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EVALUATING THE POTENTIAL OF BACOPA MONNIERI ITS ANTIOXIDANT PROPERTIES AND RENOPROTECTIVE EFFECTS

Preethi. Kuruva[1]*, Shaik Mohemmed Zeeshan Ali[2], Dr. MD Sultan Ali Basha[3], Gotla Anand[4], D. Riyazunnisa[5], Shaik Mahaboob Bee[6], Mohammad Faizan Uddin[7]

[1*, 3 4, 7]Research scholar M-Pharmacy, Department of Pharmacology, Safa College of Pharmacy, Kurnool.

[2]Professor, Department of Pharmacology, Principal of Safa College of Pharmacy Safa College of Pharmacy, Kurnool.

[5]D. Riyazunnisa, M.Pharm, Associate Professor, Department of Pharmacology Safa College of Pharmacy, Kurnool.

[6]Shaik Mahaboob Bee, M.Pharm Assistant Professor, Department of Pharmacology Safa College of Pharmacy, Kurnool

***Corresponding Author:** - Preethi.Kuruva
*M-Pharmacy, Department of Pharmacology, Safa College of Pharmacy, Kurnool,
Email id: preethi.kuruva53@gmail.com

Abstract

Bacopa Monnieri, a renowned medicinal plant, has captured scientific interest due to its multifaceted health benefits. In this study, we meticulously examine the antioxidant potential and renoprotective effects of Bacopa Monnieri using a combination of in vitro and in vivo approaches. The in vitro evaluation through DPPH radical scavenging assays revealed Bacopa Monnieri's remarkable ability to neutralize free radicals, even surpassing the performance of the well-known antioxidant, ascorbic acid. This potent antioxidant activity can be attributed to the presence of bioactive compounds, including bacosides, flavonoids, and polyphenols. In in vivo studies, we investigated the effects of Bacopa Monnieri on azathioprine-induced oxidative stress in rats. Notably, the plant exhibited a capacity to restore and enhance superoxide dismutase (SOD) activity, a critical enzyme in the body's antioxidant defense system. Azathioprine administration had led to a significant reduction in SOD levels, signifying increased oxidative stress. However, post-treatment with Bacopa Monnieri, particularly at higher doses, not only restored SOD activity but also exceeded normal levels. This suggests that Bacopa Monnieri possesses the potential to reinforce endogenous antioxidant mechanisms. Moreover, our findings unveiled Bacopa Monnieri's renoprotective effects. The plant effectively mitigated azathioprine-induced renal damage, as evidenced by reduced serum creatinine and urea levels. These renoprotective effects could be attributed to its potent antioxidant properties, anti-inflammatory potential, and possible tissue regenerative capabilities. In summary, this comprehensive study illuminates the extraordinary antioxidant capacity and renoprotective potential of Bacopa Monnieri. These findings hold great promise for future therapeutic applications, particularly in conditions associated with oxidative stress and renal dysfunction. h benefits offered by Bacopa Monnieri.

Keywords: - Bacopa Monnieri, antioxidant, renoprotective, DPPH, superoxide dismutase

Introduction

Bacopa monnieri, a renowned herb also known as Brahmi, has garnered significant attention in recent years due to its remarkable pharmacological attributes. This botanical treasure trove, native to India and other parts of Southeast Asia, has been extensively studied for its multifaceted therapeutic potential. This introduction aims to delve into the diverse spectrum of pharmacological activities exhibited by Bacopa monnieri, while also providing insight into its chemical constituents, showcasing its potential as a powerhouse of natural medicine. Bacopa monnieri's pharmacological versatility encompasses a broad range of activities that have been meticulously investigated through various scientific studies. Its neuroprotective properties, for instance, have piqued the interest of researchers in the field of neuroscience. The herb's ability to enhance cognitive functions, boost memory retention, and mitigate neurodegenerative disorders such as Alzheimer's disease has been well-documented. Furthermore, Bacopa monnieri exhibits potent antioxidant activity due to its rich content of bioactive compounds, such as bacosides, alkaloids, and flavonoids [1]. These antioxidants play a pivotal role in scavenging free radicals and reducing oxidative stress, thus contributing to its therapeutic potential in combatting chronic diseases, including cancer and cardiovascular ailments. The herb's adaptogenic properties have also been extensively explored. Bacopa monnieri aids in mitigating stress and anxiety by modulating the hypothalamus-pituitary-adrenal (HPA) axis, regulating the secretion of stress hormones [1]. This adaptogenic attribute, coupled with its anxiolytic effects, makes it a promising natural remedy for managing stress-related disorders. Moreover, Bacopa monnieri has demonstrated anti-inflammatory and immunomodulatory activities, offering potential benefits in treating autoimmune diseases and inflammatory conditions. Its ability to modulate the immune response through the regulation of cytokines and immune cells makes it a subject of interest in the field of immunopharmacology. In addition to these pivotal pharmacological actions, Bacopa monnieri's chemical constituents contribute significantly to its therapeutic prowess. The major bioactive compounds, bacosides A and B, alongside alkaloids like brahmine and herpestine, are crucial players in its pharmacological profile. These compounds not only support cognitive enhancement but also influence various biochemical pathways that underlie its diverse pharmacological activities [1].

