely, rats treated with the combination of TFP and QCT demonstrated improved body weight with an 8.65% increase compared to rats given TFP alone while rats administered TFP alongside QNP had body weight changes comparable to the control groups showing a statistically significant difference between TFP alone and TFP with QNP group.

3.2 Mean biweekly change in the feed and water intake

The mean biweekly changes in feed intake (Table 2) and water intake (Table 3) across the seven treatment groups are depicted. The control group consistently demonstrated significantly higher feed and water consumption compared to other groups. Before the administration of TFP, both control and treatment groups exhibited normal and consistent intake of feed and water, reflecting standard physiological behavior. However, rats receiving TFP 8.6 mg/kg exhibited a pronounced reduction in feed and water intake, which persisted throughout the study period,

indicating a sustained effect of TFP on these physiological functions. Importantly, rats treated with TFP combined with QCT showed improved feed and water consumption compared to the TFP-only group, while those receiving TFP with QNP displayed feed and water intake levels comparable to the control group. These findings suggest that chronic exposure to TFP cumulatively suppresses feeding and drinking behavior, whereas co-administration of QCT partially increased feed and water intake, and co-administration of QNP maintained a near-normal consumption pattern.

AOT425StatPgm					
w Test Load Data Save Data Get Report Options About AOT425 Exit					
Test / Substance: TOLFENPYRAD - MALE WISTAR ALBINO RATS					
Test Type: Main					
Limit Dose: 2000					
Assumed values at start of the main test: LD50: Default Sigma: 0.5					
Test Seq.	Animal ID	Dose mg/kg	Short-term Outcome	Long-term Outcome	Program's Data Entry Messages
1	HEAD	75	0	0	
2	BODY	99	×	×	
3	TAIL	75	0	0	
4	HEADBODY	99	×	×	
5	BODYTAIL	75	0	0	
6	TAILHEAD	99	×	×	
7		Stop Dosing			
8					
9					
10					
11					
12					
13					
14					
15					

The main test is complete.
Stopping criteria met: 5 reversals in 6 tests. LR criterion.
Estimated LD50 = 86.17 (Based on an assumed sigma of 0.5). Approximate 95% confidence interval is 75 to 99.

Fig 1: Acute oral toxicity test confirming the LD₅₀ to be 8.6 mg/kg body weight



Fig 2: Inflammation around mouth in TFP group.

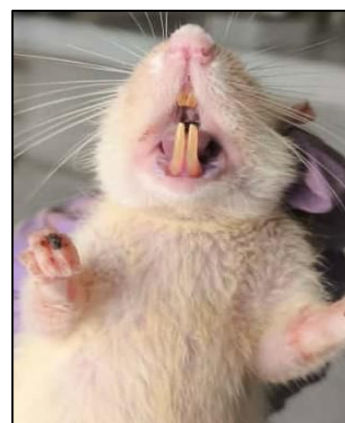


Fig 3: Rough body coat in TFP group



Fig 4: Minor oral inflammation (Arrow) and normal body coat in TFP+ QNP groups

Table 1: Body weight (g, Mean±SEM) of rat groups on seven treatments. Different uppercase superscript letters (A, B, C, D, E) indicate significant differences across days (time effect) within the same treatment group. Different lowercase superscript letters (a, b, c) within the same day indicate significant differences among treatment groups (treatment effect). Values sharing the same superscript letter are not significantly different (adjusted $p > 0.05$); those with different letters differ significantly (adjusted $p < 0.05$). Analysis was performed by two-way ANOVA followed by Tukey's multiple comparisons test. TFP-Tolfenpyrad, QCT-Quercetin, QNP-Quercetin nanoparticle

Days	Body weight (g) Mean±SEM						
	Distilled Water Control	VehicleControl	QCT	QNP	TFP	QCT+ TFP	QNP + TFP
0	208.17±3.06 ^{Aa}	210.07±1.79 ^{Aa}	207.03±2.77 ^{Aa}	211.95±2.01 ^{Aa}	208.48±3.47 ^{Aa}	209.30±1.42 ^{Aa}	211.60±1.49 ^{Aa}
15	241.98±1.61 ^{Ba}	248.37±2.97 ^{Ba}	244.28±2.30 ^{Ba}	246.25±2.40 ^{Ba}	237.78±2.51 ^{Bb}	243.22±2.79 ^{Ba}	245.20±2.50 ^{Ba}
30	290.63±2.51 ^{Ca}	289.57±2.55 ^{Ca}	289.90±2.38 ^{Ca}	291.05±1.34 ^{Ca}	273.35±2.70 ^{Cb}	280.25±4.50 ^{Cab}	289.08±2.09 ^{Ca}
45	326.75±1.60 ^{Da}	324.65±2.51 ^{Da}	323.42±3.09 ^{Da}	324.00±1.81 ^{Da}	289.07±4.18 ^{Dc}	304.53±2.67 ^{Db}	322.83±2.15 ^{Da}
60	349.88±3.16 ^{Ea}	350.45±1.36 ^{Ea}	348.22±2.22 ^{Ea}	347.05±2.1 ^{Ea}	295.26±4.21 ^{Ec}	320.80±2.29 ^{Eb}	345.18±2.17 ^{Ea}

