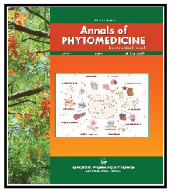
Annals of Phytomedicine 13(2): 537-543, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.2.54

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Antidiabetic, antioxidant, and hepatoprotective activities of methanolic *Tamarindus indica* L. flower extract

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Article Info

Article history
Received 12 October 2024
Revised 29 November 2024
Accepted 30 November 2024
Published Online 30 December 2024

Keywords
Antidiabetic
Antioxidant
Hepatoprotective
Oxidative stress
Tamarindus indica L.

Abstract

Liver diseases are common chronic disorders that affect millions of people and often lead to serious complications, such as diabetes mellitus, which is linked to oxidative stress. Due to the side effects associated with many conventional chemical drugs, there is increasing interest in plant-based therapeutic agents. In this context, the present study investigated the antidiabetic, antioxidant, and hepatoprotective activities of the methanolic flower extract of *Tamarindus indica* L. The antidiabetic potential of the *T. indica* methanolic crude extract was demonstrated, showing 64% and 65% inhibition of α-amylase and α-glucosidase activity, respectively, at a concentration of 5 mg/ml. Additionally, antioxidant assays, including nitric oxide, DPPH free radical scavenging, ABTS scavenging, and scavenging tests, revealed significant antioxidant properties, with scavenging activities of 72%, 76%, and 82%, respectively, after treatment. The extract also exhibited hepatoprotective effects on HepG2 cells. Based on these findings, the study suggests that *T. indica* methanolic crude extract holds potential for further research as a therapeutic option for diabetes mellitus.

1. Introduction

World wide, chronic liver disease is a non-alcoholic fatty liver disease (NAFLD) and is often the cause of a chief public health concern that affects approximately 30% of the adult population and encounters two million deaths every year (Younossi *et al.,* 2016). NAFLD comprises a huge spectrum of disease conditions characterized by simple hepatic steatosis to non-alcoholic steatohepatitis which may develop when there are no other reasons for secondary hepatic fat buildup, such as cirrhosis brought on by excessive alcohol use liver fibrosis, and elevated risk of problems from the liver, such as end-stage liver disease and hepatocellular carcinoma, liver transplantation requirement leads liver associated mortality (Chalasani *et al*., 2018). Evidence indicates the NAFLD global burden expands apart from liver-related complications which raise the chance of getting type 2 diabetes mellitus and other metabolic diseases, metabolic syndrome, heart related conditions, and dyslipidemia (Long *et al.,* 2020; Loomba *et al.,* 2020; The European Association for Liver Research, 2016). Among these, the second most common metabolic liver disorder is type 2 diabetes mellitus, which is prevalent among patients with liver diseases. Liver disorders have significantly increased mortality and morbidity rates, and their progression poses a substantial challenge to humanity (Das *et al*., 2022; Zhou *et al*., 2017; Anstee *et al.,* 2013).

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One of the most important chronic metabolic disordersdiabetes mellitus (DM) is characterized by elevated sugar levels in the blood sub sequently pancreatic deficiency like insulin production or a reduced cell sensitivity to insulin leads to diabetic conditions causing mild to serious complications up to death, if unnoticed or untreated (Wild *et al*., 2004). Worldwide, diabetes is ranked sixth leading cause of death owing to its associated complications, and diabetes patients increasing number executes an important socio-economic burden on the healthcare system and also, work loss and wages (WHO, 2016; Deshpande *et al.,* 2008). Diabetes pathogenesis is presently attributed to risk factors including metabolic abnormalities and endogenous factors like genes and also, exogenous factors including environment and behaviour (Wu *et al*., 2017). Consequently, oxidative stress is an important endogenous factor and the main cause of diabetes complications (Wright *et al*., 2006). The relationship between diabetes and oxidative stress has been explained *via* molecular mechanisms wherein the elevated reactive oxygen species production is associated with hyperglycemia causingan imbalance that leads to oxidative stress (Folli *et al.,* 2011). Therefore, a cost effective treatment option is urgently required to manage diabetes and possesses antioxidant property and also, hepatoprotective properties.

