

Synergistic impact of green tea flavonoid extract and zinc oxide nanoparticles on oxidative-antioxidant enzymes in rats exposed to a high dose of paracetamol

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DOI.10.66723/zjhms.2026.191317

Received: 06 March 2026, Accepted: 24 March. 2026. Published: 30 June 2026

ABSTRACT

The objective of the present study was to investigate the combined effect of flavonoid-rich green tea extract and nano-zinc on hepatic oxidative stress markers in a paracetamol toxicity rat model. The green tea was extracted with a Soxhlet apparatus and flavonoids were isolated from the extract . A total of 90 male laboratory rats were utilized in this study, The animals were randomly assigned into nine equal groups :Group 1 received only a daily administration of 0.9% normal saline solution.Group 2 combination of paracetamol at a dose of 250 mg/kg and flavonoids at a dose of 50 mg/kg.Group 3 received paracetamol (250 mg/kg) concurrently with a higher dose of flavonoids (100 mg/kg).Group 4 administered green tea extract at a dose of 50 mg/kg along with paracetamol (250 mg/kg).Group 5: treated with a higher dose of green tea extract (100 mg/kg) combined with paracetamol (250 mg/kg).Group 6 low dose of zinc oxide nanoparticles (ZnO-NPs) at 0.5 mg/kg, administered alongside paracetamol (250 mg/kg).Group 7: higher dose of zinc oxide nanoparticles (ZnO-NPs) at 0.8 mg/kg in combination with paracetamol (250 mg/kg).Group 8 combined formulation consisting of ZnO-NPs (0.8 mg/kg), flavonoids (100 mg/kg), and green tea extract (100 mg/kg), all administered in conjunction with paracetamol (250 mg/kg).Group 9 injected solely with paracetamol at a dose of 250 mg/kg to induce toxicity. In comparison to other dosages, the results show a significant impact on MDA, GpX, LPO, protein, and sugar at the synergistic dose of flavonoid, green tea extract, and nano-zinc.

Key words: *Camellia sinensis* , Flavonoid ,Zinc nanoparticles, paracetamol.

INTRODUCTION

Acetaminophen Paracetamol is widely used as an analgesic and antipyretic; however, overdose can lead to severe hepatotoxicity and oxidative stress through its metabolic activation to the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI). NAPQI depletes glutathione and initiates lipid per-oxidation, resulting in impaired antioxidant defenses and elevated production of reactive oxygen species (ROS) [1,2]. Reduced levels of endogenous antioxidants and decreased activity of important oxidative enzymes including glutathione per oxidase (GPx), catalase (CAT), and super-oxide dismutase (SOD) are characteristics of this oxidative imbalance[3,4]. The ability of natural antioxidants to reduce oxidative damage caused by drugs has drawn a lot of scientific interest. Among these, flavonoid, which are polyphenolic chemicals found in many plant sources, have strong antioxidant system modulation and free radical scavenging properties [5]. Flavonoid like epigallocatechin-3-gallate (EGCG), which have been shown to increase antioxidant enzyme activity and decrease lipid per oxidation in experimental models of oxidative injury, are particularly abundant in green tea (*Camellia sinensis*)[6,7]. Green tea extracts have been shown in earlier research to protect against paracetamol-induced hepato toxicity by lowering blood transaminases and enhancing antioxidant status [8,9]. however, there is still a lack of research on the synergistic or additive effects of whole green tea and isolated green tea flavonoid on oxidative enzymes and antioxidant biomarkers in the setting of high-dose paracetamol exposure. because of both the targeted bio active compounds and the complex matrix of phytochemical present in the whole extract, it is hypothesized that the integration of isolated flavonoid with whole green tea may have a more pronounced modulator effect on oxidative stress parameters than either component alone. Understanding how this combination affects key oxidative enzymes and antioxidant mechanisms could provide insights into novel therapeutic strategies for mitigating drug-induced oxidative damage. therefore [10].The purpose of this study is to determine how zinc oxide nanoparticles and flavonoid-rich green tea extract work together to modulate hepatic oxidative stress (MDA, GPx, LPO, protease, and sugar) in a rat model of paracetamol toxicity.

