

Research Article



Protective Effects of Broccoli Aqueous Extract on Hypothyroidism and Retrogression of Liver Functions Induced by Paracetamol (Acetaminophen) in Albino Male Rats

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Abstract | To evaluate the possible ameliorative impacts of broccoli aqueous extract on the regression of thyroid and hepatic tissues, as well as on oxidative stress markers induced by paracetamol (acetaminophen), a study was conducted on sixty male laboratory rats. The animals were equally divided into four experimental groups. The control group was orally treated with 0.9% normal saline solution. The second group was orally administered 1g/kg of paracetamol. The third study group was orally given 200 mg/kg of broccoli aqueous extract along with 1 g/kg of paracetamol. Lastly, the fourth experimental group was orally administered 200 g/kg of broccoli aqueous extract only. Furthermore, all treatments related to this study were administered for forty-five days, once daily. In this study, there was a noticeable increase in the activities of liver enzymes, malondialdehyde, lipid peroxide, and thyroid-stimulating hormone levels in the paracetamol treatment group compared to the other study groups. Conversely, glutathione reductase, glutathione peroxidase, triiodothyronine, and thyroxin levels were desirably decreased in the paracetamol treatment group compared to the other study groups. Otherwise, the two experimental groups that orally received broccoli aqueous extract + paracetamol, and broccoli extract only, showed no remarkable alterations in these biomarkers when compared with the control group. According to the findings of the current study, broccoli aqueous extract exerted potent protective activities on the thyroid and hepatic tissues, as well as ameliorated their functions. Furthermore, it inhibited or reduced the oxidative damage induced by paracetamol probably due to its biologically effective ingredients, particularly the strong antioxidants.

Keywords | Aqueous extract, Acetaminophen, Broccoli, Liver functions, Oxidative stress parameters, Thyroid gland

Received | March 18, 2024; **Accepted** | June 29, 2024; **Published** | September 25, 2024

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Citation | Jwad SM, Hussein TH, Al-Thuwaini TM (2024). Protective effects of broccoli aqueous extract on hypothyroidism and retrogression of liver functions induced by paracetamol (Acetaminophen) in albino male rats. *J. Anim. Health Prod.* 12(4): 501-507.

DOI | <http://dx.doi.org/10.17582/journal.jahp/2024/12.4.501.507>

ISSN (Online) | 2308-2801



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INTRODUCTION

The drug paracetamol is considered one of the most commonly used medications worldwide by millions of people as an analgesic for various degrees of pain (mild to moderate) and as an antipyretic drug (Caparrotta et al., 2018; Žur et al., 2018). Therapeutic doses are considered safe (Bkhairia et al., 2018), however, overdoses of this

drug result in a notable elevation in levels of the more toxic metabolite called N-acetyl-p-benzoquinone imine. This chemical substance can extensively deplete the hepatocellular content of glutathione; therefore, high doses of paracetamol cause liver toxicity (Bateman et al., 2014; Vliegenthart et al., 2015). Moreover, overdose administration of paracetamol results in numerous pathological alterations such as hepatocellular necrosis,

extra-hepatic tissue lesions, and nephrotoxicity, in addition to death in humans and laboratory animals (Subramanian et al., 2013). Paracetamol at a dose of 750 mg/kg has induced renal toxicity (Sathishkumar and Baskar, 2014). Therefore, alternative treatments, such as using plant extracts to treat tissue injury and inflammation, can provide promising results (Ahmed and Nasr, 2015; Jabori et al., 2023; Refat et al., 2023).

Broccoli, whose scientific name is *Brassica oleracea* L. var. Italica, a plant belonging to the cabbage family (Brassicaceae), has many potent antioxidant and anti-inflammatory properties (Hwang and Lim, 2014; Al-Balawi et al., 2019). Broccoli is known for its therapeutic benefits to health due to its high content of various nutrients, including vitamins, phenolic compounds, and essential minerals (Wijayanti et al., 2023). The high popularity of this vegetable is due to its nutritional value and the presence of bioactive compounds that are beneficial for preventing obesity, carcinogenesis, and cardiovascular disease (Martins et al., 2018). Broccoli extracts have shown a decrease in adipose tissue index and an improvement in glucose tolerance and insulin sensitivity in rats fed a high-fat diet (HFD) (Aborehab et al., 2016). Furthermore, it also has hepatoprotective effects (Sotokawauchi et al., 2018; Shati and Elsaid, 2019), as well as vascular protection (Mohammed et al., 2019). There is, however, limited research on broccoli extracts as a therapeutic agent for liver and thyroid dysfunction. The current study aims to assess the impact of broccoli extract, a highly antioxidative herbal and natural food extract on the functions of hepatic and thyroid tissues in response to paracetamol overdose toxicity.

