Journal of Chemical Health Risks

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JCHR (2025) 15(4), 954-960 | ISSN:2251-6727

Antioxidant Potential of Sesamol Versus Glyburide in DPPH and ABTS Assays: A Comparative In Vitro Study

[1]Mrs. Shabana Khatoon, [2]Shazia Usmani, [3] Mohammad Khushtar

1Assistant Professor, Pharmacology, Department of Pharmacy

2Professor, Pharmacognosy, Department of Pharmacy

Correspondence Author

3Professor, Pharmacology, Department of Pharmacy

(Received: 16 May 2025 Revised: 20 June 2025 Accepted: 12 July 2025)

KEYWORDS

Sesamol, Glyburide, Antioxidant activity, DPPH assay, ABTS assay, Osteoporosis

ABSTRACT:

The free radical scavenging capacity of compounds is often evaluated using the ABTS assay, which measures their ability to neutralize the ABTS radical cation. Sesamol, a natural phenolic compound present in sesame seeds, has been shown to display strong antioxidant activity in both in vitro and in vivo studies.

One antioxidant that has garnered attention is glyburide, a commonly used medication for the management of type 2 diabetes. Growing evidence indicates that glyburide may also exhibit antioxidant properties, which could offer potential benefits in the prevention or treatment of osteoporosis.

Objectives:"Investigation of protective effect of sesamol and glyburide against osteoporosis in ovariectomised rats".

Material and methods:

DPPH and ABTS method was evaluated for potential for the prevention and treatment of osteoporosis by mitigating oxidative stress.

Method: DPPH and ABTS exhibited prevention and treatment of osteoporosis activity by sesamol and glyburide. Result: Sesamol demonstrated strong antioxidant activity in both assays, showing a significantly greater free radical scavenging ability compared to glyburide.

Conclusion: These findings indicate that sesamol's antioxidant properties may play a role in its therapeutic potential, especially in managing Oxidative stress-related conditions, including diabetes. Further research is required to explore the potential synergistic effects of combining sesamol and glyburide to enhance both antioxidant and antidiabetic efficacy.

Introduction

Oxidative stress refers to an imbalance between the production of reactive oxygen species and the body's antioxidant defence systems, has been implicated in the pathogenesis of osteoporosis. Antioxidants are essential in maintaining the balance between free radicals and the body's natural defense systems, helping to protect against a range of oxidative stress-related diseases (Koleva et al., 2002). One such antioxidant compound is phenolic derivative found in sesame seeds, which has been extensively studied for its potent antioxidant properties (Kerasioti et al., 2019) (Piccinelli et al., 2013) (Luo et al., 2010). Osteoporosis is a common bone disorder marked by decreased bone mineral density and the deterioration of bone microarchitecture, leading to increased fracture risk. This debilitating condition disproportionately affects postmenopausal women due to estrogen deficiency resulting from ovarian hormone depletion. The ovariectomized model has emerged as a valuable tool for investigating pathogenesis and potential treatments for postmenopausal osteoporosis, as it closely mimics the human condition (Kalu et al., 1989)

Studies employing the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assays are widely

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JCHR (2025) 15(4), 954-960 | ISSN:2251-6727

utilized techniques for assessing antioxidant activity, Williams et al;1995.Through these assays, the antioxidant potential of sesamol and glyburide has been demonstrated. Both assays assess the ability of antioxidants to donate electrons or hydrogen atoms to neutralize stable free radicals, thereby reducing their oxidative activity Pellegrini et al;1999 .

Free radicals and reactive oxygen species (ROS) are highly reactive molecules generated during normal cellular metabolism and in response to environmental stressors such as UV radiation. These ROS can interact with and damage essential cellular components, including DNA, proteins, lipids, and carbohydrates, ultimately resulting in cellular and tissue damage Sakanaka et al; 2004.

Excessive production of ROS is linked to inflammation, accelerated aging, and the onset of various diseases, including cancer, diabetes, and atherosclerosis Valko et al 2007) Uttara et al;2009,Kruk et al;2014.

While organisms possess complex antioxidant defence systems to counteract oxidative stress, excessive ROS can overwhelm these defences, resulting in significant damage Sakanaka et al;2004. Assessing antioxidant activity is essential for evaluating naturally occurring or synthetic compounds that may be used as dietary supplements, topical protectants, or therapeutic agents Anilakumar et al;2006.

This study aims to evaluate the antioxidant potential of sesamol and glyburide in an ovariectomized rat model of osteoporosis using DPPH and ABTS assays. The results may offer valuable insights into the possible therapeutic roles of these compounds in the prevention and management of osteoporosis.