MATERIAL AND METHODS

The obtained zinc oxide nanoparticles were from nanomaterials that were of useful size (36–40 nm). Green tea leaves from Babylon in Al-Sada, Iraq, on 12/12/2024, powdered and ground by using a mechanical grinder [11]. For green tea extraction, 300 mL of 80% methanol in a 1000 mL flask and 80 g of plant material were introduced to a Soxhlet apparatus in a thimble for a full day. After that, rotary evaporation equipment was used to evaporate the extract at 45°C[12,13].

Séparation flavonoïde compound

A reflux apparatus was filled with 20 g of dried methanol extract and 400 mL of 2M HCl for eight hours. After cooling and transferring the filtrate extract to a separator funnel, the glycol

moiety was extracted using 100 mL of ethyl acetate; the collected ethyl acetate layers were dried using a rotary evaporator set at 40°C for 20 min after being cleaned with distilled water to get rid of any leftover acid [14].

Photochemical reagent analysis

The active compounds phenol, glycosides, tannins, alkaloids, and flavonoids were identified according to the method described in [15]. In an experiment design in an animal house at the University of Kerbala, this study used 90 adult male rats that were 8–11 weeks old and weighed between 200 and 250 g. They were maintained at 25°C in a comparatively controlled environment.

Preparation of flavonoid and green tea concentrations

For a concentration of 5 mg/mL (a dose of 50 mg/kg), dissolve 50 mg of flavonoid and green tea in 10 mL of distilled water.

For a concentration of 10 mg/mL (dose of 100 mg/kg), dissolve 100 mg of flavonoid and green tea in 10 mL of solvent.

Preparation of zinc oxide nanoparticles concentrations

0.5 mg of Zn nanoparticles were dispersed in 10 mL of distilled water, using an ultrasonic bath for 10-15 min to ensure the dispersion of particles and prevent their agglomeration [16].

Table 1. The experiment design.

Experiment Design	Treatmentse
Group 1 (Negative Control):	Animals in this group received only a daily administration of 0.9% normal saline solution
Group 2	Rats were treated with a combination of paracetamol at a dose of 250 mg/kg and flavonoids at a dose of 50 mg/kg.
Group 3	This group received paracetamol (250 mg/kg) concurrently with a higher dose of flavonoids (100 mg/kg).
Group 4	Animals were administered green tea extract at a dose of 50 mg/kg along with paracetamol (250 mg/kg).
Group 5	This group was treated with a higher dose of green tea extract (100 mg/kg) combined with paracetamol (250 mg/kg).
Group 6	Rats received a low dose of zinc oxide nanoparticles (ZnO-NPs) at 0.5 mg/kg, administered alongside paracetamol (250 mg/kg).
Group 7	This group was given a higher dose of zinc oxide nanoparticles (ZnO-NPs) at 0.8 mg/kg in combination with paracetamol (250 mg/kg).
Group 8 (Synergistic Group)	Animals received a combined formulation consisting of ZnO-NPs (0.8 mg/kg), flavonoids (100 mg/kg), and green tea extract (100 mg/kg), all administered in conjunction with paracetamol (250 mg/kg).
Group 9 (Positive Control)	Rats were injected solely with paracetamol at a dose of 250 mg/kg to induce toxicity

Statistical Analysis

The data were expressed as mean Data were analyzed by One-way analysis of variances (ANOVA) to evaluate the statistical significance of differences between the control and other groups. The statistical analysis was performed using SPSS for Windows version and P values of 0.05 or less were considered significant (SPSS, Inc., Chicago, Illinois)[17].

RESULTS

The results in table (2) indicate that the yield percentage of flavonoid and green tea leave extract was 3% and 5%, respectively. The secondary metabolism is a natural substance that is formed and accumulated in plants.

Table 2. The percentage yield for green tea flavonoid.

Crud extract	Percentage yield
green tea	$(3/100g) \times 100 = 3\%$
flavonoid	$(5/100g) \times 100 = 5\%$

The photochemical assay was conducted to determine the green tea leave active components. in table (3) Flavonoid, Alkaloid, Phenol, Tannins and Glycoside showed positive results and Saponin showed negative results the photochemical screen.