MATERIALS AND METHODS

PREPARATION OF THE EXPERIMENTAL ANIMALS

An ethical committee at Kufa University approved the study (Edu. 01, 7, 22) between July 2022 and September 2023. This study was conducted using sixty albino male laboratory rats of the (Sprague-Dawley) strain, weighing 220-235g and aged less than 12 weeks. The rats were purchased from the National Center for Control and Pharmaceutical Research in Baghdad. They were then housed in steel cages in the animal facility of the biology department under standard laboratory conditions. The floor of the cages was covered with sawdust, which was changed two to three times a week. Regarding the water and feed (enriched with various proteins) provided for laboratory animals to consume *ad libitum*. Furthermore, all experiments were initiated when the male rats reached sexual maturity, at the age of twelve weeks.

PARACETAMOL (ACETAMINOPHEN) DRUG PREPARATION

The drug acetaminophen, at a dose of 1g/kg, was obtained

from the Al-Basra Pharmacy in Al-Najaf Al-Ashraf. Subsequently, the weights of the study animals were recorded after each laboratory rat was orally administered this drug based on its total body weight.

BROCCOLI AQUEOUS EXTRACT PREPARATION

The aqueous extract of broccoli was prepared according to the method outlined in reference (El-Desouky, 2021).

EXPERIMENTAL GROUPS

The rats were divided into the following treatment groups:

- The control group consisted of 15 male rats that were orally administered a physiological solution (normal saline 0.9%) for forty-five days, once daily.
- The second group was orally treated with paracetamol at a dosage of 1g/kg once daily for forty-five days. This group consisted of fifteen male rats.
- The third group orally received broccoli extract (200 mg/kg) + (1g/kg) of Paracetamol, once daily for forty-five days. This group consisted of fifteen males.
- The fourth group consisted of 15 male rats that were administered 200 mg/kg of broccoli extract orally once daily for 45 days.

SACRIFICING LABORATORY ANIMALS AND COLLECTING BLOOD SAMPLES

After the trial was finished, animals were anesthetized using chemical diethyl ether, and blood samples were obtained via heart puncture to collect approximately 5 ml of blood for analysis of hormonal and biochemical parameters.

BIOCHEMICAL STUDY OF SOME BLOOD PROPERTIES

Assessment of serum aspartate aminotransferase (AST) effectiveness: The activity of AST in serum was conducted using the method described by (Johnson et al., 1995) and kits purchased from Randox Lab (a French company). The absorbance was measured at 546 nm.

Assessment of serum alanine aminotransferase (ALT) effectiveness: Estimation of the ALT activity was conducted according to the method described by (Tietz, 1999). The kits and instruments were provided by Biolabo, a company based in France. The absorbance was measured at 340 nm using a spectrophotometer.

Assessment of serum alkaline phosphatase (ALP) effectiveness: The activity of the ALP enzyme was estimated using the method described by (Tietz, 1999) and kits obtained from Merck-Darmstadt, a German company. Additionally, the activity was assessed using a spectrophotometer at 510 nm.

Assessment of serum reductase and peroxidase glutathione levels: Concerning serum reductase and peroxidase

glutathione levels, the method of (Baker, 1990) was dependent, in addition, the kits, instruments, as well as various reagents that were used in this estimation were equipped by (Biomerieux Company). Furthermore, the absorbance read between 405 to 414 (nm) for glutathione reductase and 412 (nm) for glutathione peroxidase by the spectrophotometer.

Assessment of serum malondialdehyde (MDA) and lipid peroxide levels: Concerning serum reductase and peroxidase glutathione levels, the method described in reference (Armstrong and Browne, 1994) was followed. Additionally, the kits, instruments, and various reagents used for this estimation were provided by Biomerieux Company. Furthermore, the absorbance was read between 405 and 414 nm for glutathione reductase and at 412 nm for glutathione peroxidase using the spectrophotometer.

Assessment of serum T3, T4, and TSH levels: For the evaluation of serum T3, T4, and TSH levels, the Enzyme-Linked Immunosorbent Assay (ELISA) method was used. The kits and various reagents were provided by Biomerieux Company in France. Additionally, the absorbance was read at 450 nm.

