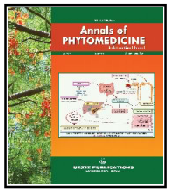
Annals of Phytomedicine 13(1): 793-798, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.1.83

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

# Marrubin natural immunomodulator: Studies on the suppressive effect on phagocytic nitric oxide and pharmacokinetic prediction

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**Article Info**

**Article history**
Received 11 February 2024
Revised 29 March 2024
Accepted 30 March 2024
Published Online 30 June 2024

**Keywords**
Respiratory burst
Inflammation
Cytotoxic
ADMET

**Abstract**

Oxidative stress is an imbalance between free radicals and antioxidants in your body. This can cause damage to organs and tissues and result in various inflammatory diseases. This study aimed to evaluate the therapeutic potential of the naturally occurring compound Marrubin (1R,4S,8S,9R,10R,12R)-9-[2-(furan-3-yl) ethyl]-9-hydroxy-4,8,10-trimethyl-2-oxatricyclo [6.3.1.04,12] dodecan-3-one, which have been extensively studied for its biological properties.

Immunomodulation studies were conducted using various techniques, including cell-based assay, spectrophotometry, chemiluminescence, and predictive analyses using web-based tools. Results indicated that MBN slightly suppressed superoxide anion production by 22.85%, MBN exhibited no inhibition of the myeloperoxidase-dependent pathway-producing hypochlorite anion, with an $IC_{50}$ value of >100 µg/ml. Additionally, MBN demonstrated significant inhibition of nitric oxide (NO) production by 40.7% at 25 µg/ml and $IC_{50}$ value of 35 µg/ml. Furthermore, MBN exhibited no cellular toxicity, with an $IC_{50}$ value of >25 µg/ml, tested on 3T3 fibroblast cell lines. Physicochemical and pharmacokinetic prediction studies indicated high blood-brain barrier (BBB) permeability and gastrointestinal (GI) absorption. Additionally, no toxicity was predicted against normal cell lines also. MBN was predicted to possess anticancer effects on various cancer cell lines. In conclusion, this study highlights the moderate biological potential of Marrubin at a tested concentration of 25 µg/ml. However, MBN's cytotoxicity results suggest that if, the concentration is increased above 25 µg/ml keeping in view the toxicity profile, desirable results can be achieved reflecting the immunomodulating potential of the molecule.

## 1. Introduction

Oxidative stress and inflammation are interconnected processes implicated in various diseases. Oxidative stress arises from an imbalance between free radicals and antioxidants in the body (Segu Prathyusha, 2023). These free radicals, including reactive oxygen species (ROS), can damage cellular components, leading to tissue injury and inflammation (Valko *et al.,* 2007).

Inflammation is the body's response to harmful stimuli, such as pathogens or tissue damage, characterized by redness, swelling, heat, and pain (Afroz Alam and Adnan Ahmad Khan, 2022). Chronic inflammation and hyper immune activation can result from persistent oxidative stress and are associated with numerous health conditions, including acute bacterial or viral infections specifically SARS-CoV-2 infection, cardiovascular diseases, diabetes, and neurodegenerative disorders (Khushi Parnami and Sarla Lakhawat, 2022; Biswas, 2016). Free radicals play a significant role in inflammation by contributing to oxidative stress, which damages cells and tissues (Ghezzi, 2000; Pizzino *et al.,* 2017). Inflammation, characterized by the body's immune response to injury or infection, leads to the production of free radicals (Li and Kong, 2009). Excessive free radicals can cause oxidative damage to biomolecules like lipids, proteins, and DNA, exacerbating inflammation (Butterfield *et al.,* 2002).

Suppression of free radicals is crucial because oxidative stress-induced inflammation is implicated in various diseases, including cardiovascular diseases, cancer, diabetes, and neurodegenerative disorders (Segu Prathyusha, 2023). Antioxidants counteract the harmful effects of free radicals by neutralizing them or promoting their decomposition (Kim and Park, 2016). They are essential in maintaining cellular homeostasis and preventing oxidative damage (Forni *et al.,* 2019). Suppressing free radicals through antioxidants can help mitigate inflammation-associated tissue damage and reduce the risk of chronic diseases (Greanious *et al.,* 2021). Hence, understanding the role of free radicals in inflammation and the necessity of their suppression is crucial for developing therapeutic strategies to combat inflammation-related pathologies. Natural compounds, derived from plants or other natural sources, help combat oxidative damage by scavenging free radicals and reducing oxidative stress levels (Chaturvedi *et al.,* 2012). Substances like resveratrol and pterostilbene have been identified as potent

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antioxidants, capable of modulating oxidative damage and mitigating the effects of oxidative stress (Awaneet Kaur *et al.,*2023). Additionally, natural compounds induce cytoprotective effects by ameliorating oxidative stress through their inherent antioxidant activities (Subramoniam, 2014).