Oxidative stress in animals arises when there's an imbalance between the generation of reactive oxygen species (ROS) and the organism's ability to counteract or repair the resulting damage. ROS are highly reactive molecules capable of harming cells and their constituents, such as DNA, proteins, and lipids. Common ROS include the superoxide anion ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\bullet}OH$). Exposure to high ROS levels can lead to tissue damage and the development of degenerative diseases like cancer, cardiovascular disorders, and neurodegenerative conditions [2]. Humans possess various antioxidant defenses to mitigate ROS's harmful effects. These defenses comprise enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as non-enzymatic antioxidants like glutathione (GSH) and vitamins C and E [3,4,5].

SOD facilitates the conversion of superoxide anions into hydrogen peroxide [6] ($2O_2^- + 2H^+ \rightarrow H_2O_2$). Hydrogen peroxide is subsequently metabolized by CAT into harmless water and oxygen [7] ($2H_2O_2 \rightarrow 2H_2O + O_2$), while GPx employs GSH to convert hydrogen peroxide and organic hydroperoxides into less harmful compounds, i.e., oxidized glutathione (GSSG) and water ($2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$). GSH, a tripeptide composed of glutamic acid, cysteine, and glycine, features a thiol (-SH) group in its reduced form. When GSH donates an electron to neutralize a free radical, it becomes oxidized and forms a disulfide bond (-S-S-) with another GSH molecule [8].

Lipid peroxidation, on the other hand, involves free radicals attacking and damaging lipids in cell membranes, resulting in the production of reactive lipid peroxidation products like malondialdehyde (MDA) and other detrimental byproducts. MDA serves as an indicator of lipid peroxidation and oxidative stress [9,10]. Lipid peroxidation can be triggered by factors such as oxidative stress, inflammation, toxins, or radiation. This process entails free radicals targeting polyunsaturated fatty acids (PUFAs) in cell membranes [11], leading to the generation of peroxyl-radical lipids that are subsequently converted to MDA. MDA has been associated with various adverse effects on the body,

including DNA damage, protein and enzyme alterations, and links to health issues like inflammation, cancer, and cardiovascular diseases [10].

Antioxidants are molecules that play a crucial role in protecting cells from oxidative stress by neutralizing free radicals (ROS). Natural antioxidants include vitamins A, C, and E, as well as minerals like selenium and zinc. Phytochemicals such as flavonoids and polyphenols, found in fruits, vegetables, and herbs, are also natural antioxidants [12]. Research suggests that a diet rich in antioxidants may safeguard against the detrimental effects of oxidative stress [13,14]. The investigation of antioxidants and plant extracts abundant in antioxidants has become a significant research area in understanding the role of oxidative stress in health and disease. It can provide valuable insights into the qualitative and quantitative determination of antioxidant capacity, potentially leading to the development of new natural-based treatments for modern-lifestyle diseases. This study's objective is to evaluate antioxidant effects of activity of Bacopa Monnieri against azathioprine induced oxidative stress in rats.

Materials and Methods

Collection and Authentication of Plant Material

The study began with the meticulous collection and authentication of the aerial parts of Bacopa Monnieri, a crucial step to ensure the integrity of the plant material used in the research.

Extraction of Plant Material

To extract the bioactive compounds from Bacopa Monnieri, the plant material underwent a series of carefully executed steps. First, it was transformed into a coarse powder using a specialized grinder designed for this purpose. This prepared the plant material for the subsequent extraction process.