In these circumstances, the natural product is highly considered for its long lasting history of medical and health benefits on human health (Dias *et al*., 2012). Amongst, plants are the most examined source due to their wide distribution and their variety range of biological properties like antibacterial, antiageing, anti-inflammatory, anticancer, and antiviral (Xu *et al.,* 2017). Consequently, *T. indica* generally known as tamarind is an evergreen leguminous tree from Caesalpiniaceae. The pharmaceutical values of the different plant parts are mainly associated with various phytochemicals including

saponin, tannins, flavonoids, steroids, polyphenols, and alkaloids (Komakech *et al.,* 2019). Hence, our study investigated the antidiabetic, antioxidant, and hepatoprotective properties of *T. indica* flower methanolic extract.

2. Materials and Methods

2.1 Plant authentication

The plant used in the present study was identified as *T. indica* by Dr. Shamna, Department of Herbal Medicine, Deseeya Ayurvedic Pharmacy, Calicut, India with authentication number DAP/22-20/2024.

2.2 *T. indica* methanolic crude extracts preparation

The gathered *T. indica* flowerwas sundried and a roughly powered 20 g. *T. indica* flower was added to a fresh cellulose thimble that was inserted into the Soxhlet device as per standard protocol (Harley *et al.,* 2022). Adding methanol to the flask initiated the reaction and continued for many hours until it wasa clear solvent and the obtained resultant was used for further studies.

2.3 Alpha-amylase inhibition activity

The ability of *T. indica* flower methanolic extract to inhibit alpha-amylase activity was studied as explained earlier (Alqahtani *et al*., 2019). The range of concentrations, in short is (1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, and 5 mg/ml) of *T. indica* flower methanolic extract as well as the enzyme amylase in 20 mM for10 min; the buffer of sodium phosphate (pH 6.8) was kept at 25°C. A 1% starch solution was added to a 0.02 M sodium phosphate buffer (pH 6.9) to the pre-incubated above mixtures and allowed for 15 min. After adding 1.0 ml of dinitrosalicylic acid to halt the reaction, the mixtures were incubated for 5 min in a water bath. The dilution was carried out in the response mixtures and the optical density was measured at 540 nm to calculate amylase activity inhibition percentage.

2.4 Glucosidase activity inhibition assay

The ability of *T. indica* flower methanolic extract to inhibit glucosidase activity was examined as shown previously (Elya *et al*., 2008). In short, the varying ranges of amounts of (1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml) of *T. indica* flower methanolic extract was incubated along with α-glucosidase (0.01 mg/ml) for 10 min. The reaction was started with 5 mM of p-nitrophenyl-α-D-glucopy ranoside (pNPG) addition to the above mixture and continued for 60 min. The 0.1 M Na_2CO_3 was included to stop the response and the final product was measured at 400 nm to calculate the glucosidase action inhibition percentage.

2.5 DPPH free radical scavenging assay

T. indica flower methanolic extract the ability to scavenge radicals was investigated using the DPPH (2, 2-diphenyl-1-picryhydrazyl) assay as stated previously (Gayathri and Sathish Kumar, 2016). In short, the varying concentrations (1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml) of *T. indica* flower methanolic extract (3 ml) were added in DPPH solution and allowed for 30 min in dark situation. The radical scavenging activity percentage was calculated after reading the final product at 517 nm:

$$\text{Scavenging effect} = 100 \times \frac{(\text{blank OD} - \text{sample OD})}{\text{blank OD}}$$

2.6 ABTS scavenging assay

The ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) scavenging activity of *T. indica* flower methanolic extract was evaluated, as previously reported by Pacifico *et al.* (2018). Briefly, ABTS solution was prepared by mixing 7 mM ABTS in water with 2.45 mM potassium persulfate (KSO) and allowing the reaction to proceed in the dark for 16 h. The resultant ABTS solution was then diluted with 0.1 M sodium phosphate buffer (pH 7.4) to achieve an absorbance of 0.750 ± 0.025 at 734 nm. Subsequently, *T. indica* flower methanolic extract at various concentrations (1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, and 5 mg/ml) was mixed with 1 ml of ABTS solution and allowed to react for 6 min. The absorbance of the final reaction product was measured at 734 nm, and the percentage of ABTS scavenging activity was calculated:

$$\text{ABTS scavenging effect (\%)} = 100 \times \frac{(\text{control OD} - \text{sample OD})}{\text{control OD}}$$