Marrubin, a compound found in *Marrubium vulgare* (white horehound), exhibits various biological activities as reported in research papers. Pharmacological studies have highlighted its antinociceptive properties, demonstrating effectiveness against pain as evidenced by the writhing test (Adesina, *et al.,* 2017). Additionally, *M. vulgare* extracts containing marrubin have been associated with antispasmodic effects (Sultana *et al.,* 2016). This compound contributes to the plant's therapeutic effects, including its use as an expectorant and antispasmodic agent in treating respiratory conditions like bronchitis and whooping cough (Adesina *et al.,* 2017; Alamgeer *et al.,* 2018). The biological activities of marrubin contribute to the medicinal properties attributed to *M. vulgare* in traditional medicine (Amri *et al.,* 2017; Alamgeer *et al.,* 2018).

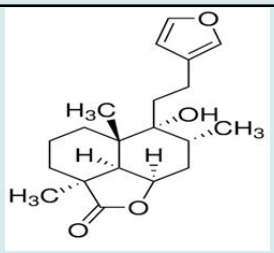
In this study, we evaluated if, the marrubin holds promise for therapeutic interventions targeting oxidative stress-related conditions. Additionally, *in silico* physicochemical and ADMET (absorption, distribution, metabolism, excretion, and toxicity) studies were conducted to predict the biological potential of these compounds to identify their selectively in cancer cells, leading to cell death, thus showing potential in cancer therapy.

## 2. Materials and Methods

### 2.1 Collection of compounds and sample preparation (1mg/ml stock solution)

Marrubin was selected based on available literature (Amri *et al.,* 2017; Popoola *et al.,* 2013) for their potential biological activity and purchased from Bench Chem and Sigma Aldrich (Table 1). We used 1 mg/ml of the stock solution by the addition of one milligram of the compound in 50 µl DMSO, solubilised, and if needed sonication is done till the compound is solubilised completely. 950 µl phosphate buffer saline was added to get the final concentration of 1mg/ml and stored in a freezer at 4°C until use.

**Table 1: Selected marrubin compounds reported for their biological activity**

| S. No. | Structure | IUPAC name | Compound code |
|---|---|---|---|
| 1 |  | (1R,4S,8S,9R,10R,12R)-9-[2-(furan-3-yl) ethyl]-9-hydroxy-4,8,10-trimethyl-2-oxatricyclo[6.3.1.04,12] dodecan-3-one | MBN |

### 2.2 Determination for oxidative burst by chemiluminescence assay

A chemiluminescence assay was performed as published before (Choudhary *et al.,* 2013). Different concentrations of test compounds 1 µg/ml, 10 µg/ml and 100 µg/ml were set in 96 well white flat bottom plates, incubated at 37ºC for 15 min in the thermostat chamber of luminometer with 1:20 dilution of whole blood in HBSS$^{++}$. Control wells established HBSS $^{++}$ and cells without the test compound. Following incubation luminol ($7\times10^{-5}$ M), and serum opsonized zymosan (SOZ) were added into each well except blank wells that contain HBSS$^{++}$ only to detect free radicals. Intracellular reactive oxygen detecting probe luminometer was used to detect oxidative burst reactive oxygen species (ROS) production. The level of the ROS was measured as relatively light units for 50 min using repeat scan mode.

### 2.3 NBT assay for superoxide production

Macrophages J774 ($1 \times 10^7$) cells/ml were suspended in a medium, and 100 µl of macrophages were placed in 96-well plates in the RPMI media with FBS. Nitro blue tetrazolium assay was done as prescribed earlier (Soomro *et al.,* 2023). Cells and test compound were incubated for 30 min at 37ºC, after incubation 100 ul of 1mg/ml NBT solution was added and 50 ul of phorbol-12- myristate-13-acetate (PMA). Diphenyleneiodonium was used as a negative control after addition at a concentration of 10 µg/ml to activate the cells and after an additional 90 min incubation. Blue formazan was dissolved in DMSO and the absorbance was measured at 570 nm. Controls were set as cells in a medium rather than without a test compound. Percent inhibition of NBT reduction was calculated.