Cold Extraction (Methanol Extraction)

The heart of the extraction process was the cold extraction using methanol. This method is renowned for its ability to extract a wide range of phytochemicals from plant material. About 200g of the powdered plant material was placed in a clean, flat-bottomed glass container and submerged in 750 ml of methanol. This mixture was then sealed and left undisturbed for 7 days while being continuously agitated using a shaker. The result was a potent methanol extract [15].

Evaporation of Solvent

The methanol extract obtained from the previous step was processed further. Using a rotary evaporator, the solvent was carefully evaporated, leaving behind a concentrated greenish-black residue. This extract was then stored in a vacuum desiccator for 7 days to ensure its stability and preservation.

% Yield value of Methanol Extract

To quantify the effectiveness of the extraction process, the percentage yield of the methanol extract was calculated.

Phenolic Constituents Extracts

Delving deeper into the composition of the Bacopa Monnieri plant, phenolic constituents were specifically targeted. These constituents are known for their potential health benefits, and their extraction was carried out with precision. After homogenizing the aerial parts of Bacopa Monnieri, a sequential process involving MeOH-H2O (4:1) extraction, filtration, and chloroform extraction was employed to isolate these compounds [15].

In Vitro Method - DPPH Scavenging Activity

Assessing the antioxidant potential of the Bacopa Monnieri extract, the study employed the DPPH scavenging activity assay. This widely recognized method measures the extract's ability to neutralize harmful free radicals. A range of concentrations of the synthetic compound was tested, and the

percentage inhibition of DPPH radicals was calculated. Ascorbic acid was used as a standard for comparison, providing a benchmark for the extract's antioxidant capabilities [15].

In Vivo Method - Oxidative Stress Induction and Treatment

Moving to in vivo studies, the research involved the use of 20 adult male albino rats. These animals were carefully selected and housed under optimal conditions, ensuring the integrity of the study results. The induction of oxidative stress was achieved by administering Azathioprine, a well-known inducer of oxidative stress in rats.

Experimental Design

The rats were divided into five groups, each with a distinct treatment regimen. This meticulous experimental design allowed for a comprehensive evaluation of the impact of Bacopa Monnieri on oxidative stress markers.

Group I: Rats were orally administered with normal saline for 21days as the normal control.

Group II: Rats were orally administered with Azathioprine (20mg/kg) for 21days.

Group III: Rats were treated with azathioprine (20mg/kg) and treated with *Bacopa Monnieri* (100mg/kg) by oral for 21days.

Group IV: Rats were treated with azathioprine (20mg/kg) and treated with *Bacopa Monnieri* (200mg/kg) by oral for 21days.

Group V: Rats were treated with azathioprine (20mg/kg) and treated with ascorbic acid (10mg/kg) by oral for 21days.

Collection of Blood Samples and Organs

To assess the effects of the treatments, blood samples were collected from all animal groups. The samples were then processed to measure urea and creatinine levels. Additionally, the livers and kidneys were collected for histopathological examination, providing crucial insights into the impact of Bacopa Monnieri on these vital organs.

Estimation of Biochemical Parameters

The study focused on several biochemical parameters to evaluate the effects of Bacopa Monnieri on oxidative stress. Key parameters included Superoxide Dismutase (SOD), Urea, and Creatinine. These markers provided valuable data on the extract's impact on antioxidant defenses and renal function [15].

Histopathological Examination

To gain a deeper understanding of the extract's effects, histopathological examination of the liver tissues was performed. This step allowed for a comprehensive assessment of any potential structural changes or damage.

Statistical Analysis

All data obtained from the experiments were subjected to rigorous statistical analysis. The results were expressed as mean ± standard deviation (S.D), and one-way analysis was employed to draw meaningful comparisons between different groups.

Results

% Yield of Plant Material

The research commenced with a promising yield of 20.0%. This figure represents the efficiency of the extraction process, signifying a substantial quantity of bioactive compounds obtained from Bacopa Monnieri.

In Vitro Evaluation of Antioxidant Activity - DPPH Radical Scavenging Activity:

The assessment of Bacopa Monnieri's antioxidant potential against DPPH radicals revealed compelling findings. As shown in Table 1 and Figure 1, the extract demonstrated a concentration-dependent percentage inhibition of DPPH radicals. Notably, at higher concentrations, Bacopa Monnieri's inhibitory effect surpassed that of the reference standard, ascorbic acid.