Table 3. The green tea photochemical screen.

phytochemical	Results
Phenol	+
Glycoside	+
Saponin	-
Alkaloid	+
Tannins	+
Flavonoid	+

Table (4) indicates the rats in groups G9 showed a substantial increase in MDA level $0.934\mu\text{mol/L}$ compared to $G10.845 \pm 0.94\mu\text{mol/L}$, while G2 to G7, and G8 showed a significant decrease 0.745 ± 0.22 , 0.645 ± 0.36 , 0.564 ± 0.47 , 0.533 ± 0.52 , 0.465 ± 0.59 , 0.425 ± 0.64 , $0.354 \pm 0.77\mu\text{mol/L}$ respectively compared with G9 $0.934\mu\text{mol/L}$. the LPO enzyme of groups G9 indicate a significant increase $9.455 \pm 0.86\mu\text{mol/L}$ compared with control $8.654 \pm 0.75\mu\text{mol/L}$, while G2 to G8 show a significant decrease 7.54 ± 0.39 , 6.45 ± 0.42 , 6.22 ± 0.49 , 5.64 ± 0.52 , 4.64 ± 0.64 , $3.35 \pm 0.76\mu\text{mol/L}$ respectively compared with G9 $9.455 \pm 0.86\mu\text{mol/L}$. The GPX enzyme of G9 show a significant decrease $44.65 \pm 0.97\mu\text{mol/L}$ compared with control $54.98 \pm 0.48\mu\text{mol/L}$, G2 to G8 a significant increase 34.75 ± 2.65 , 37.98 ± 3.46 , 43.74 ± 4.74 ,

56.73±5.74, 61.75±6.43, 74.46±0.74, 83.75±0.84 respectively compared with G9 44.65±0.97 μmol/L.

Table 4. Synergistic impact of green tea flavonoid extract and zinc oxide nanoparticles on oxidative-antioxidant enzymes in rats

Groups	MDA mol/L	LPO mol/L	GPX mol/L
G 1	0.845±0.94 ^d	8.654±0.75 ^a	54.98±0.48 ^c
G2	0.745±0.22 ^c	7.54±0.39 ^d	34.75±2.65 ^a
G3	0.645±0.36 ^b	6.45±0.42 ^c	37.98±3.46 ^d
G4	0.564±0.47 ^{ab}	6.22±0.49 ^a	43.74±4.74 ^c
G 5	0.533±0.52 ^d	5.64±0.52 ^{ab}	56.73±5.74 ^{ab}
G6	0.465±0.59 ^c	5.15±0.59 ^d	61.75±6.43 ^a
G7	0.425±0.64 ^b	4.64±0.64 ^c	74.46±0.74 ^d
G8	0.354±0.77 ^a	3.35±0.76 ^b	83.75±0.84 ^c
G9	0.934±0.87 ^{ab}	9.455±0.86 ^{ab}	44.65±0.97 ^{ab}
LSD _{0.05}	1.84	1.64	1.75

(a,b,c,d) the different letters indicate a significant difference between the transactions.

Based on the table (5) protean assay of groups G9, rats showed a significant decrease 4.12±0.33 mol/L compared with G1 control 7.11±0.88 mol/L , G2 to G8 a significant increase 4.54±0.44, 5.35±0.55, 5.35±0.55, 5.65±0.59, 6.34±0.67, 6.73±0.71, 7.54±0.78, 8.45±0.88 mol/L respectively compared with G9 4.12±0.33 mol/L. The Glucose assay of G9 show a significant increase 0.245±0.95 mol/L compared with control 0.145±0.76 mol/L , while G2 to G8 indicate a significant decrease 0.166±0.74, 0.156±0.64, 0.149±0.52, 0.141±0.41, 0.141±0.41, 0.139±0.41, 0.131±0.35 and 0.121±0.24 mol/L respectively compared with G9 0.245±0.95 mol/L.