### 2.4 Measurement of NO in cell culture supernatant

J774 macrophage cells and U 138MG neuronal cells were collected and adjusted to a concentration of 10^6 cells/ml, and 200 µl of cells were added to a 96-well microtiter plate. Lipopolysaccharide from *E. coli* was added at a final concentration of 30 µg/ml to activate the macrophage cells, inducing NO production. Test compound was added initially at a concentration of 25 µg/ml in activated J774 cells and neuronal cells for detection of % inhibition of nitric oxide release. Identification of $IC_{50}$ value J774 cells were incubated with three different concentrations 25, 50 and 100 µg/ml, then incubated at 37°C, 5% $CO_2$ for 24 h. After incubation, cell culture supernatants were collected and immediately processed for nitrite accumulation studies using the Griess method. The Griess method used 50 µl of 1% sulfanilamide in 2.5% phosphoric acid added to 50 µl of the cell culture supernatant, followed by 50 µl of 0.1% naphthyl ethylenediamine dihydrochloride in 2.5% phosphoric acid. Absorbance was read at 550 nm after incubation for 10 min at 23°C. The micromolar concentration of sodium nitrite was calculated from a standard curve generated using sodium nitrite as the reference compound. The findings were described as means ± standard deviation (SD) of triplicate readings. This method provides a simple and reliable way to evaluate the ability of test compounds to modulate NO production in activated J774 macrophage cells, which can be useful in screening for potential anti-inflammatory or immunomodulatory agents (Soomro *et al.,* 2020).

### 2.5 *In vitro* cytotoxicity assay

The MTT assay is a commonly used method to measure cell viability and proliferation. In this assay, 3T3 fibroblast cells were plated in a 96-well plate and treated with different concentrations of test compound for 48 h. After that, the cells were washed and MTT was added to the wells. MTT is reduced by mitochondrial dehydrogenases in viable cells to form a coloured formazan product. The dissolution of formazan was done in DMSO, and then the absorbance was measured at 570 nm using a microplate reader. The $IC_{50}$ values, which represent the concentration of the compounds required to hinder cell viability by fifty per cent, were calculated. Cyclohexamide, a known inhibitor of protein synthesis, was used as a positive control to compare the effect of the test compounds on cell viability. The percentage of cell viability was calculated using the formula:

$$\% \text{ Cell viability} = \frac{\text{Total viable cells(unstained)}}{\text{Total cells(viable + dead)}} \times 100$$

The viable cell count was calculated as the average viable cell count per square x dilution factor x 10 (Sreenivasagan *et al.,* 2020).

### 2.6 Physicochemical and pharmacokinetic predictions of thiourea compounds

Swiss ADME (http://www.swissadme.ch/index.php) and PreADMET (https://preadmet.bmdrc.kr/) predicted the physicochemical and pharmacokinetic profiles of marrubin. The cellline cytotoxicity was predicted by CLC-Pred (http://way2drug.com/Cell-line/) and toxicity by protox II.

### 2.7 Statistics

To determine the statistical significance of the results, a paired *t*-test was used to compare the mean values of the experimental test conditions with their respective controls. A *p*-value of 0.05 was regarded to signify a considerable difference between the two groups, denoted as * $p<0.05$. A *p*-value of less than 0.001 (1%) was regarded to signify a highly considerable difference, denoted as ** $p<0.001$.

## 3. Results

### 3.1 Effect of compounds on superoxide production $O_2$

Phagocytic NADPH oxidase is activated by phorbol myristate acetate (PMA) in mouse macrophages and initiates the protein kinase C (PKC) pathway. The PKC pathway led to the generation of superoxide anions (Soomro *et al*., 2019). PMA-activated cells were treated and exposed to 25 µg/ml of compound and the effect on superoxide was detected by NBT reduction assay. MBN was found to suppress the superoxide production slightly with a per cent inhibition of 22 % (Table 2).

### 3.2 Effect of compounds on HOCl production

During the myeloperoxidase-dependent intracellular killing process of phagocytosis HOCl, hypochlorous acid is produced that can be detected with Luminol enhanced chemiluminescence technique (Soomro *et al*., 2019). The study demonstrated that comp does not possess inhibitory activity against ROS production in whole blood cells activated with zymosan. The $IC_{50}$ value for MBN was found to be >100 µg/ml, $IC_{50}$ value of the standard anti-inflammatory drug ibuprofen (11.8 ± 1.2 µg/ml) was used for comparison (Table 2).