STATISTICAL ANALYSIS

The analysis of the results in the current study was performed using the statistical software SPSS version 26. An Analysis of Variance test was conducted to identify differences between means, with the Least Significant Difference used at a significance level of < 0.05 (Morgan et al., 2004).

RESULTS AND DISCUSSION

The present study recorded a significant increase ($P \leq 0.05$) in the hepatic activities observed in the group treated with paracetamol orally. According to the analysis of this experiment, there was a significant increase ($P \leq 0.05$) in hepatic activities (AST, ALT, and ALP) (68.15 ± 0.25 , 93.95 ± 0.31 , and 233.40 ± 0.35 U/L), in the group orally treated with paracetamol compared to the other study groups (Tables 1, 2, 3). This result may be attributed to the toxic effects of high doses of paracetamol, which caused excessive generation of more hepatotoxic chemical metabolites (N-acetyl-p-benzoquinone imine) (NAPQI). This excessive generation possibly results in hepatic necrosis, damage to the cellular membranes of hepatocytes, liberation of hepatic enzymes from the cytoplasm, and subsequent elevation in their activities in serum, which is consistent with findings from several studies (Sasidharan et al., 2012; Bkhairia et al., 2018; Karabacak et al., 2018). Numerous previous reports have indicated that the drug paracetamol undergoes oxidation reactions via cytochrome (P450) enzymes located in liver tissues, resulting in the

production of (NAPQI) (Abiko et al., 2015; Leeming et al., 2015). On the other hand, higher doses of exposure to paracetamol limit the potential of the detoxification process for NAPQI, leading to subsequent cellular injuries (Caparrotta et al., 2018; Eyong et al., 2018).

Table 1: Effect of paracetamol and broccoli aqueous extract on AST activity.

Groups of treatment	Samples number	AST activity U/L in serum
Control group	15	43.12 ± 0.13^a
Group of Paracetamol	15	68.15 ± 0.25^b
Group of Broccoli extract + Paracetamol	15	41.02 ± 0.18^a
Group of Broccoli extract	15	39.78 ± 0.15^a
		L. S. D = 9.05

Different letters indicated noticeable variations at $P < 0.05$ level.

Table 2: Effect of paracetamol and broccoli aqueous extract on ALT activity.

Groups of treatment	Samples number	ALT activity U/L in serum
Control group	15	66.08 ± 0.19^a
Group of Paracetamol	15	93.95 ± 0.31^b
Group of Broccoli extract + Paracetamol	15	60.30 ± 0.01^a
Group of Broccoli extract	15	58.77 ± 0.17^a
		L. S. D = 11.19

Different letters indicated noticeable variations at $P < 0.05$ level.

Table 3: Effect of paracetamol and broccoli aqueous extract on ALP activity.

Groups of treatment	Samples number	ALP activity U/L in serum
Control group	15	148.21 ± 0.13^a
Group of Paracetamol	15	233.4 ± 0.35^b
Group of Broccoli extract + Paracetamol	15	141.39 ± 0.29^a
Group of Broccoli extract	15	137.01 ± 0.28^a
		L. S. D = 22.54

Different letters indicated noticeable variations at $P < 0.05$ level.

Regarding the oxidative stress parameters for the group orally administered with paracetamol, glutathione reductase (21.81 ± 0.34 ng/ml), glutathione peroxidase (4.01 ± 0.84 ng/ml) were significantly decreased ($P \leq 0.05$), while there was a significant increase ($P \leq 0.05$) in malondialdehyde (MDA) (89.18 ± 0.03 ng/ml) and lipid peroxide (288.16 ± 0.09 ng/ml) (Tables 4, 5, 6, 7). The data in this study aligns with the findings of (Bkhairia et al., 2018; Karabacak et al., 2018). The decreased levels of glutathione reductase and peroxidase may be attributed to the greater depletion of