2.7 Nitric oxide scavenging activity

The methanolic extract of *T. indica* flowers demonstrated nitric oxide scavenging activity, as previously reported by Alam *et al.* (2013). Briefly, a reaction mixture containing *T. indica* flower methanolic extract at various concentrations (1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, and 5 mg/ml) and 10 mM sodium nitroprusside in 0.5 M phosphate buffer (pH 7.4) was incubated for 60 min. After incubation, an equal volume of Griess reagent, composed of 1% sulfanilamide and 0.1% naphthyl ethylenediamine dihydrochloride in 2.5% phosphoric acid (1:1, v/v), was added to the reaction mixture. The resulting pink-colored product was measured at 540 nm to determine the percentage of nitric oxide scavenging activity:

$$\text{Scavenging activity (\%)} = 100 \times \frac{(\text{control OD} - \text{sample OD})}{\text{control OD}}$$

2.8 Hepatoprotective activity of *T. indica* methanolic crude extract

The cytotoxicity of *T. indica* methanolic crude extract on HepG2 cells was evaluated using the MTT assay, as described by Meiyazhagan *et al*. (2015). HepG2 cells cultured in DMEM medium were treated with varying concentrations of *T. indica* methanolic crude extract (1 mg ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, and 5 mg/ml). After incubation, MTT solution was added to facilitate the formation of formazan crystals, followed by the addition of DMSO to solubilize the crystals and produce a purple-colored product. The absorbance of the product was measured at 570 nm to assess cell viability.

2.9 Statistical analysis

The standard and mean deviations were used for calculating error bars for all the experiments.

3. Results

3.1 Alpha-amylase inhibition activity

The ability of *T. indica* flower methanolic extract to inhibit α-amylase activity was assessed, and the percentage inhibition of α-amylase activity is presented in Figure 1. The figure shows that α-amylase activity was inhibited by 26%, 31%, 43%, 52%, and 64% following treatment with *T. indica* flower methanolic extract at concentrations of 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, and 5 mg/ml, respectively.