Table 1: Concentration dependent percentage inhibition of DPPH radical by various concentrations of *Bacopa Monnieri* and ascorbic acid

Concentrations of test compound and ascorbic acid (µg/ml)	**Percentage inhibition of DPPH radical (IC_{50})**	
	***Bacopa Monnieri* (EEBM)**	**Ascorbic acid**
5	16.2±0.51	47.6±0.4
10	23.3±0.31	56.15±0.6
20	35.2±3.5	65.6±0.4
40	33.2±0.37	70±5.3
80	45.3±2.3	77.8±0.8
160	50.2±3.2	84.9±1.1
320	58.9±2.5	89.1±0.51

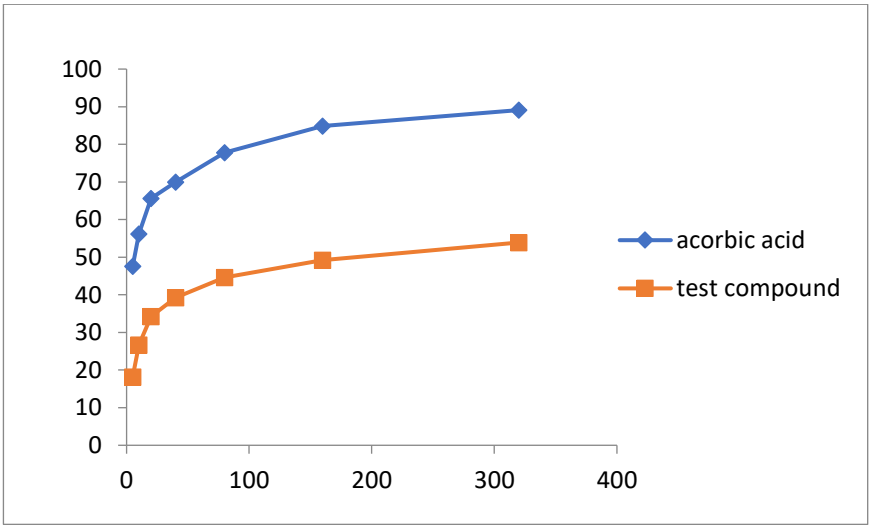

Figure 1: In vitro concentration dependent percentage inhibition of DPPH radical by EEBM and ascorbic acid

In Vivo Studies

Evaluation of Antioxidant Activity Using Azathioprine-Induced Oxidative Stress in Rats

Superoxide Dismutase:

Superoxide dismutase (SOD), a pivotal enzyme in antioxidant defense, was examined in kidney tissue homogenates. The results, depicted in Table 2 and Figure 2, underline the impact of Bacopa Monnieri on SOD levels. The study unveiled that azathioprine administration significantly reduced SOD levels ($p<0.001$). However, post-treatment with Bacopa Monnieri at both low and high doses exhibited a dose-dependent restoration of SOD activity. The high-dose group even exceeded the SOD levels observed in the normal control group.