**Table 2: Effect of compounds on ROS, NO, and $O_2^-$ production in activated macrophages and neutrophils and cytotoxicity analysis**

| Compounds | ROS (HOCl) $IC_{50}$ ± SD µg/ml | Superoxide $O_2^-$ %inhibition ± SD | Nitric oxide (NO) %inhibition ± SD | Nitric oxide (NO) $IC_{50}$ | Cytotoxicity $IC_{50}$ ± SD µg/ml |
|---|---|---|---|---|---|
| **MBN** | >100 | 22.85 ± 0.6 | 40.7 ± 1.7 | 35.3 ± 2* | >25 |
| **Standard drugs** | Ibuprofen 11.8 ± 1.2 ug/ml | Diphenyl iodonium 92 ± 0.94 % | monomethyl L $N^G$ arginine 64 ± 0.05% | $N^G$ monomethyl L arginine 24 ± 0.2% | Cyclohexamide 0.13 ± 0.02 µg/ml |

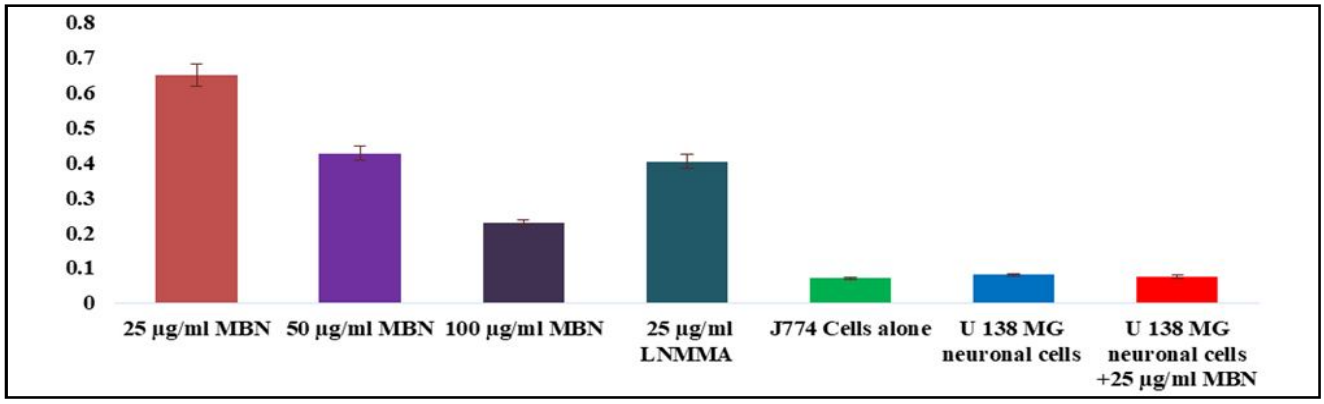



**Figure 1: Effect of Marrubin (MBN) on nitric oxide (NO) release as optical density (nm) by LPS-activated J774 mouse macrophage cells ($2 \times 10^5$) incubated in the presence (25, 50, 100 µg/ml) or absence of the compound (J774 cells alone), in presence of inhibitor of nitric oxide LNMMA and U 138MG neuronal cells incubated with and without MBN for 48 h. The supernatant was analyzed for the presence of nitrite using the Griess method. Values are expressed as mean ± SD for triplicate experiments.**

In this experiment, three different concentrations were used. The minimum concentration used was 1 µg/ml and the maximum was 100 µg/ml, did not depict the real bilogical potential of the compound as reported earlier. However, we can assume that increasing the compunds concentration may inhibt the HOCl production.

### 3.3 Effect of compounds on nitric oxide (NO)

Lipopolysaccharide (LPS) activates toll-like receptor 4 (TLR4) on macrophages, leading to the production of inducible nitric oxide synthase (iNOS) and subsequent production of nitric oxide (NO) (Forte *et al.,* 2016). In the study, we investigated the effect of the test compound on NO production in macrophages activated with LPS. The results showed that test compound had moderate inhibition of NO at 25 µg/ml with 40% inhibition. To further investigate the inhibitory concentration, we used three concentrations increasing from 25 to 100 µg/ml and the results were intriguing that increasing concentration suppressed NO in a dose-dependent manner showing potential therapeutic effect with $IC_{50}$ 35.3 ± 2 and possessing no impact on the constitutional form of nitric oxide release by neuronal cells (Figure 1).