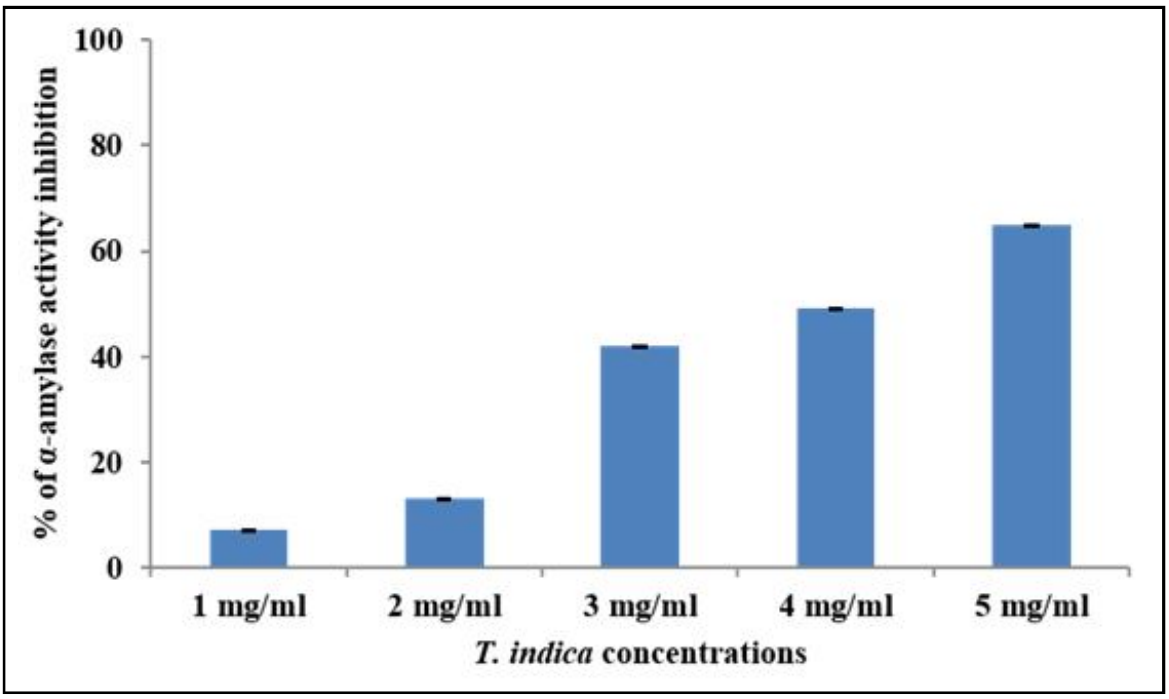

Figure 1: Effect of *T. indica* flower methanolic extract on α-amylase activity inhibition.

3.2 Glucosidase activity inhibition assay

The inhibitory effect of *T. indica* flower methanolic extract on glucosidase activity is shown in Figure 2. The graph illustrates the percentage inhibition of glucosidase activity following treatment with varying concentrations of *T. indica* flower methanolic extract (1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, and 5 mg/ml). The extract effectively inhibited glucosidase activity, with inhibition rates of 7%, 13%, 42%, 49%, and 65%, respectively.