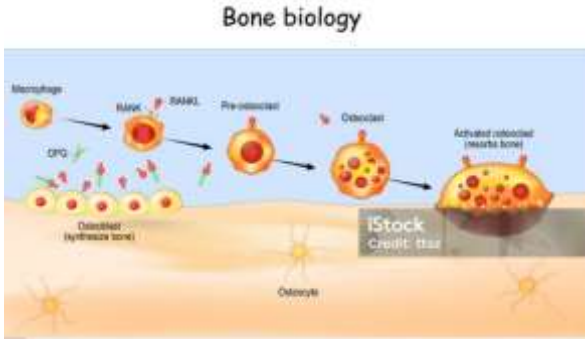

Fig.1 Illustration features of bone biology

Antioxidant Assays DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical Scavenging assay
Procedure :

The radical scavenging activity of sesamol and glyburide was assessed using the DPPH assay, based on the method outlined by Chang et al; (2001). The decrease in absorbance of the DPPH solution, reflecting free radical scavenging activity, was measured at 517 nm following the addition of the antioxidant samples. Ascorbic acid (10 mg/mL prepared in DMSO) served as the reference standard.

Various volumes (2–20 µL) of sesamol were diluted to a total volume of 40 µL with DMSO, followed by the addition of 2.96 mL of DPPH solution (0.1 mM). After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The % radical scavenging activity of thesesamol and glyburide was calculated using the following formula,

$$\%\ DPPH\ Scavenging = \frac{\Delta\ \text{Abs. of control} - \Delta\ \text{Abs. of test drug}}{\Delta\ \text{Abs. of control}} \times 100$$

Furthermore, the IC_{50} value, representing the concentration of sesamol and glyburide required to achieve 50% inhibition of DPPH radicals, was determined by interpolating data through linear regression analysis using Origin Professional for Windows. The percentage (%) of DPPH free radical scavenging was calculated using the following formula:

$$\%\ DPPH\ Scavenging = \frac{\Delta\ \text{Abs. of control} - \Delta\ \text{Abs. of test drug}}{\Delta\ \text{Abs. of control}} \times 100$$

Additionally, the IC_{50} value, indicating the concentration of sesamol and glyburide required to inhibit 50% of DPPH radicals, was determined through interpolation using linear regression analysis.

Principle 1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical in its powdered form, characterized by a deep red colour that changes to yellow upon reduction by an antioxidant. The DPPH assay utilizes this property to assess free radical scavenging activity. The scavenging reaction between DPPH and an antioxidant (HA) can be represented as follows:

(DPPH) + (H-A) = DPPH-H + (A)

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

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Reagent preparation: 0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100 ml of ethanol **Blois et al;1958**.

ABTS free radical scavenging assay

The radical-scavenging activity of sesamol and glyburide was assessed following a previously established protocol. ABTS radicals were generated by mixing equal volumes of 4.9 mM potassium persulfate solution and 14 mM ABTS solution, followed by incubation in the dark for 16 hours. The resulting solution was then diluted with distilled water to achieve an absorbance of 0.90 at 734 nm and used for the antioxidant assay. Various concentrations of sesamol and glyburide (0.0–250 µM) were added to this pre-activated ABTS solution for analysis. Ascorbic acid (0.0–250 µM) was used as a reference standard. The reaction mixture was vortexed for 10 seconds, and the decrease in absorbance was measured at 734 nm using distilled water as a blank, with readings taken on an Eppendorf UV-visible spectrophotometer (Germany). The percentage (%) of DPPH free radical scavenging was calculated using the following formula: **Pellegrini et al; 1999**

$$\%\ \text{ABTS Scavenging} = \frac{\Delta\ \text{Abs. of control} - \Delta\ \text{Abs. of test drug}}{\Delta\ \text{Abs. of control}} \times 100$$

Results

DPPH free radical scavenging activity of sesamol :

The DPPH radical scavenging assay is a widely used method for evaluating antioxidant activity within a short period (Alvi et al;2016).

DPPH is a stable free radical capable of accepting an electron or hydrogen atom, transforming into a stable diamagnetic molecule.In this study, Sesamol exhibited strong antioxidant activity, with an IC_{50} value of 19.98 ± 0.03 µM, which is comparable to the reference standard, ascorbic acid, which had an IC_{50} of 14.07 ± 1.49 µM (**Fig. 2**).

This significant ability to scavenge DPPH free radicals highlights the potent antioxidant potential of sesamol.

Our findings are in accordance with the previously published report which also reported similar antioxidant potential of ascorbic acid (Fig. 2.2.1) (Sreeramulu and Raghunath, 2010; Sharifi-Rad et al; 2020) .

