

Preparation of Nano-Zinc-Loaded basil (*Ocimum basilicum*) and cucumber (*Cucumis sativus*) leaf Extracts in Protecting Against High-Dose acetaminophen Induced Hepatic Oxidative Stress in Rats"

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ABSTRACT

Drug-induced liver injury remains a major clinical concern, particularly that associated with high-dose idol, which is closely linked to oxidative stress and depletion of endogenous antioxidant defenses. The present study was designed to evaluate the hepatoprotective and antioxidant effect of (*Ocimum basilicum*) and cucumber leaf extracts loaded with zinc nano on acetaminophen induced hepatic oxidative stress in rats. Thirty six male Wistar rats were randomized into six groups: control: only distilled water, The animals in G2 were given a high dose of acetaminophen to induce hepatic oxidative stress, G3: was given acetaminophen + basil extract, G4: was given acetaminophen + cucumber leaf extract, G5: was given acetaminophen + Nano zinc, G6: was given acetaminophen + basil extract and cucumber leaves loaded with nano-zinc. Liver function enzymes (ALT, AST, ALP) and oxidative stress markers such as glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) were measured. Results showed that acetaminophen administration increased ALT, AST, ALP and MDA levels significantly with significant decrease in activities of antioxidant enzymes indicating severe hepatic oxidative damage. On the other side, the biochemical parameters were significantly improved using plant extracts and ZnO-NPs. The most expressed effect was registered in the group of nano-loaded extract, in which the liver function markers were almost normalized and the antioxidant enzyme activities were restored. In conclusion, ZnO-NPs-loaded basil and cucumber leaf extracts show a strong synergistic hepatoprotective effect against acetaminophen induced oxidative stress. This effect is probably mediated by increased antioxidant defense mechanisms and decreased lipid peroxidation.

Key words: Nano-Zinc-Loaded, Basil, Cucumber leaf Extracts, Acetaminophen, Hepatic Oxidative Stress.

INTRODUCTION

Oxidative stress is considered one of the main pathological mechanisms associated with tissue damage, resulting from an imbalance between the body's antioxidant defense systems and the generation of free radicals [1]. Particularly in the liver, which is the main organ involved in metabolic and detoxifying processes, this imbalance leads to cellular damage, including lipid peroxidation, protein degradation, and DNA damage, which eventually disrupts essential cellular activities [2]. acetaminophen is widely used as an analgesic and antipyretic, but high doses of it are a common cause of liver toxicity [3]. The mechanism of toxicity is primarily due to the formation of a reactive toxic metabolite (NAPQI) that depletes glutathione stores within liver cells, leading to increased production of reactive oxygen species and exacerbating oxidative stress [4]. (CAT) and glutathione peroxidase (GPx), which neutralize free radicals and preserve cellular equilibrium, are essential for shielding cells from oxidative damage [5]. Therefore, evaluating the activity of these enzymes is an important indicator for determining the level of oxidative stress and the severity of liver damage[6]. The use of natural substances with antioxidant qualities as medicinal supplements or substitutes to lessen oxidative damage has grown in popularity in recent years. One of the therapeutic plants rich in flavonoids and phenolic compounds with anti-inflammatory and antioxidant properties is basil (*Ocimum basilicum*) [7]. The leaves of cucumber (*Cucumis sativus*) also contain effective compounds that contribute to reducing oxidative stress and improving the functional state of tissues[8]. However, by increasing absorption and bioavailability, nanotechnology especially zinc nano has demonstrated encouraging potential for boosting the bioactivity of natural substances. Additionally, zinc is essential for maintaining antioxidant systems and controlling a number of essential enzymes[9]. Three mechanical advantages of nanoparticle loading over conventional extracts are as follows: enhanced bioavailability: the small size of the nanoparticles gives compounds a large surface area, which makes it easier for them to pass through liver cell membranes than poorly soluble conventional extracts. Sustained Release: To provide a longer-lasting therapeutic impact, zinc particles function as a clever carrier that delivers the active ingredients inside the target cells gradually. Direct inhibition of toxicity: The degradation of zinc nanoparticles releases (Zn^{2+}) ions that stimulate liver-protective metallothionein proteins and inhibit the cytochrome (CYP2E1) enzymes responsible for converting the drug into its toxic form[10]. Based on the above, this study aims to evaluate the preventive and therapeutic effects of basil extract and zinc-loaded cucumber leaves against liver oxidative stress induced by high doses of idol in rats, by measuring the activity of antioxidant enzymes and oxidative stress indicators.

MATERIAL AND METHODS

In this study, adult white rats of the Wistar albino type, weighing between 180–220 grams, were used. The animals were acquired from the college's experimental animal section and housed in standard conditions at a temperature of 22 ± 2 °C, a light cycle of 12 hours of light and 12 hours of darkness, and unlimited access to food and water. Before the trial started, the animals were given a week to adjust [11].