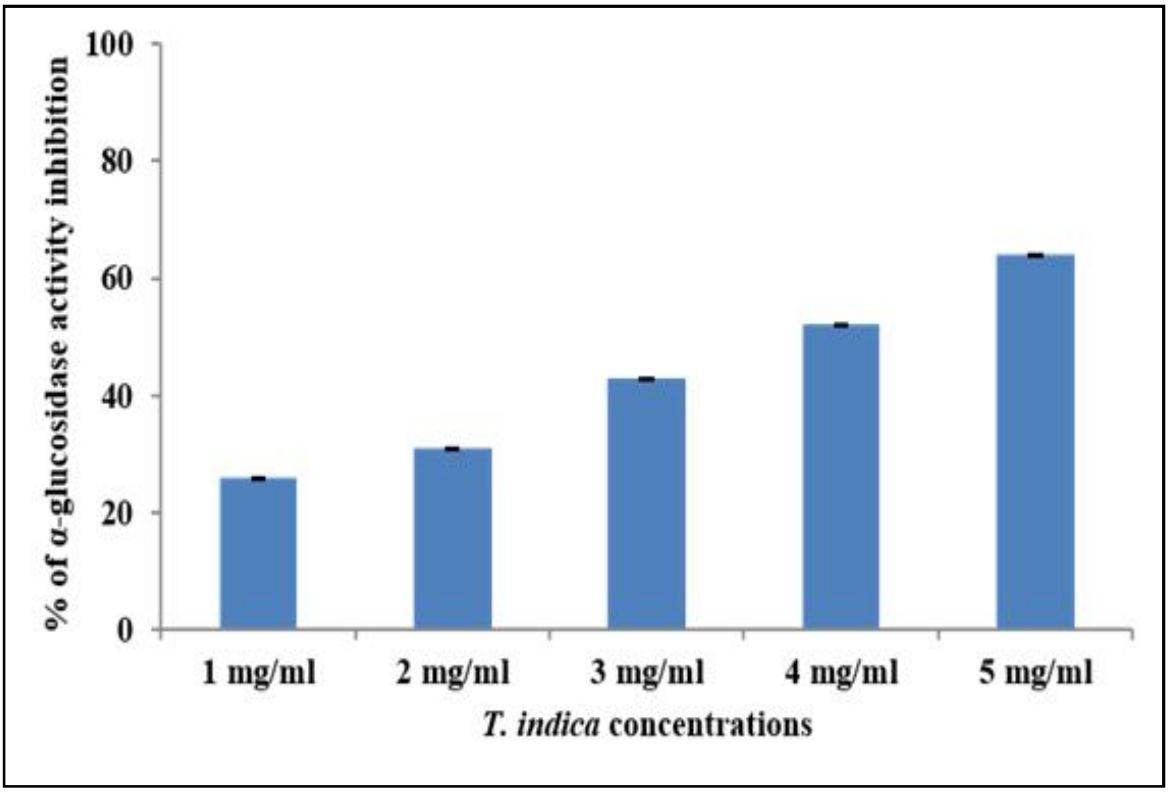

Figure 2: Effect of *T. indica* flower methanolic extract on α-glucosidase activity inhibition.

3.3 DPPH free radical scavenging assay

The radical scavenging capacity of *T. indica* flower methanolic extract was evaluated using the DPPH assay, and the calculated scavenging activity percentages are presented in Figure 3. The figure demonstrates that *T. indica* flower methanolic extract exhibited scavenging activities of 41%, 48%, 60%, 69%, and 72% at varying concentrations, highlighting its potent antioxidant activity.

3.4 ABTS scavenging assay

The effect of *T. indica* flower methanolic extract on ABTS scavenging activity is shown in Figure 4. The graph illustrates the calculated scavenging activity percentages after treatment with various concentrations of *T. indica* flower methanolic extract (1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, and 5 mg/ml). The extract exhibited ABTS scavenging activities of 47%, 49%, 58%, 70%, and 76%, respectively, demonstrating its strong antioxidant potential.

3.5 Nitric oxide scavenging activity

The effect of *T. indica* flower methanolic extract on nitric oxide scavenging activity was evaluated, and the calculated scavenging activity percentages are presented in Figure 5. The graph indicates that *T. indica* flower methanolic extract exhibited nitric oxide scavenging activities of 15%, 51%, 58%, 69%, and 82% at various concentrations, demonstrating its potent ability to neutralize nitric oxide radicals.

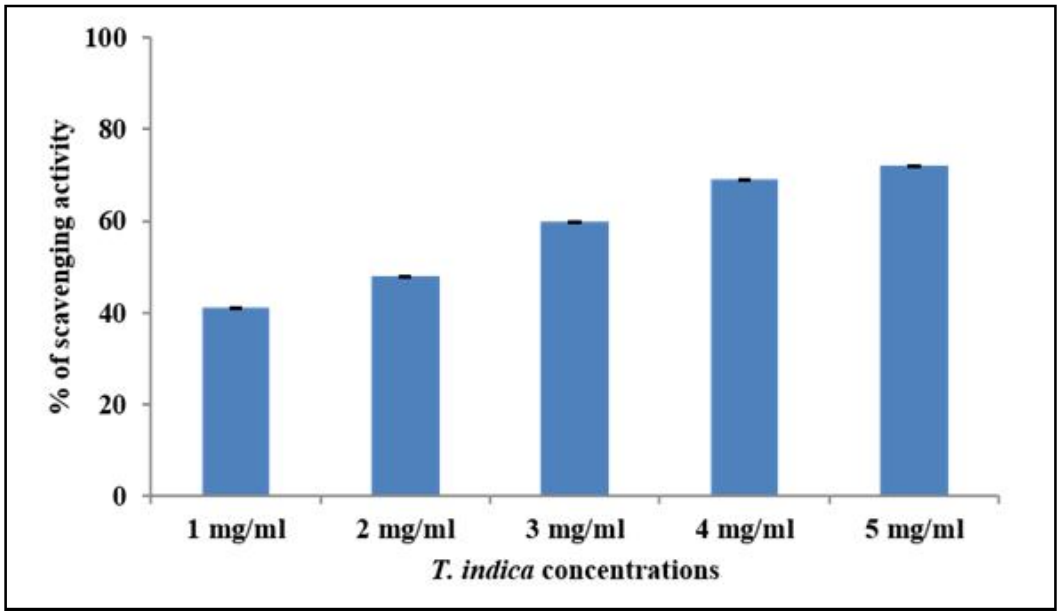

Figure 3: Effect of *T. indica* flower methanolic extract on free radical scavenging activity.

