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Pharmacological evaluation of rice varieties, Hassawi and Njavara

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Abstract

Hasswai and Njavara are two notable international and Indian red rice cultivars that provide a variety of nutrients and phytochemicals. An effort was made to investigate the pharmacological properties of these rice types. In conclusion, both types of rice exhibit lipid peroxidation, acetylcholinesterase, thrombolytic, anti-arthritic, antioxidant, and anti-inflammatory properties. Methanol extracts showed increased acetylcholinesterase, thrombolytic, and antioxidant activity in the current investigation. On the other hand, aqueous extracts have stronger anti-inflammatory, anti-arthritic, lipid peroxidation, protease inhibition, and amylose inhibitory properties. Of these two varieties, Hasawi showed good activities concerning amylose inhibition, thrombolytic activity, antioxidant activity, and anti-inflammatory and lipid peroxidation. However good protease inhibition and acetylcholinesterase activities were noted in Njavara than in Hasawi. This points to a specific phytochemical composition that these two rice varieties contain. More detailed investigations are warranted to score the exact phytochemical basis for the observed activities.

Keywords: Hasswai; Njavara; Pharmacology; Aqueous extracts; Methanol extracts

1. Introduction

Hippocrates advocated "food as a medicine, medicine as a food" for health and nourishment over 2500 years ago, which faded into obscurity by the nineteenth century. Later, in the early twentieth century, the discovery of vital vitamins and elements provides a solution, particularly in the context of nutrient deficiency in various disorders Mark (2004). The "Indian Materia Medica" reveals the presence of numerous medicinal rice types grown in ancient times Das and Oudhia (2003). Rice has become a staple diet for over 75% of the world's population on most continents, including Asia. There are 4,000 rice types in India Boominathan and Lakshmi (2016), and 40,000 have been cultivated worldwide Ricepedia (2018).

White rice accounts for half of the world's calorie intake, although it is nutritionally inferior to pigmented rice variants Mbanjo (2020). In pigmented rice cultivars, bran proteins are more abundant in albumin (66%), globulin (7%), prolamin, and glutelin (27%) than endosperm proteins. Traditional rice varieties contain a diverse spectrum of phytoconstituents engaged in various pharmacological activities, including anthocyanins, alkaloids, xanthones, and flavonols such as quercetin. Polyphenol intake through diet has been linked to a lower risk of some age-related neurological illnesses such as macular degeneration and dementia Bastianetto (2002). Both Hasswai and Njavara contain nearly 25 bioactive components that exhibit anti-rheumatic, anti-inflammatory, hypocholesterolaemia, antiarthritic, and antiandrogenic properties Boominathan and Lakshmi (2016). It also has a glucose-lowering effect followed by increased vitamin supplementation (A, B, C, D, and especially E) Reshmi (2018). Reductase inhibitors found in pigmented rice types are utilized to treat prostatic hyperplasia and relieve urinary tract symptoms (Sulochana and Singaravadivel (2015).

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Anthocyanins in black rice have a strong anti-cancer activity Chen (2006). Hasswai possesses low CHO (carbohydrate) content, high protein, fiber, and ash, as well as less RAG (rapidly accessible glucose) and SAG (slowly available glucose) with a low glycemic index, implying that it may prevent type II diabetes Al-Mssallem (2011); McMillan (2006); Bahrany *(*2002). Surprisingly, native rice types contain high fiber content (22% - 32%) Shylaraj (2017). Pigmented rice-extracted bran oil contains these fibers, which serve to improve oxidative stability during storage while also lowering saturated fatty acids (SFA), unsaturated fatty acids (USF), and cholesterol levels Kim (1997). The current study sought to investigate a few more pharmacological efficiencies of Hasswai and Njavara rice varieties and perform a comparative analysis of their pharmacological abilities.

2. Materials and methods

2.1. Sample collection

Njavara and Hassawi rice germplasm was obtained during the year 2022 from U. Kottahapalli local farmer near Kakinada, Andhra Pradesh and a local farmer of Saudi Arabia respectively and preserved at room temperature.

2.2. Extractions of plant material

The dehulled rice germplasm samples were processed into fine powder using a food processor. The powders were subjected to cold extraction with water and methanol for 72 hours while shaking continuously. These extracts were further filtered, and crude extracts were produced using a rota evaporator. Extracts were kept at 4° C to avoid pathogen contamination until further study.