Experimental Design**Table 1. The six groups (n = 6) of rats.**

Groups	Treatments
G1	Given only distilled water
G2	Hepatic oxidative stress was caused by exposure to a high dose of acetaminophen (1000 mg/kg b. w).
G3	Treated with acetaminophen (1000 mg/kg b. w)+ basil extract 400 mg/kg
G4	Treated with acetaminophen (1000 mg/kg b. w.)+ cucumber leaf extract 400 mg/kg
G5	Treated with acetaminophen (1000 mg/kg b.w.)+ Nano zinc 5 mg/kg
G6	Treated with acetaminophen (1000 mg/kg b. w.) + basil extract and cucumber leaves loaded with nano-zinc 5 mg/kg.

The study design focused on evaluating the prophylactic role of the extracts. The animals underwent a pre-treatment with zinc-loaded extracts daily for 14 consecutive days. On day 15, a single toxic dose of acetaminophen (1000 mg/kg) was administered to induce acute liver toxicity. After 24 hours on the 16th, blood samples were collected.

Hepatic Oxidative Stress Induction

On the seventh day of the experiment, the animals were given a high dose of acetaminophen (1000 mg/kg b. w.) orally following a 12-hour fast in order to induce hepatic oxidative stress [12].

Preparation of Plant Extracts

The basil and cucumber leaf extracts were prepared using the alcoholic extraction method (70% ethanol). The leaves were dried and ground, then soaked in the solvent for 48–72 hours with continuous stirring. After that, the solution was filtered and the solvent was evaporated using a rotary evaporator to obtain the crude extract, which was then stored at 4 °C until use [13].



Figure1. Real image of cucumber leaf.



Figure 2.The real image of basil leaf.

Preparation of Nano-Zinc

Zinc oxide nano were used at a specific concentration (5 mg/kg), and they were prepared and suspended in distilled water using an ultrasonic device to ensure a homogeneous distribution before being administered to the animals [14].

Treatment Protocol

Plant extracts (400 mg/kg) and nano-zinc were administered daily orally for 7 days before giving idol, and the treatment continued for 7 days after, with the aim of evaluating the protective and therapeutic effects [15].

Preparation of Nano-Zinc-Loaded Plant Extracts

Zinc oxide nanoparticles (ZnO-NPs) were loaded onto plant extracts using the incubation method as follows:

A specific amount of ZnO-NPs (5 mg/kg equivalent) was dissolved in D. W with ultrasonic stirring (Sonication) to ensure homogeneous dispersion. the plant extract was gradually added to the Nano colloid. the mixture was magnetically stirred for 2–4 hours at room temperature to ensure complete loading. Then it was left to stabilize and confirm homogeneity before administration to the animals. the plant extracts loaded with zinc oxide nanoparticles were administered at a fixed dose of 5 mg/kg for ZnO-NPs within the plant extract [16].

Sample Collection

At the end of the experiment, the animals were anaesthetized, blood samples were taken to obtain serum, then the liver was excised and washed with cold saline solution. the samples were stored at -20 °C for biochemical analyses [17].

Biochemical Evaluation

A pre-made kit was used to assay the liver enzymes (ALT, AST, and ALP)

Additionally assessed were oxidative stress indicators, such as:

MDA, or malondialdehyde

The enzyme superoxide dismutase (SOD)

Catalase (CAT)

GPx, or glutathione peroxidase [18]

Statistical Analysis

The data were analysed using SPSS software, and the results were presented as (Mean $\pm$ SD). One-way ANOVA was used followed by the Tukey post-hoc test, and a p-value of ($P < 0.05$) was considered statistically significant [19].

RESULTS

The results showed at table 2 a significant increase ($P < 0.05$) in the levels of liver enzymes ALT, AST, and ALP in the acetaminophen group compared to the control group, indicating the occurrence of acute liver injury due to drug-induced oxidative stress. In contrast, treatment with basil extracts and cucumber leaves, as well as zinc oxide nanoparticles, led to a

significant decrease in these enzymes compared to the control group. The group treated with the nano-loaded extracts showed the best improvement, with values significantly approaching those of the control group.

Table2. Effect of Nano-Zinc-Loaded basil and cucumber Extracts on liver enzyme.