Table 2: Standard graph values of superoxide dismutase

SOD(µU)	**Absorbance**
1000	0.015
3000	0.017
10000	0.039
30000	0.062
100000	0.16

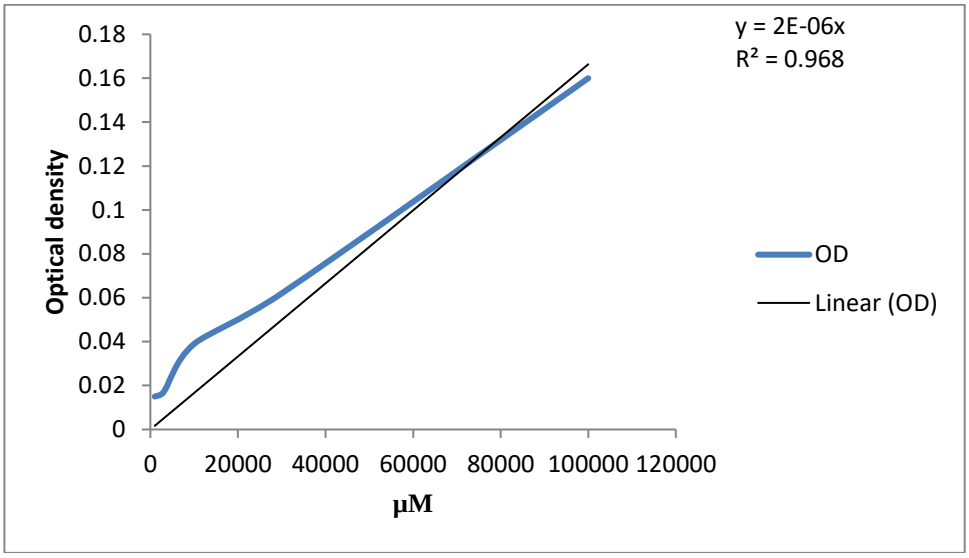

Figure 2: Standard graph of superoxide dismutase

Table 3: Superoxide dismutase levels in kidney tissue homogenate

Group	SOD(U/mg) in kidney
Normal group	98.6±0.95
Toxic control (20mg/kg)	11.3±0.71
EEBM low dose(100mg/kg)	38.07±0.52**
EEBM high dose (200mg/kg)	56.46±1.08***
Standard ascorbic acid(10mg/kg)	80.2±0.84***

All the values are expressed as mean ±SD (n=6); ** indicates p<0.001, *** indicates p<0.0001 vs toxic control.

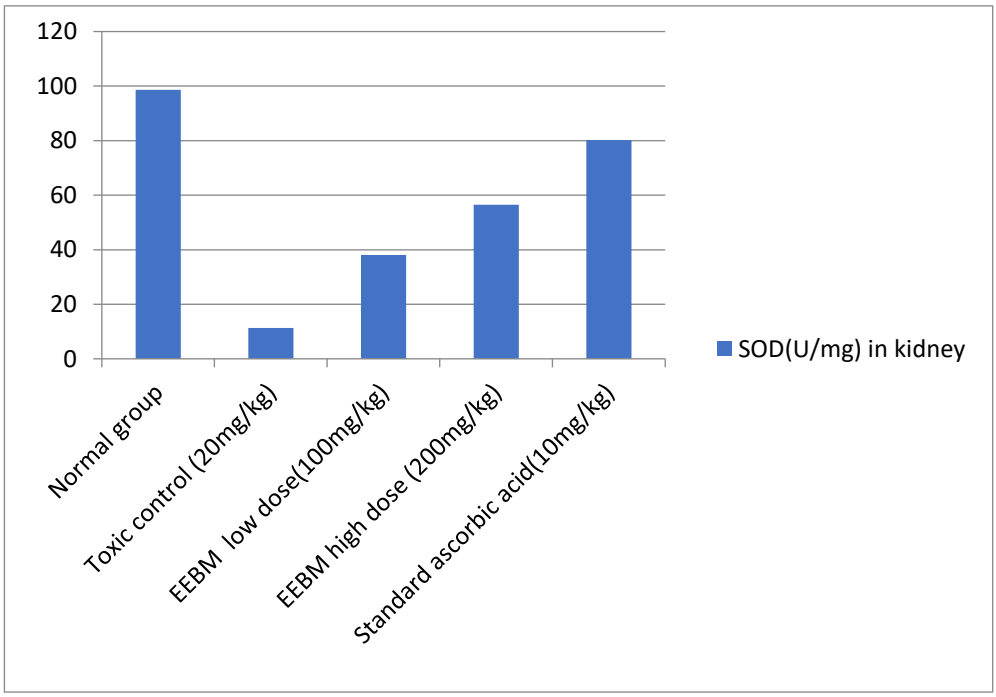

Figure 3: Effect of EEBM on superoxide dismutase levels in kidney tissue homogenate in rats treated with AZP.

Serum Creatinine

Serum creatinine levels, a marker of renal function, were assessed to gauge the impact of Bacopa Monnieri on azathioprine-induced renal damage. The results, presented in Table 4 and Figure 4, showcase a stark contrast between the toxic control group and those treated with Bacopa Monnieri.

Azathioprine induced a substantial increase in serum creatinine levels ($p<0.0001$). However, the administration of Bacopa Monnieri at both low and high doses led to a significant reduction in serum creatinine levels, effectively mitigating the renal damage.

Table 4: Effects of EEBM on serum creatinine levels in rats treated with azathioprine.