### 3.4 Effect of compound on cytotoxicity

The viability of 3T3 Fibroblast cells was studied in the presence of compounds at several concentrations of 50, 5 and 0.5 µg/ml. Results were compared with cyclohexamide which is used as a standard cytotoxic drug (Rochette *et al.,* 2013). The tested MBN showed no toxicity up to 25 µg/ml suggesting that increasing concentration to get possible biological effect is applicable (Table 3 and Figure 2). 3T3 NIH mouse fibroblast cell lines were incubated in the absence and presence of varying concentrations of MBN for 48 h. The cytotoxic effects of MBN were determined using the MTT assay, as described in the text. The effect of the cytotoxic drug cyclohexamide was also determined for comparison.

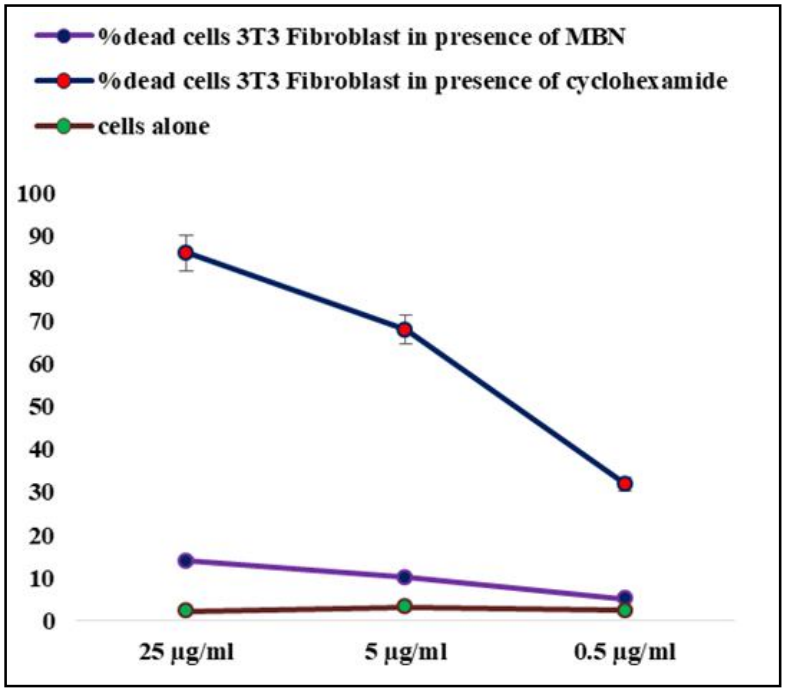



**Figure 2: Effect of MBN on cellular toxicity.**

### 3.5 Physicochemical and pharmacokinetic predictions of MBN compounds

Marrubin, underwent comprehensive *in-silico* analysis, including ADMET, physicochemical properties, and evaluation of their anticancer effects, utilizing web-based tools (Soomro *et al.,* 2019). The predictive studies revealed that compound possess significant potential as therapeutic agents, with favourable gastrointestinal absorption and permeability across the blood-brain barrier. The predicted profile suggested that the compound was non-carcinogenic, nephrotoxic, and non-mutagenic. Its predicted lethal dose ($LD_{50}$) was moderate (244 mg/kg), indicating that this compound was not lethal. Since the compound had a molecular weight, of 332.43 g/mol it violated no rule of Lipinski's rule of five, which applies to oral drugs. Gastrointestinal absorption measures were high and bioavailability measures were significant with a score of 0.55. The compound showed cross blood-brain barrier permeability. The skin permeation of the compound was also -5.68. The compound does not act as a P-glycoprotein substrate and it does not act as an inhibitor of CYP1A2, CYP2C19, or CYP2C9. However, it is a CYP2D6 inhibitor and not a CYP3A4 inhibitor. The compound depicted no pan assay interference compounds (PAIN) alerts or brenkcriteria, suggesting it was safe. The cytotoxicity of different cancer cell line predictions showed that it could exhibit activity toward Colon adenocarcinoma (DLD-1), Adult B acute lymphoblastic leukaemia (NALM-6), and non-small cell lung cancer. 3 stage (NCL-H838), and Metastatic melanoma (SK-MEL-1), while it shows activity on nontumor cell lines toward embryonic lung fibroblast (WI-38 VA13).