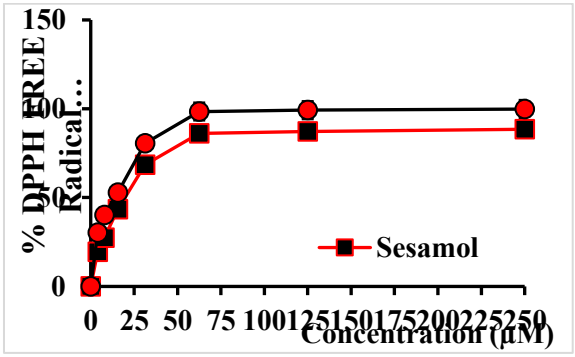

Fig. 2 SESAMOL'S DPPH FREE RADICAL SCAVENGING ACTIVITY

ABTS radical scavenging activity of sesamol

In addition to assessing DPPH free radical scavenging activity, the ABTS radical quenching ability of sesamol was also evaluated. The ABTS assay is another well-established and widely used method for determining the antioxidant potential of various phenolic, and their bioactive secondary metabolites **Arnao et al;2001.**

In this attempt, reported that sesamol exhibited strong ABTS radical scavenging potential with an IC_{50} of 3.49 ± 0.03 µM. The ABTS radical quenching ability of sesamol was far better than that of reported in case of standard ascorbic acid (27.14 ± 0.03 µM) (Fig. 3)

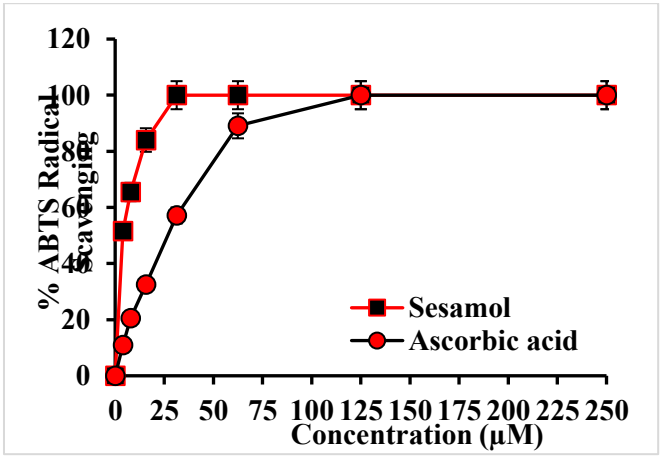

Fig. 3 ABTS radical scavenging activity of sesamol

DPPH free radical scavenging activity of glyburide

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The DPPH radical scavenging assay is a commonly employed method for rapidly evaluating antioxidant activity. DPPH is a stable free radical that becomes a stable diamagnetic molecule upon receiving an electron or hydrogen atom **Molyneux et al;2004**.

In the same context, this research also analyzed the DPPH free radical scavenging activity of glyburide. The result of study indicated IC_{50} value of glyburide 0.55µg/ml (Fig. 4) and the IC_{50} value or reference standard (ascorbic acid) 1.37µg/ml (Fig. 6), that means glyburide has better antioxidant power than ascorbic acid.This substantial scavenging of DPPH was then attempted for co-relation with osteoporotic principles.The Glyburide showed excellent antioxidant potential as compared to ascorbic acid in terms of both DPPH as well as ABTS scavenging assay.

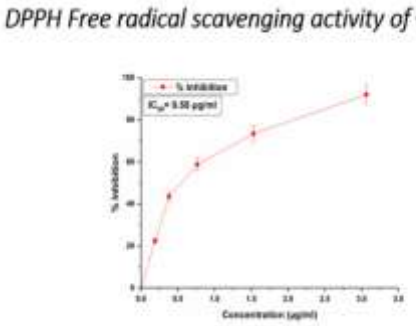

Fig. 4 DPPH free radical scavenging activity of Glyburide

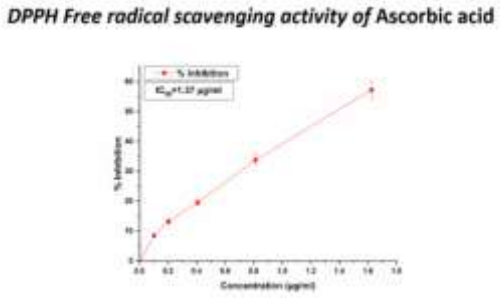

Fig. 5 DPPH free Radical Scavenging Activity of Ascorbic acid

ABTS Free Radical Scavenging Activity of Glyburide

In addition to evaluating DPPH free radical scavenging activity, the ABTS radical quenching capacity of glyburide was also assessed, as the ABTS assay is one of the most widely used methods for determining the antioxidant potential of various plant extracts and their bioactive secondary metabolites **Pellegrini et al;1999**.