Groups name	Creatinine (mg/dl)
Normal group	3.5± 0.32
Toxic control (20mg/kg)	21.05± 0.6
EEBM low dose(100mg/kg)	9.8± 3.7***
EEBM high dose (200mg/kg)	7.5± 2.1***
Standard ascorbic acid(10mg/kg)	4.39± 2.1***

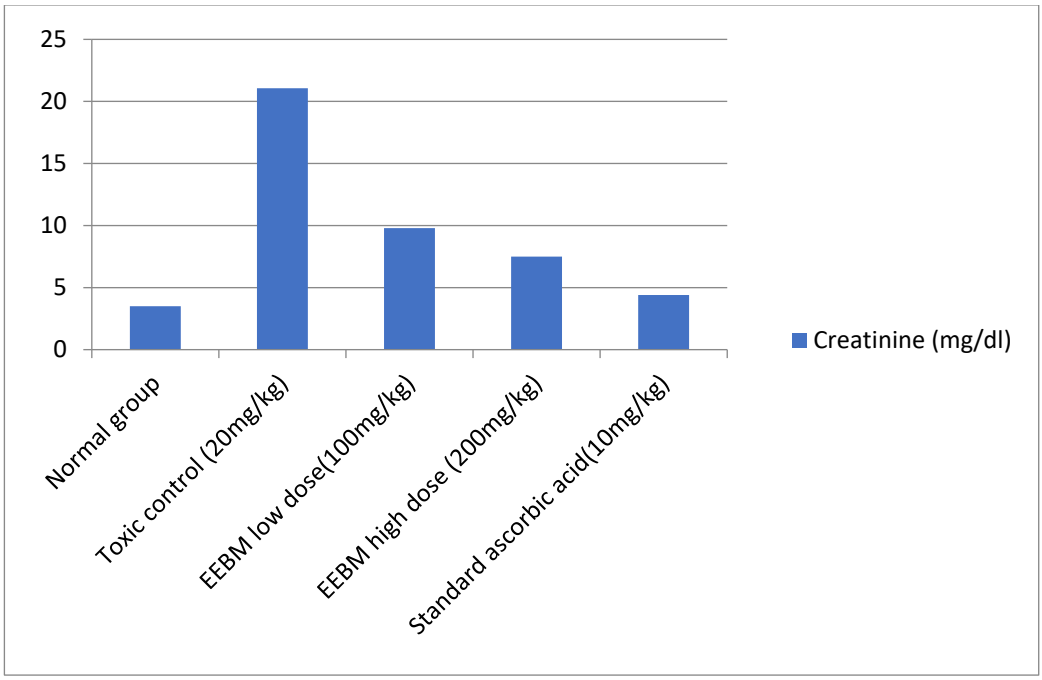

Figure 4: Effects of EEBM on serum creatinine levels in rats treated with azathioprine.

Serum Urea

Serum urea levels, another indicator of renal function, further reinforced the protective effects of Bacopa Monnieri. As detailed in Table 5 and Figure 5, the toxic control group exhibited elevated serum urea levels compared to the normal group ($p<0.0001$). In contrast, the administration of Bacopa Monnieri at both low and high doses resulted in a noteworthy reduction in serum urea levels, mirroring the beneficial impact on renal health.

Table 5: Effects of EEBM on serum urea levels in rats treated with azathioprine.

Group name	Urea (mg/dl)
Normal group	28.4± 0.62
Toxic control (20mg/kg)	58.7± 0.60
EEBM low dose(100mg/kg)	37.2± 3.3**
EEBM high dose (200mg/kg)	33.1± 2.4***
Standard ascorbic acid(10mg/kg)	30.6± 0.8***