**Table 3: Prediction studies of marrubin**

| | |
|---|---|
| **Physicochemical properties** | **Formula:** $C_{20}H_{28}O_4$, Molecular weight: 332.43 g/mol, Num. heavy atoms: 24, Num. arom. heavy atoms: 5, Fraction Csp3: 0.75, Num. rotatable bonds: 3, Num. H-bond acceptors:4, Num. H-bond donors:1, Molar Refractivity:91.40, TPSA: 59.67 A2. |
| | **Lipophilicity**<br>Log $P_{o/w}$ (iLOGP): 2.99, Log $P_{olw}$ (XLOGP3): 3.73, Log $P_{o/w}$ (WLOGP): 3.72, Log $P_{olw}$ (MLOGP):2.76, Log $P_{olw}$ (SILICOS-IT):3.96, Consensus Log $P_{olw}$: 3.43. |
| | **Water solubility**<br>Log S (ESOL): -4.21, Solubility: 2.06e-02 mg/ml; 6.21e-05 mol/l, Class: Moderately soluble, Log S (Ali): -4.67, Solubility 7.03e-03 mg/ml; 2.11e-05 mol/l, Class: Moderately soluble, Log S (SILICOS-IT): -4.93, Solubility: 3.90e-03 mg/ml; 1.17e-05 mol, Class: Moderately soluble. |
| **Pharmacokinetics properties** | **GI absorption:** High; BBB permeation: Yes, P-gp substrate: No, CYP1A2 inhibitor: No, CYP2C19 inhibitor: No, CYP2C9 inhibitor: No, CYP2D6 inhibitor: Yes, CYP3A4 inhibitor: No, Log Kp (skin permeation): -5.68 cm/s. |
| **Drug likeness** | **Lipinski:** Yes; 0 violation, Ghose: Yes, Veber: Yes, Egan: Yes, Muegge: Yes, Bioavailability Score: 0.55. |
| **Medicinal chemistry** | **PAINS:** 0 alert, Brenk: 0 alert, Lead likeness: No; 1 violation: XLOGP3>3.5, Synthetic accessibility: 4.86. |
| **Toxicity** | Non-mutagenic, carcinogenicity = None Predicted. $LD_{50}$: 244 mg/kg, Hepatotoxicity = No, Nephrotoxicity = yes, Respiratory toxicity = yes. |
| **Anticancer** | Colon adenocarcinoma (DLD-1), Adult B acute lymphoblastic leukaemia (NALM-6), non-small cell lung cancer. 3 stage (NCL-H838), and Metastatic melanoma (SK-MEL-1), Nontumor cell lines embryonic lung fibroblast (WI-38 VA13). |

## 4. Discussion

In our investigation, the effect of marrubin on a series of free radicals in activated phagocyte cells was observed. The activation of NADPH oxidase by phorbol myristate acetate (PMA), leads to superoxide anion generation through the protein kinase C (PKC) pathway (Soomro *et al.,* 2019). Marrubin possesses minimal inhibitory effect towards superoxide production. In contrast, the modulation of ROS production particularly HOCl detected by chemiluminescence technique in response to zymosan activation (Lu *et al.,* 2006) showed no significant activity. Despite the lack of inhibitory activity against ROS production, there is a need for further investigation because the minimum concentration was used in these experiments and could be increased to identify therapeutic effects keeping in view the toxicity profile.

Furthermore, our study identified the physiological effects of compounds to specifically modulate inflammatory responses on nitric oxide (NO) production in macrophages activated by lipopolysaccharide (LPS) (Soomro *et al.,* 2019). The dose-dependent increase in the inhibitory effect of marrubin implies a correlation between concentration and effectiveness. This suggests that higher concentrations of the compound result in stronger inhibition, indicating a potential for therapeutic application. By effectively modulating NO levels, the compound may offer therapeutic benefits in managing cardiovascular conditions and other inflammatory disorders. These findings emphasise the compound's potential as a targeted intervention for NO-related pathologies (Moncada and Higgs, 1991).

Furthermore, our evaluation of cytotoxicity in fibroblast cells provided valuable insights into the safety profile of MBN compounds. This assessment is crucial in determining the potential toxicity of compounds intended for therapeutic use, especially considering fibroblast's role in tissue repair and maintenance. The results indicating a lack of cytotoxic effects on fibroblast cells suggest that MBN compound may exhibit a favourable safety profile, a critical aspect for their consideration in therapeutic applications (Rochette *et al.,* 2013).