In this study, glyburide exhibited potent ABTS radical scavenging activity, with an IC_{50} value of 0.35 µg/ml. The ABTS radical quenching ability of Glyburide was far better than that of reported in case of standard ascorbic acid (1.08µg/ml) (Fig.6).

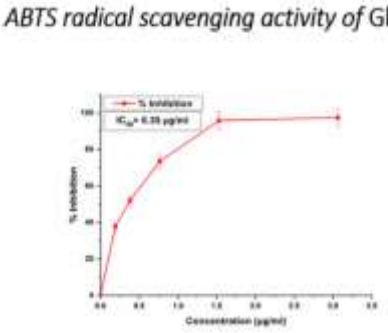

Fig. 6 ABTS Radical Scavenging Activity of Glyburide

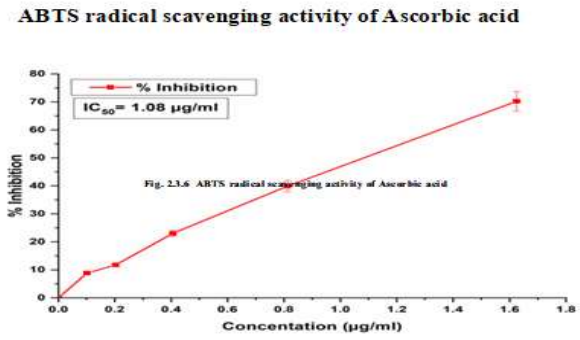

Table 1 IC_{50} of DPPH and ascorbic acid

S.NO.	Chemical name	IC_{50} (µg/ml)
Sesamol		
1.	DPPH of sesamol	19.98
2.	DPPH of Ascorbic acid	14.49
3.	ABTS of sesamol	3.49
4.	ABTS of ascorbic acid	27.14
Glyburide		
1.	DPPH of glyburide	0.55
2.	DPPH of Ascorbic acid	1.37
3.	ABTS of glyburide	0.35
4.	ABTS of ascorbic acid	1.08

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JCHR (2025) 15(4), 954-960 | ISSN:2251-6727

Discussion

The present study offers experimental evidence that sesamol exhibits strong antioxidant properties in addition to its well-documented pharmacological activities. This finding may help explain its potential therapeutic benefits in various diseases where oxidative stress plays a key role in pathogenesis. The scavenging activity was evaluated using the stable free radical DPPH, which displays an EPR signal and a prominent absorption band at 517 nm that diminishes upon reduction by antioxidants **Parihar et al;2008**

Notably sesamol demonstrated comparable antioxidant potential (IC_{50} = 19.98µg/ml) with ascorbic acid (IC_{50} = 14.49µg/ml) in terms of DPPH frree radical scavanging activity.where as interms of ABTS free radical scavenging activity, antioxidant potential of sesamol(IC_{50} = 3.49µg/ml) is far better than ascorbic acid (IC_{50} = 27.14µg/ml) which is a reference standard.The data on the antioxidant activity of sesamol suggests that it acts as an antioxidant. Sesamol may have a beneficial role as an antioxidant.Our result reported that sesamol exhibited strong ABTS radical scavenging potential with an IC_{50} of 3.49 ± 0.03 µM. The ABTS radical quenching ability of sesamol was far better than that of reported in case of standard ascorbic acid (27.14 ± 0.03 µM).

The Glyburide showed far better antioxidant activity as compared to ascorbic acid in terms of both DPPH as well as ABTS scavenging assay.IC_{50} value of glyburide in terms of DPPH was 0.55µg/ml and of ascorbic acid was 1.37µg/ml. ABTS scavanging activity of glyburide was 0.35 µg/ml and of ascorbic acid was 1.08 µg/ml.Finally it is concluded that sesamol shows comparable antioxidant with ascorbic acid on scale of DPPH free radical scavanging activity and better antioxidant potential than ascorbic acid on scale of ABTS free radical scavanging activity.Whereas glyburide shows better antioxidant potential than ascorbic acid on scale of both DPPH as well as ABTS free radical scavanging activity.

Conclusion

This study investigated the antioxidant potential of sesamol and glyburide. Our findings demonstrated that both sesamol and glyburide exhibited significant antioxidant activity, as evidenced by their ability to scavenge DPPH and ABTS radicals. These results suggest that sesamol and glyburide may have therapeutic potential for the prevention and treatment of osteoporosis by mitigating oxidative stress. Further research is warranted to elucidate the underlying mechanisms of action. The strong free radical scavenging activity demonstrated by these compounds suggests their potential therapeutic value. Since oxidative stress and free radicals are known to contribute to the development of osteoporosis, the antioxidant properties of these drugs may help justify their use in the prevention and treatment of osteoporosis. However, further studies are needed to confirm their efficacy in preclinical as well as clinical settings.

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