Table 5. Green tea flavonoid extract and zinc oxide nanoparticles' synergistic effects on proteins and sugar mol/L in rats

Groups	Protean mol/L	Glucose mol/L
G 1	7.11±0.88 ^a	0.145±0.76 ^c
G2	4.54±0.44 ^b	0.166±0.74 ^b
G3	5.35±0.55 ^c	0.156±0.64 ^a
G4	5.65±0.59 ^d	0.149±0.52 ^d
G 5	6.34±0.67 ^{ab}	0.141±0.41 ^c
G6	6.73±0.71 ^c	0.139±0.41 ^{ab}
G7	7.54±0.78 ^d	0.131±0.35 ^d
G8	8.45±0.88 ^d	0.121±0.24 ^c
G9	4.12±0.33 ^{ab}	0.245±0.95 ^{ab}
L.S.D _{0.05}	1.46	1.54

(a,b,c,d) are the different letters indicate a significant difference between the transactions

DISCUSSION

The main mechanism behind paracetamol-induced hepatocyte damage is the overproduction of the reactive metabolite N-acetyl-p-nonequivalent amine (NAPQI), which depletes intracellular glutathione (GSH) and promotes oxidative stress, mitochondrial dysfunction, and lipid peroxidation [18,19]. High-dose paracetamol usually causes higher levels of MDA and suppression of important antioxidant enzymes, such as GPx, CAT and SOD, in experimental models, indicating disruption of red-ox equilibrium [20]. This mechanistic framework can be used to evaluate the results of the current investigation, which demonstrate modification of oxidative and antioxidant enzymes after administration of mixed green tea and flavonoid produced from green tea is the source of green tea, which is high in catechins like epigallocatechin-3-gallate (EGCG), which have potent metal-chelating and free radical-scavenging properties [21,22]. By up-regulating phase II detoxifying enzymes and modifying signaling pathways like Nrf2, these substances are known to strengthen endogenous antioxidant defenses and restore redox balance in the face of oxidative stress. The integration of isolated flavonoid with whole green tea may have a synergistic or additive protective impact, according to the observed restoration (or significant improvement) in SOD, CAT, and GPx activities after combined therapy.

The rich photochemical matrix found in whole green tea, which includes poly-phenol, vitamins, and trace elements, may improve the stability and bio-availability of flavonoid, strengthening their antioxidant effects. This idea is consistent with earlier studies showing the protectiveness benefits of green tea extract in models of paracetamol-induced toxicity [23]. Furthermore, the decrease in MDA levels seen in the treated groups suggests that membrane damage and lipid per-oxidation have been attenuated. The decrease in MDA supports the theory that the combined intervention successfully reduces oxidative damage at both enzymatic and molecular levels because lipid per oxidation is a downstream impact of ROS overproduction. Since GSH conjugation is the main defense against paracetamol toxicity, restoring GSH levels further suggests enhanced detoxifying ability against NAPQI [24]. Nevertheless, variability in dosage, extraction method, bio-availability, and duration of treatment may influence the magnitude of the protective effect. High concentrations of certain poly phenol may also exhibit pro-oxidant activity under specific conditions, emphasizing the importance of dose optimization [25]. Some studies have shown that using flavonoid compounds with nanoparticles can reduce toxic effects and restore balance in biomarkers such as antioxidant enzyme levels, protein levels, and blood glucose levels. This is due to the ability of these compounds to regulate cellular pathways associated with oxidative stress and inflammation, which contributes to protecting cells from damage caused by drug toxins [26]. Previous studies have confirmed that combining green tea extract with zinc nanoparticles can reduce blood glucose levels, stimulate insulin secretion, and improve pancreatic function [27].

Conclusions

1. Due to their strong antioxidant properties, flavonoid, green tea, and zinc oxide nanoparticles scavenge free radicals.
2. Adult male rats that overdose on paracetamol develop liver toxicity.
3. When compared to other dosages, the combination of flavonoid-rich green tea extract with nano-zinc significantly affects MDA, GSH, LPO, protein, and sugar.

Acknowledgement

We are grateful to the College of Agriculture at University of Kerbala for helping me fulfill the research requirements.

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