*In silico* analysis of marrubiin revealed its pharmacokinetic and toxicological properties, indicating its potential as a therapeutic agent. The compound demonstrated high gastrointestinal absorption and significant bioavailability, suggesting favourable pharmacokinetics. Furthermore, marrubiin exhibited cross-blood-brain barrier permeability, enhancing its potential for central nervous system-related applications. Toxicity predictions suggested marrubin to be noncarcinogenic, nonmutagenic, and with moderate nephrotoxicity, with a predicted $LD_{50}$ of 244 mg/kg, indicating low acute toxicity. Additionally, marrubiin showed inhibitory effects on CYP2D6 but not on CYP3A4, highlighting its potential interactions with certain drug-metabolizing enzymes. Overall, these findings suggest that marrubin possesses promising pharmaco-kinetic and safety profiles for further therapeutic development (Soomro and Sangi, 2019).

## 5. Conclusion

The findings regarding natural compounds MBN and its potential as therapeutic agents for inflammation are ameliorating. The observed significant suppression of the nitric oxide release by activated phagocytes by marrubin, along with prediction studies indicating its potential to reduce inflammation by inhibiting nitric oxide free radical generation, holds promise.

## Acknowledgements

I am extending my sincere thanks to the Deanship of Scientific Research, Northern Border University, Arar, Saudi Arabia, for providing the research fund with project number NBU-FFR-2024-2615-01 and also to the Department of Pharmaceutics, Faculty of Pharmacy, Northern Border University Rafha 91431, Saudi Arabia.

## Conflict of interest

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

## References

**Adesina, S. K.; Johnny, I. I. and Olayiwola, G. (2017).** Plants in respiratory disorders I-anti-asthmatics: A review. Br. J. Pharm. Res., **16**(2):1-22.

**Afroz Alam and Adnan Ahmad Khan. (2022).** Antiviral potential of frequently used plants in herbal formulations as immunity enhancers against SARS-CoV-2. Ann. Phytomed., **11**(3):S12-S17.

**Alamgeer; Younis, W.; Asif, H.; Sharif, A.; Riaz, H.; Bukhari, I. A. and Assiri, A. M. (2018).** Traditional medicinal plants used for respiratory disorders in Pakistan: A review of the ethnomedicinal and pharmacological evidence. Chin. Med., **13**:1-29.

**Amri, B.; Martino, E.; Vitulo, F.; Corana, F.; Ben-Kaâb, L. B.; Rui, M. and Collina, S. (2017).** *Marrubium vulgare* L. leave extract: Phytochemical composition, antioxidant, and wound healing properties. Molecules, **22**(11):1851.

**Awaneet Kaur; Md. AftabAlam; Tarique Mahmood and Farogh Ahsan (2023).** Herbal medicinal plants for the treatment of memory impairment: A futuristic approach for neurological studies, Ann. Phytomed., **12**(2):29-40.

**Biswas, S. (2016).** Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? Oxid. Med. Cell. Longev., pp:401-413.

**Butterfield, D. A.; Castegna, A.; Lauderback, C. M.; and Drake, J. (2002).** Evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death. Neurobiol. Aging, **23**(5):655-664.

**Chaturvedi, S.; Hemamalini, R.; and Khare, S. K. (2012).** Effect of processing conditions on saponin content and antioxidant activity of Indian varieties of soybean (*Glycine max* Linn.). Ann. Phytomed., **1**(1):62-68.

**Choudhary, M. I.; Khan, N.; Ahmad, M.; Yousuf, S.; Fun, H. K.; Soomro, S. and Shaheen, F. (2013).** New inhibitors of ROS generation and T-cell proliferation from Myrtuscommunis. Org. Lett., **15**(8):1862-1865.

**Forni, C.; Facchiano, F.; Bartoli, M.; Pieretti, S.; Facchiano, A.; D'Arcangelo, D. and Jadeja, R. N. (2019).** Beneficial role of phytochemicals on oxidative stress and age-related diseases. Biomed Res. Int., pp:11-22.

**Forte, M.; Conti, V.; Damato, A.; Ambrosio, M.; Puca, A. A.; Sciarretta, S. and Carrizzo, A. (2016).** Targeting nitric oxide with natural derived compounds as a therapeutic strategy in vascular diseases. Oxid. Med. Cell. Longev., pp:612.

**Ghezzi, P. (2000).** Regulation of protein functions by glutathionylation. Free Radic. Res., **34**(6):649-657.

**Greanious, A. M. N. M.; Ncube, N.; Sibanda, A.; Dube, D.; Farai, F. C. and Makoni, P. (2021).** Phytotherapeutics attenuation of oxidative stress, inflammation and lipid peroxidation in severe and chronic diseases. Accenting Lipid Peroxidation, pp:77.