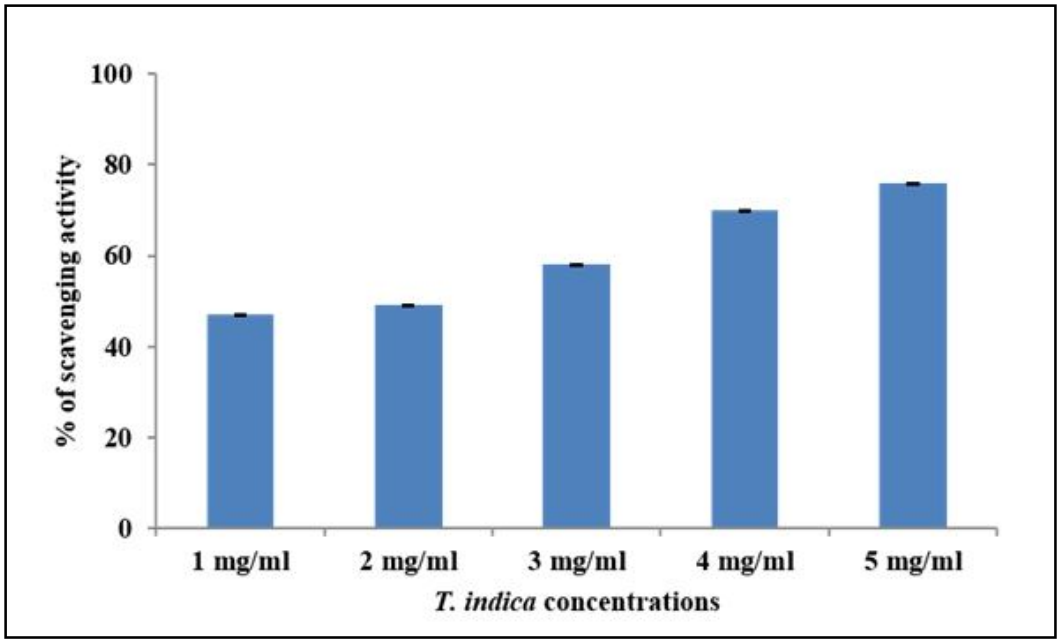

Figure 4: Effect of *T. indica* flower methanolic extract on ABTS scavenging activity.

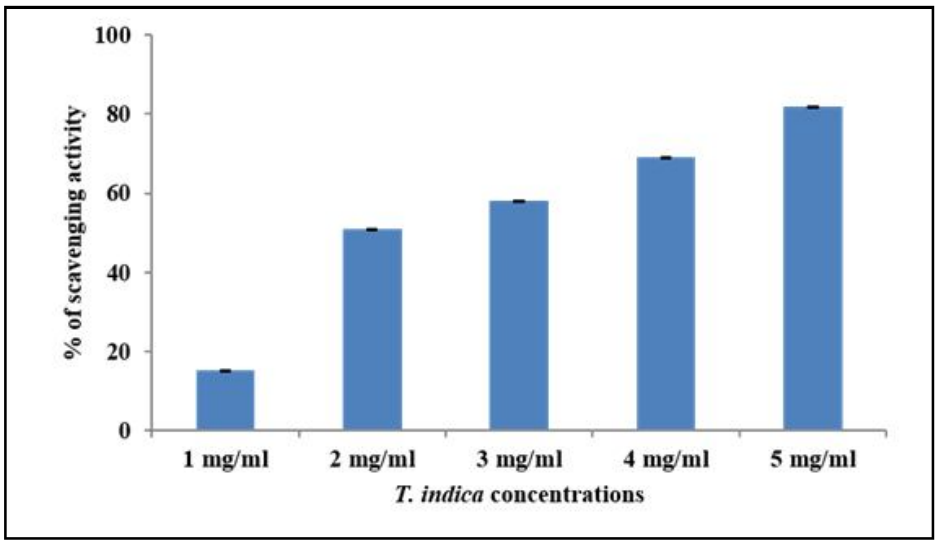

Figure 5: Effect of *T. indica* flower methanolic extract on *nitric oxide scavenging activity.*