Table 2: Feed intake (g, Mean±SEM) of rat groups on seven treatments. Different uppercase superscript letters (A, B, C, D, E) indicate significant differences across days (time effect) within the same treatment group. Different lowercase superscript letters (a, b, c) within the same day indicate significant differences among treatment groups (treatment effect). Values sharing the same superscript letter are not significantly different (adjusted $p>0.05$); those with different letters differ significantly (adjusted $p<0.05$). Analysis was performed by two-way ANOVA followed by Tukey's multiple comparisons test. TFP-Tolfenpyrad, QCT-Quercetin, QNP-Quercetin nanoparticle

Days	Feed Intake (g) Mean±SEM						
	Distilled Water Control	Vehicle Control	QCT	QNP	TFP	QCT+ TFP	QNP + TFP
0	11.43±0.31 ^{Aa}	11.17±0.10 ^{Aa}	11.22±0.19 ^{Aa}	11.53±0.13 ^{Aa}	11.23±0.18 ^{Aa}	11.12±0.07 ^{Aa}	11.02±0.09 ^{Aa}
15	14.00±0.37 ^{Ba}	13.77±0.21 ^{Ba}	13.43±0.14 ^{Ba}	13.32±0.11 ^{Ba}	13.07±0.14 ^{Ba}	13.05±0.14 ^{Ba}	13.53±0.13 ^{Ba}
30	15.68±0.36 ^{Ca}	15.57±0.21 ^{Ca}	15.33±0.28 ^{Ca}	15.30±0.07 ^{Ca}	13.92±0.28 ^{Cb}	15.03±0.21 ^{Ca}	15.62±0.22 ^{Ca}
45	16.75±0.23 ^{Da}	16.62±0.10 ^{Da}	16.45±0.10 ^{Da}	16.42±0.08 ^{Da}	15.42±0.22 ^{Db}	15.92±0.21 ^{Dab}	16.73±0.19 ^{Da}
60	18.37±0.24 ^{Ea}	18.33±0.35 ^{Ea}	17.77±0.45 ^{Ea}	18.00±0.52 ^{Ea}	14.92±0.51 ^{Ec}	16.98±0.09 ^{Eb}	18.27±0.18 ^{Ea}

Table 3: Water intake (mL, Mean±SEM) of rat groups on seven treatments. Different uppercase superscript letters (A, B, C, D, E) indicate significant differences across days (time effect) within the same treatment group. Different lowercase superscript letters (a, b, c) within the same day indicate significant differences among treatment groups (treatment effect). Values sharing the same superscript letter are not significantly different (adjusted $p>0.05$); those with different letters differ significantly (adjusted $p<0.05$). Analysis was performed by two-way ANOVA followed by Tukey's multiple comparisons test. TFP-Tolfenpyrad, QCT-Quercetin, QNP-Quercetin nanoparticle

Days	Water intake(mL) Mean±SEM						
	Distilled Water Control	Vehicle Control	QCT	QNP	TFP	QCT+ TFP	QNP + TFP
0	24.97±0.37 ^{Aa}	25.52±0.63 ^{Aa}	24.92±0.82 ^{Aa}	23.83±0.75 ^{Aa}	25.77±0.72 ^{Aa}	24.50±0.22 ^{Aa}	24.50±0.76 ^{Aa}
15	33.78±0.56 ^{Bab}	32.87±0.80 ^{Bab}	32.77±0.26 ^{Bab}	33.50±0.43 ^{Bab}	30.95±0.39 ^{Bc}	31.05±0.53 ^{Bc}	33.83±0.31 ^{Ba}
30	39.33±0.48 ^{Ca}	38.97±0.59 ^{Ca}	38.97±0.51 ^{Ca}	40.40±0.74 ^{Ca}	35.85±0.22 ^{Cb}	37.27±0.51 ^{Cab}	40.12±0.65 ^{Ca}
45	43.42±0.54 ^{Da}	43.25±0.65 ^{Da}	43.72±0.45 ^{Da}	43.92±0.55 ^{Da}	36.75±0.55 ^{Dc}	39.78±0.46 ^{Db}	43.33±0.48 ^{Da}
60	45.25±0.71 ^{Ea}	44.92±1.21 ^{Ea}	45.05±0.49 ^{Ea}	44.63±0.53 ^{Ea}	37.33±0.79 ^{Ec}	40.78±0.61 ^{Eb}	45.00±0.58 ^{Ea}