**Kim, J. and Park, H. Y. (2016).** Association of serum ferritin with metabolic syndrome and diabetes mellitus in the South Korean general population according to the Korean National Health and Nutrition Examination Survey 2008. Metabolism, **65**(2):306-314.

**Li, W. and Kong, A.N. (2009).** Molecular mechanisms of Nrf2-mediated antioxidant response. Molecular Carcinogenesis: Published in cooperation with the University of Texas MD Anderson Cancer Center., **48**(2):91-104.

**Lu, C.; Song, G. and Lin, J. M. (2006).** Reactive oxygen species and their chemiluminescence-detection methods. Trends Anal. Chem., **25**(10):985-995.

**Moncada, S. and Higgs, E. A. (1991).** Endogenous nitric oxide: Physiology, pathology and clinical relevance. Eur. J. Clin. Invest., **21**(4):361-374.

**Pizzino, G.; Irrera, N.; Cucinotta, M.; Pallio, G.; Mannino, F.; Arcoraci, V. and Squadrito, F. (2017).** Oxidative stress: harms and benefits for human health. Oxid. Med. Cell. Longev., pp:13-22.

**Popoola, O. K.; Elbagory, A. M.; Ameer, F. and Hussein, A. A. (2013).** Marrubiin. Molecules., **18**(8):9049-9060.

**Khushi Parnami and Sarla Lakhawat (2022).** Practices of using home remedies as an immunity booster during COVID-19. Ann. Phytomed., **11**(3):S64-S71.

**Rochette, L.; Lorin, J.; Zeller, M.; Guilland, J. C.; Lorgis, L.; Cottin, Y. and Vergely, C. (2013).** Nitric oxide synthase inhibition and oxidative stress in cardiovascular diseases: Possible therapeutic targets. Pharmacol. Ther., **140**(3):239-257.

**Segu Prathyusha (2023).** Formulation, phytochemical and antioxidant activity evaluation of selected Indian medicinal plants, Ann. Phytomed., (1) AU Pharmacon (TRIPS-2022):S66-S71.

**Soomro, S. and Sangi, S. (2019).** Oxidative stress: Implications in chronic Inflammation-cancer and aging. J. North Basic Appl. Sci. **4**(1).

**Soomro, S.; Aljameeli, M. A. and Sangi, S. (2020).** *In-vitro* immunomodulatory and cytotoxicity potential of natural compounds from *Fragari anubicola*. Int. J. Pharm. Sci. Res., **11**(11):5679-5684.

**Soomro, S.; Chidrawar, V. R.; Imran, M. and Alshammari, H. O. (2019).** Immunomodulating, antibacterial and anticancer potential of Za'atar (*Thymus vulgaris*) and its combination with essential oil (Olive and Balsam Oil). Int. J. Pharm. Sci., **10**(8):3721-3726.

**Soomro, S.; Mesaik, M. A.; Shaheen, F.; Khan, N.; Halim, S. A.; Ul-Haq, Z. and Choudhary, M. I. (2019).** Inhibitory effects of *Myrtu commua* cetalone 1 (MCA-1) from *Myrtus communis* on inflammatory response in mouse macrophages. Molecules, **25**(1):13.

**Soomro, S.; Sangi, S. and Nayeem, N. (2023).** Antioxidative burst, cytotoxicity, and ADME studies of thiourea compounds. Pharmacophore, **14**(2).

**Sreenivasagan, S.; Subramanian, A. K. and Rajeshkumar, S. (2020).** Assessment of antimicrobial activity and cytotoxic effect of green mediated silver nanoparticles and its coating onto mini-implants. Ann. Phytomed., **9**(1):207-212.

**Subramoniam, A. (2014).** Phytomedicines for healthcare. Ann. Phytomed., **3**:1-3.

**Sultana, S.; Khan, A.; Safhi, M. M. and Alhazmi, H. A. (2016).** Cough suppressant herbal drugs: A review. Int. J. Pharm. Sci. Invent., **5**(5):15-28.

**Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M. T.; Mazur, M. and Telser, J. (2007).** Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol., **39**(1):44-84.

**Citation** **Samreen Soomro, Aleya Khalaf Alshammari, Ryoof Saleh Almutrafi, Qamar A. Alshammari, Amani A. Ali., Mashael Alanazi and Naira Nayeem (2024). Marrubin natural immunomodulator: Studies on the suppressive effect on phagocytic nitric oxide and pharmacokinetic prediction. Ann. Phytomed., 13(1):793-798. http://dx.doi.org/10.54085/ap.2024.13.1.83.**