3.6 Hepatoprotective activity of *T. indica* methanolic crude extract

The hepatoprotective activity of *T. indica* methanolic crude extract on HepG2 cells was assessed using the MTT assay, and the calculated cell viability percentages are presented in Figure 6. As shown in the figure, the graph illustrates the hepatoprotective activity of *T. indica* flower methanolic extract at varying concentrations (1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, and 5 mg/ml), with cell viability percentages of 93%, 87%, 41%, 40%, and 25%, respectively, after treatment.

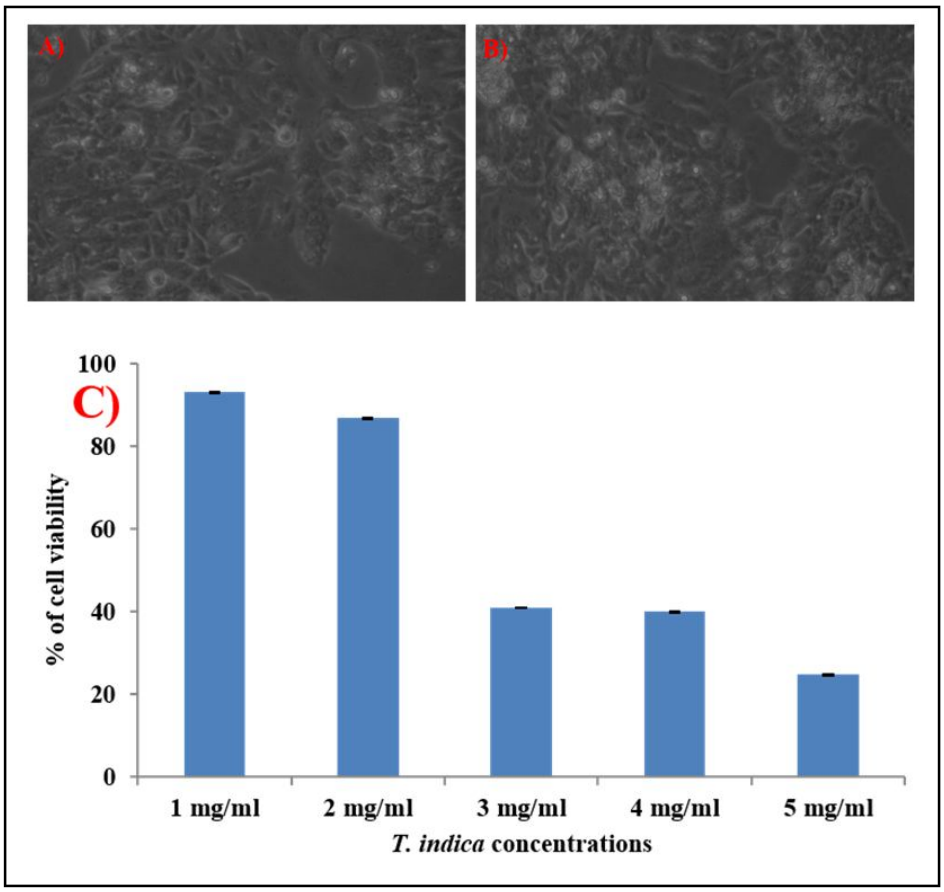

Figure 6: Effect of *T. indica* flower methanolic extract on hepatoprotective activity. (A) Untreated HepG2 cells, (B) HepG2 cells treated with *T. indica* flower methanolic extract, and (C) Graph represents the cell viability percentage after treatment.