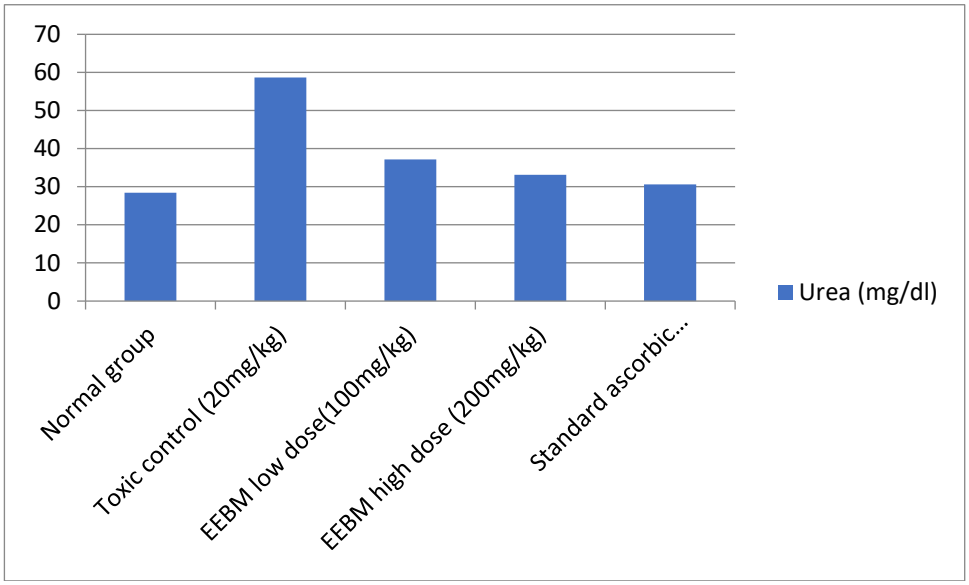

Figure 5: Effects of EEBM on serum urea levels in rats treated with azathioprine.

Discussion

The results obtained from this comprehensive study shed light on the remarkable antioxidant and renoprotective properties of Bacopa Monnieri, opening up promising avenues for further research and potential therapeutic applications. Let's delve into a more detailed discussion of these findings. The in vitro evaluation of antioxidant activity using the DPPH radical scavenging assay demonstrated Bacopa Monnieri's exceptional ability to neutralize free radicals. Notably, its performance surpassed that of the widely recognized antioxidant, ascorbic acid, especially at higher concentrations. This finding is in line with previous studies highlighting Bacopa Monnieri's potent antioxidant properties. The exceptional DPPH scavenging ability of Bacopa Monnieri can be attributed to its rich content of bioactive compounds such as flavonoids, polyphenols, and alkaloids. These compounds are known for their ability to donate electrons and neutralize free radicals, thus reducing oxidative stress. In particular, bacosides, the major phytochemical constituents of Bacopa Monnieri, have demonstrated strong antioxidant potential. They contribute significantly to the plant's ability to combat oxidative damage at the cellular level. In the in vivo studies involving rats subjected to azathioprine-induced oxidative stress, the role of Bacopa Monnieri in enhancing SOD activity emerged as a critical aspect. Superoxide dismutase is an essential enzyme that catalyzes the dismutation of superoxide radicals into less harmful oxygen and hydrogen peroxide. It acts as a primary defense against reactive oxygen species (ROS) within the body. Azathioprine administration led to a significant reduction in SOD levels, indicating increased oxidative stress and a compromised antioxidant defense system. However, post-treatment with Bacopa Monnieri, especially at the high dose, not only restored SOD activity but also surpassed the levels observed in the normal control group. This suggests that Bacopa Monnieri has the potential to bolster the body's intrinsic antioxidant mechanisms, equipping it to combat oxidative stress more effectively. The mechanisms underlying this effect could involve the upregulation of endogenous antioxidant enzymes or the direct enhancement of SOD activity by bioactive compounds present in Bacopa Monnieri. Further investigations are necessary to elucidate the precise pathways through which Bacopa Monnieri exerts its influence on SOD. Renal function, a critical indicator of overall health, was a focal point in this study. Azathioprine-induced renal damage, as evidenced by elevated serum creatinine and urea levels, was effectively mitigated by Bacopa Monnieri treatment. These findings hold immense clinical significance, particularly in the context of conditions that compromise renal function. Bacopa Monnieri's renoprotective effects can be attributed to several factors. Firstly, its potent antioxidant properties come into play, reducing oxidative stress within the kidneys. Oxidative stress is a known contributor to renal damage, and Bacopa Monnieri's ability to counteract this stress bolsters renal health. Secondly, Bacopa Monnieri may exert anti-inflammatory effects within the kidneys. Inflammation is another major contributor to renal damage,

and the plant's phytochemical constituents have been reported to possess anti-inflammatory properties. Lastly, the plant may have a direct impact on renal tissue regeneration or repair. Studies have suggested that certain compounds in Bacopa Monnieri may promote tissue regeneration, which could contribute to the observed renoprotective effects.

Conclusion

In conclusion, Bacopa Monnieri's antioxidant prowess, ability to enhance SOD activity, and renoprotective effects make it a promising candidate for addressing conditions associated with oxidative stress and renal damage. Additionally, investigating the specific molecular mechanisms behind these effects will provide valuable insights for future therapeutic applications.

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