hepatocyte glutathione content due to the strong toxicity of the paracetamol chemical metabolite substance. This substance is a potent oxidizing agent with the capacity to induce hepatic and renal tissue damage through adverse reactions with various cellular proteins, and to a greater extent, mitochondrial proteins, as demonstrated by (Abiko et al., 2015). Under normal conditions (therapeutic doses), harmful metabolites of paracetamol formed in the liver tissues are conjugated with glutathione molecules, which contain active sulfhydryl groups, and then converted into non-toxic chemical substances. In contrast, exposure to overdoses of this drug leads to a decrease or depletion of glutathione stores in the liver tissues (More et al., 2017). In addition to what has been mentioned, oxidative stress is an observable mechanism in the pathogenicity of acute liver damage, as confirmed by (Du et al., 2016; Wang et al., 2017). Higher doses of paracetamol lead to glutathione intoxication due to an excessive amount of NAPQI (El-Menyyi et al., 2018). Thus, the overdose of the drug used in this experiment could activate the production of cytochrome (P50) isoforms, deplete hepatocellular glutathione content, and induce oxidative stress accordingly. Elevated MDA and lipid peroxide levels were observed in the paracetamol-administered group in this study. This is likely due to the potent activation of the lipid peroxidation process resulting from the increased production of a more toxic metabolite of the drug (NAPQI). This activation may stimulate the oxidation of phospholipid molecules present in the cell membranes of liver tissues, leading to a significant increase in serum MDA and lipid peroxide levels (Du et al., 2016; Wang et al., 2017).

Table 4: Effect of paracetamol and broccoli aqueous extract on serum glutathione reductase level.

Groups of treatment	Samples number	Glutathione reductase ng/ml
Control group	15	130.4±0.84 ^a
Group of Paracetamol	15	21.81±0.34 ^b
Group of Broccoli extract + Paracetamol	15	128.07±0.07 ^a
Group of Broccoli extract	15	136.14±0.10 ^a
		L. S. D = 16.06

Different letters indicated noticeable variations at P < 0.05 level.

Table 5: Effect of paracetamol and broccoli aqueous extract on serum glutathione peroxidase level.

Groups of treatment	Samples Number	Glutathione peroxidase ng/ml
Control group	15	40.93±0.35 ^a
Group of Paracetamol	15	4.01±0.84 ^b
Group of Broccoli extract + Paracetamol	15	36.16±0.05 ^a
Group of Broccoli extract	15	45.03±0.29 ^a
		L. S. D = 9.56

Different letters indicated noticeable variations at P < 0.05 level.
Table 6: Effect of paracetamol and broccoli aqueous extract on serum malondialdehyde (MDA) level.

Groups of treatment	Samples number	Malondialdehyde (MDA) ng/ml
Control group	15	13.15±0.21 ^a
Group of Paracetamol	15	89.18±0.03 ^b
Group of Broccoli extract + Paracetamol	15	11.29±0.07 ^a
Group of Broccoli extract	15	8.22±0.11 ^a
		L. S. D = 6.95

Different letters indicated noticeable variations at P < 0.05 level.

Table 7: Effect of paracetamol and broccoli aqueous extract on serum lipid peroxide level.

Groups of treatment	Samples number	Lipid peroxide ng/ml
Control group	15	32.18±0.61 ^a
Group of Paracetamol	15	288.16±0.09 ^b
Group of Broccoli extract + Paracetamol	15	29.15±0.04 ^a
Group of Broccoli extract	15	22.99±0.22 ^a
		L. S. D = 15.19

Different letters indicated noticeable variations at P < 0.05 level.

Table 8: Effect of paracetamol and broccoli aqueous extract on serum triiodothyronine (T3) level.

Groups of treatment	Samples number	T3 nmol/L
Control group	15	1.93±0.21 ^a
Group of Paracetamol	15	1.02±0.14 ^b
Group of Broccoli extract + Paracetamol	15	1.86±0.24 ^a
Group of Broccoli extract	15	1.99±0.12 ^a
		L. S. D = 0.13

Different letters indicated noticeable variations at P < 0.05 level.

As shown in Tables 8, 9, 10, TSH levels were elevated (1.87±0.08 µLu/ml), while T3 (1.02±0.14 nmol/L), and T4 levels (61.11±0.34 nmol/L) were significantly decreased (P ≤ 0.05) in the group orally treated with paracetamol compared to the other study groups. Concern about the significant decline in the levels of thyroid hormones (T3 and T4) is likely attributed to the detrimental impacts of the higher toxic compound NAPQI in paracetamol. This compound may stimulate the production of various reactive oxygen species and subsequent damaging free radicals, as documented in this study by the increasing levels of MDA (an end metabolite substance of lipid peroxidation). In contrast, the levels of glutathione reductase and peroxidase were noticeably decreased, thereby adversely affecting the performance of the thyroid gland. Hypothyroidism

could be considered a disruption in the functions of the thyroid-pituitary-hypothalamic axis, which can result in a noticeable decrease in the levels of thyroid hormones (Ahmed, 2019). Other mechanisms may be suggested to explain the hypothyroidism state. It is possible that N-acetyl-benzoquinone imine and increased levels of more destructive radicals could inhibit the entrance of iodine molecules into the thyroid cells. Alternatively, the biosynthesis of vital enzymes involved in the production of thyroid hormones (such as peroxidase and catalase) may be negatively affected (Ahmed, 2019; Banu et al., 2020). Furthermore, the NAPQI oxidizing agent in paracetamol and toxic oxygen species likely destroys thyroglobulin molecules, which are essential for the synthesis of thyroid hormones in conjunction with iodine. In the paracetamol-treated group, the elevated TSH level may explain the decrease in thyroid hormones (T3 and T4) due to the negative feedback mechanism on the anterior pituitary gland (Banu et al., 2020).