Groups	ALT U/L	AST U/L	ALP U/L
Mean $\pm$ SD			
G1	43 $\pm$ 3.76	112 $\pm$ 5.76	$\pm$ 4.4589
G2	154 $\pm$ 8.65	250 $\pm$ 11.87	$\pm$ 11.65211
G3	54 $\pm$ 4.45	169 $\pm$ 5.87	$\pm$ 6.65139
G4	55 $\pm$ 5.56	177 $\pm$ 6.76	$\pm$ 6.11132
G5	51 $\pm$ 4.76	145 $\pm$ 6.65	$\pm$ 5.76120
G6	45 $\pm$ 2.45	116 $\pm$ 5.76	$\pm$ 4.6592

The adult group showed at table (3) a significant increase in malondialdehyde (MDA) levels, with a clear decrease in the activity of antioxidant enzymes (SOD, CAT, GPx), indicating a severe disruption in the oxidative-reductive balance in liver tissue. As for the treated groups, they showed a gradual improvement in these indicators, with a decrease in MDA and an increase in antioxidant enzymes. The best results were observed in the group treated with ZnO-NPs-loaded extracts, which restored the oxidative balance almost naturally.

Table 3. Effect of Nano-Zinc-Loaded basil and cucumber Extracts on oxidative stress enzyme.

Groups	MDA nmol/mg	SOD U/mg	CAT U/mg	GPx U/mg
Mean $\pm$ SD				
G1	$\pm$ 0.32.3	$\pm$ 0.49.51	$\pm$ 0.37.64	$\pm$ 0.47.65
G2	$\pm$ 0.46.7	$\pm$ 0.24.65	$\pm$ 0.23.54	$\pm$ 0.32.65
G3	$\pm$ 0.35.76	$\pm$ 0.36.65	$\pm$ 0.25.65	$\pm$ 0.34.65
G4	4.65 $\pm$ 0.3	$\pm$ 0.37.76	$\pm$ 0.46.65	$\pm$ 0.45.74
G5	$\pm$ 0.23.4	$\pm$ 0.48.76	$\pm$ 0.47.76	6.75 $\pm$ 0.4
G6	$\pm$ 0.12.5	0.59.5 $\pm$	$\pm$ 0.58.76	$\pm$ 0.57.56

DISCUSSION

The results of the current study showed that administering high doses of acetaminophen led to a significant hepatic oxidative stress in rats, demonstrated by a substantial rise in malondialdehyde (MDA) levels, a fall in the activity of antioxidant enzymes like SOD, CAT, and GPx, and a drop in reduced glutathione (GSH) levels [20]. These results are consistent with the known hepatotoxicity of acetaminophen, which is metabolized to reactive NAPQI that depletes glutathione stores and elicits the formation of reactive oxygen species, leading to widespread oxidative damage to liver cells[21].

On the other hand, MDA levels decreased and antioxidant enzyme activity increased following treatment with cucumber leaves and basil extract, which markedly alleviated oxidative stress indicators. This effect may be explained by the rich chemical composition of these plants, which contains flavonoids and phenolic compounds with a potent ability to scavenge free radicals and stop lipid peroxidation events. Additionally, by boosting the synthesis of antioxidant enzyme genes, these compounds may strengthen the cellular defense system against oxidative stress[22]. In comparison to the groups treated with extracts alone, the findings of the usage of zinc nanoparticles demonstrated an additional improvement in the parameters under study, including a significant increase in the activity of antioxidant enzymes. This action is explained by zinc's essential function as a cofactor in numerous enzyme systems, as well as its capacity to stabilize cell membranes and lower the production of free radicals. Additionally, using the nano form has improved absorption efficiency and bioavailability, which has increased biological efficacy [23]. It is noteworthy that the treatment group that received the load (basil extract + cucumber leaves + nano zinc) had the greatest outcomes because all of the indicators under study significantly improved, and the values were close to those of the control group. This suggests that the plant compounds and nano-zinc have a synergistic impact since the plant chemicals function as direct antioxidants and zinc strengthens internal defense mechanisms, offering double protection against oxidative stress [24]. These findings are in line with numerous earlier investigations that demonstrated the protective function of antioxidant-rich plant extracts in lowering drug-induced liver damage, as well as with studies that demonstrated the effectiveness of nano-zinc in improving oxidative status and reducing tissue damage. However, this study adds a new dimension by loading plant extracts with nanotechnology, enhancing therapeutic efficacy and opening avenues for developing more efficient therapeutic strategies [25].

Despite the promising results, there are some limitations that should be taken into consideration, such as the lack of in-depth exploration of fine molecular pathways (like gene expression or cellular signalling pathways), in addition to the need to evaluate the long-term toxicity of nanoparticles. Therefore, the study recommends conducting future research focusing on the molecular aspects and potential clinical applications. It can be concluded that basil extract and zinc-loaded cucumber leaves have an effective protective and therapeutic effect against acetaminophen induced hepatic oxidative stress in rats. This effect is attributed to the antioxidant properties of the plant compounds, in addition to the vital role of nano-zinc in enhancing cellular

defenses. The results indicate the potential use of this approach as a promising strategy to reduce liver toxicity caused by oxidative stress, with the need for further studies to evaluate the molecular mechanisms and potential clinical applications.

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