4. Discussion

Liver disease is a significant chronic metabolic condition that can lead to mild to severe complications due to various factors. Among these complications, diabetes mellitus (DM) is a major metabolic disorder, often linked to risk factors such as oxidative stress. Therefore, our study investigated the antidiabetic, antioxidant, and hepatoprotective activities of the methanolic crude extract of *T. indica* and found it to exhibit potent antidiabetic, antioxidant, and hepatoprotective properties. α-amylase is a key enzyme responsible for breaking down dietary carbohydrates into simple sugars in the digestive tract, while α-glucosidases further degrade these simple sugars into glucose, which is then absorbed into the bloodstream. Given this, our study aimed to assess the ability of the methanolic *T. indica* crude extract to inhibit both α-amylase and α-glucosidase activities. Our results revealed the antidiabetic activity of *T. indica* crude extract. In favor of this, the study explored the methanolic raw extract of *Nuxia oppositifolia* antidiabetic and antioxidant of purified components. The purified compounds, katononic acid and 3-oxolupenal were examined for α-glucosidase and α-amylase enzyme inhibition and revealed the potent inhibitory activity of both enzymes and also, the molecular docking highest binding affinity with active sites of both enzymes which provide primary information about *N. oppositifolia* use for diabetes mellitus (Alqahtani *et al.,* 2019). The *in vitro* analysis of various solvents (hexane, ethanol, ethyl acetate, and aqueous extracts) extracted *Bridelia ferruginea* investigated for antidiabetic and antioxidant analysis. The plant extract had the antioxidant property by scavenging the free radicals and exposed antidiabetic activity by inhibiting α-glucosidase, α-amylase, and lipase enzymes activities and showed good binding affinity with enzymes which exposed the antioxidant and antidiabetic potential of the plant (Oyebode *et al.,* 2022).

Diabetes mellitus (DM) is caused by various risk factors, including metabolic abnormalities, endogenous factors, and exogenous influences. Oxidative stress, a significant endogenous factor, plays a major role in the complications associated with hyperglycemia, leading to an imbalance that triggers oxidative stress. In this context, the antioxidant properties of the methanolic *T. indica* crude extract were investigated, and the results demonstrated that *T. indica* extract exhibits excellent antioxidant activity, as evidenced by various assays. In support of this, the antioxidant, α-glucosidase, and α-amylase inhibitory properties of *Canarium tramdenum* fruits were also studied. Different solvent extracts of *C. tramdenum* revealed the presence of various phytochemicals and exhibited the most effective antioxidant properties. Additionally, the extract showed promising inhibitory effects on α-amylase and α-glucosidase, highlighting the biological potential of *C. tramdenum* (Quan *et al.,* 2019; Segwatibe *et al.,*2023). Similarly, the methanolic extracts of *Ageratum lanatum* (L.) were analyzed for their chemical composition, revealing high concentrations of phenolic acids (PAs), which contributed to their significant antioxidant properties, as demonstrated through various assays. The identified fractions showed promising antidiabetic effects by inhibiting α-amylase activity (Pieczykolan *et al.,* 2021). Additionally, the hepatoprotective ability of *T. indica* was studied, and no toxic effects on cells were observed. Overall, these studies highlight the promising antidiabetic, antioxidant, and hepatoprotective activities of the extracts, suggesting that they could serve as effective agents for managing diabetes mellitus (DM).

5. Conclusion

As the prolonged use of many antidiabetic drugs can lead to severe side effects, researchers are exploring plant-based alternatives. The present study evaluated *T. indica* for its antidiabetic, antioxidant, and hepatoprotective activities. The methanolic crude extract of the plant demonstrated significant antidiabetic effects by inhibiting α-amylase and α-glucosidase activities. Various assays confirmed its antioxidant properties, and the extract showed promising hepatoprotective effects when tested on liver cells. Based on these findings, the study recommends further detailed investigations of *T. indica* crude methanolic flower extract as a potential alternative to chemical drugs in the treatment of diabetes mellitus.

Acknowledgments

The authors are grateful to the Deanship of Scientific Research, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia for its support and encouragement in conducting the research and publishing this report.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **Md. Nadeem Bari, Md. Rizwan Ansari, Imran Mohammad and Mohammad Anwar (2024). Antidiabetic, antioxidant, and hepatoprotective activities of methanolic *Tamarindus indica* L. flower extract. Ann. Phytomed., 13(2):537-543. http://dx.doi.org/10.54085/ap.2024.13.2.54.**