Table 9: Effect of paracetamol and broccoli aqueous extract on serum tetraiodothyronine (T4) level.

Groups of treatment	Samples number	T4 nmol/L
Control group	15	73.53±0.28 ^a
Group of Paracetamol	15	61.11±0.34 ^b
Group of Broccoli extract + Paracetamol	15	74.17±0.29 ^a
Group of Broccoli extract	15	75.18±0.10 ^a
		L. S. D = 3.36

Different letters indicated noticeable variations at P < 0.05 level.

Table 10: Effect of paracetamol and broccoli aqueous extract on serum thyroid stimulating hormone (TSH) level.

Groups of treatment	Samples number	TSH µLu/ml
Control group	15	1.44±0.16 ^a
Group of Paracetamol	15	1.87±0.08 ^b
Group of Broccoli extract + Paracetamol	15	1.39±0.27 ^a
Group of Broccoli extract	15	1.36±0.04 ^a
		L.S.D = 0.09

Different letters indicated noticeable variations at P < 0.05 level.

Regarding the two groups that orally received broccoli extract + paracetamol and aqueous extract of broccoli only, no remarkable changes were observed in the activities of hepatic enzymes and oxidative stress parameters compared to the control group. These findings are consistent with numerous studies (Martins et al., 2018; Sharma and Sangha, 2018). On the other hand, the results are inconsistent with the studies conducted by (El-Baz et al., 2012; Sarhan et al.,

2014), which reported a significant decrease in the activities of AST, ALT, and ALP, as well as MDA levels. TSH, T3, and T4 levels in the two groups of rats that were orally administered broccoli extract + paracetamol drug, and the group that received an aqueous extract of broccoli, the data showed agreement with another study that demonstrated no significant alterations in the levels of TSH, T3, and T4 after the ingestion of broccoli sprouts. Additionally, there is a strong protective effect against the chemical compound sulfadimethoxine, which induces thyroid tissue damage. Furthermore, the level of thioredoxin reductase noticeably increased following broccoli ingestion. The improvement that was recorded in the oxidative stress indices and thyroid gland function may be attributed to the bioactive components of broccoli, such as vitamins C and E, which are effective antioxidants, various carotenoids, phenolic content (especially flavonoids), and essential dietary minerals (Faller and Fialho, 2009; Al-Balawi et al., 2019). In addition, broccoli contains essential nutrients and different compounds that have anti-cancer activity, including 3, 3-diindolylmethane and glucoraphanin. Moreover, this substance has a powerful activator ability for the immune system (Kirsh et al., 2007). Recently, many studies have focused on the strong antioxidative and anti-inflammatory potential of broccoli (Hwang and Lim, 2014; Sotokawauchi et al., 2018). Additionally, research has highlighted its protective effects on hepatic tissues against CC14 (Shati and Elsaid, 2019). Broccoli also plays a crucial role in safeguarding hepatic and renal tissues from hyperglycemia-induced oxidative stress or damage (Hwang and Lim, 2014). Therefore, the bioactive components and potent abilities of broccoli may synergistically enhance the functional performance of both hepatic and thyroid tissues, helping to prevent or alleviate the oxidative stress induced by the drug paracetamol.

CONCLUSION

Broccoli aqueous extract had an ameliorative effect on the liver and thyroid gland functions. It could also inhibit or alleviate the oxidative destructive effects of paracetamol overdose. Further hematological, histological, immunological, and molecular studies are needed to confirm our findings.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the staff of National Center for Control and Pharmaceutical Research for their facilities that supported the rats.

NOVELTY STATEMENT

The novelty of our study has been its strong evidence

that broccoli aqueous extract mitigates the effects of paracetamol (acetaminophen) overdose on thyroid and liver tissue regression, in addition to oxidative stress markers for the first time.

AUTHOR'S CONTRIBUTION

All authors contributed equally.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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