4. Discussion

Using Up-and-Down Procedure of OECD TG 425, the approximate LD₅₀ of TFP was estimated to be 86 mg/kg. The sub-chronic study for 60-day evaluated the ability of QNP to mitigate the subchronic physiological toxicity induced by the pesticide TFP in male Wistar rats. TFP exposure at a sublethal dose did not cause overt mortality but induced clear physiological disturbances, including perioral inflammation, a roughened hair coat, and mild diarrhea. While QCT co-administration moderately alleviated these clinical signs, the QNP co-treatment was markedly more effective, restoring the physical appearance of the rats to a state comparable to the control group. This enhanced efficacy aligns with findings that QNP can accelerate cutaneous wound healing through the targeted modulation of inflammatory and proliferative pathways (Choudhary *et al.*, 2020) [6].

A significant reduction in body weight gain was a primary consequence of TFP exposure, consistent with reports on other pyrazole-based acaricides. For instance, fenpyroximate exposure led to similar weight loss (Ayed-Boussema *et al.*, 2024) [3], and a prior 13-week study on TFP itself documented a marked decrease in body weight at doses comparable to those used in this study (JMPR, 2013) [11]. In this study, QCT only partially attenuated this weight loss. This limited protective effect is likely due to QCT's well-documented poor bioavailability and rapid metabolism (Mullen *et al.*, 2008; Zhang *et al.*, 2023) [14, 27]. In contrast, co-administration of QNP with TFP resulted in body weight gains statistically similar to the control group and significantly greater than in the QCT co-administration group. This superior performance of the nanoformulation is supported by analogous studies where QNP counteracted weight loss induced by toxicants like aluminum oxide nanoparticles and ethion (Abo-EL-Sooud *et al.*, 2023; Ranjith *et al.*, 2024) [1, 20]. The primary toxic mechanism of TFP is the inhibition of mitochondrial Complex I, disrupting cellular energy metabolism. This metabolic disruption can

lead to systemic toxicity, including effects on the liver and kidneys, which may contribute to gastrointestinal distress and reduced appetite (JMPR, 2013) [11]. The observed weight loss was strongly associated with a significant reduction in feed and water intake in TFP-treated rats, suggesting toxicity-induced anorexia. This pattern is common with pesticide exposures, such as carbendazim, where as QCT was shown to partially restore feed and water intake (Patil *et al.*, 2018) [19]. Notably, in the present study, the QNP co-treatment restored consumption levels to those of the control group. QCT is known to exert protective effects on gastrointestinal epithelia and mitigate oxidative stress (Patil *et al.*, 2018) [19], but its efficacy is limited by pharmacokinetic constraints. The superior therapeutic outcome of QNP is attributable to the properties of its chitosan matrix. Synthesized with sodium tripolyphosphate, this nano-carrier system effectively delivers hydrophobic QCT (Nan *et al.*, 2018) [15]. The positively charged chitosan enhances mucoadhesion and transiently opens tight junctions in the intestinal epithelium, facilitating paracellular transport and significantly improving the bioavailability of the encapsulated QCT (Onsoyen & Skaugrud, 1990; Rehman *et al.*, 2011) [18, 21]. This enhanced delivery directly translates to the observed physiological recovery, as the more bioavailable QCT can more effectively counteract the mitochondrial and oxidative disruptions caused by TFP.

5. Conclusion

The alterations observed in this study, along with evidence from earlier research, suggest that TFP exposure can interfere with key physiological functions responsible for controlling appetite, growth, and water homeostasis. These adverse effects may be mitigated through the use of QCT, particularly in their nanotherapeutic formulations. Given that rodents and humans share comparable mammalian physiology, similar protective outcomes might be

achievable in humans, warranting extensive investigation to minimize the physiological impacts of TFP exposure.

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