2.3. Alpha-amylase inhibition activity (α-AIA)

The anti-amylase assay was carried out in accordance with Bernfeld (1955), with minor changes. To initiate the reaction, 1 mL of rice extracts were added to an equal amount of sodium phosphate buffer (0.02M, pH 6.9) containing 0.5 mg/mL pig pancreatic α-amylase solution (Sigma, USA) and incubated at 25 °C for 10 minutes. The reaction was halted by adding 2 mL of 3,5-Di-nitro-salicylic (DNS) acid color reagent and heating it in a water bath at 100 °C for 5 minutes. It was then diluted with 10 ml of double distilled water and detected at 540 nm with a Perkin Elmer lambda 40 UV spectrophotometer. The absorbencies were estimated using the equation below.

$$\% \textbf{ Inhibition } = \frac{\textbf{Abs Control } (\textbf{540}) - \textbf{ Abs Extract } (\textbf{540})}{\textbf{Abs Control } (\textbf{540})} \times \textbf{ 100}$$

2.4. Thrombolytic activity

Human blood samples (n=10) were obtained from volunteers who had not used oral contraception or blood thinners. 1.5 ml of blood was collected, and 500 ul of blood was subjected to clot lysis at 37°C for an hour until a clot formed. Loaded serum was emptied from the tubes without disturbing the clot, and the empty Eppendorf tubes were weighed to determine the exact clot weight using the formula below. Each sample contains 100 ul of MESAL, normal saline as a negative control, and lyophilized streptokinase (30,000 IU and 15,000 IU) as a positive control. After 90 minutes of correct incubation at 37° C, gently eliminate the residual chemicals from the clots without disturbing them, and weigh the clots once again. The proportion of clot lysis was estimated by subtracting the pre- and post-clot weights.

$$\textbf{W = C-T}$$

Whereas,
W= Weight of clot,
C= Clot weight with tube and
T= Tube weight.

2.5. DPPH radical scavenging assay

The oxygen-bleaching rate of rice methanolic and aqueous extracts is studied with standard samples using the stable free radical DPPH. Briefly, a 0.004% (0.1 mM) DPPH solution was prepared in methanol, and 900 ul of this solution was combined with 100 ul of extract solution of varied strengths (100 ug - 500 ug/mL) of dried extracts. These solutions were completely vortexed and incubated for 30 minutes at room temperature before measuring absorbance at 517 nm against a blank. Quercetin and ascorbic acid were employed as positive controls. The antioxidant activity of the extract

was quantified as IC_{50}, with extracts inhibiting the generation of DDPH radicals by 50%. The free radical scavenging activity was determined using the following formula.

$$\text{DPPH radical scavenging activity (\%)} = [(A_0\text{-}A_s)/A_s] \times 100$$

Whereas,
A_o= Absorbance of the control and
A_1= Absorbance of the extract or standard.

2.6. Determination of anti-inflammatory activity

Total total protein denaturation in traditional rice varieties was measured, followed by an egg albumin denaturation method with minor changes. The whole reaction mixture (5 mL) contained 0.5 mL of fresh hen's egg albumin, 2.5 mL of phosphate-buffered saline (PBS, pH 6.4), and 2 mL of various methanolic and aqueous extraction concentrations (25, 50, 75, 100, 125, 150,175 and 200 ug/mL). Milli-Q water was utilized as a control. These mixes were incubated at 37 °C in a BOD incubator for 15-20 minutes before being heated to 70 °C for 5 minutes. After the samples had reached room temperature, their absorbance was measured at 660 nm in the UV spectrophotometer against a blank. The same diclofenac concentrations were utilized as reference samples.

$$\text{\% inhibition} = 100 \times [V\,t\,/\,V\,C - 1]$$

Where,
Vt = absorbance of the test sample,
Vc = absorbance of control.

2.7. Determination of lipid peroxidation

Total lipid peroxidation products in both rice cultivars were calculated using the approach of Heath and Packer (1998). Freshly produced methanolic and aqueous rice extracts were vigorously shaken before incubating for 15 minutes at 37 °C. Later, a reaction mixture of 0.25% Thiobarbituric acid (TBA) in 10% trichloroacetic acid (TCA) and phosphate-buffered saline (PBS) at a 1:4 (w/v) ratio was added, followed by 0.5 mL of 24 mM ferrous sulfate and 0.5 mL of PBS and heated at 95 °C. After 30 minutes, the mixture was centrifuged at 10,000×g for 20 minutes at 25 °C. The UV spectrophotometer measured the absorbance at 532 nm. The proportion of total lipid peroxidation was estimated using the formula.

$$\text{\% Inhibition} = \frac{\text{Abs (sample)} - \text{Abs (control)}}{\text{Abs Control}} \times 100$$

The experiment results were expressed as the sample concentration that provided 50% inhibition (IC50), indicating that lipid peroxidation was 50% inhibited.

2.8. Determination of protease inhibition

The protease inhibition experiment was carried out according to Bijina (2011). 1 mL of trypsin (in 0.1 M Phosphate buffer pH 7) and 1 mL of protease inhibitor were pre-incubated at 37°C for 30 minutes. The reaction was then stopped with 2.5 mL of 0.44 M trichloroacetic acid. To remove precipitated proteins from the samples, centrifuge them at 10,000 rpm for 15 minutes. In a UV spectrophotometer, the absorbance was measured at 280 nm against a blank control. Casein production by trypsin reaction in the presence or absence of the inhibitor, quantified using tyrosin as a standard. The inhibitory activity of proteases is given as a percentage inhibition. The total inhibitory activity of proteases is estimated using the following formula.

$$\text{Inhibitory activity (\%)} = \frac{B - A}{B} \times 100$$

Where,
A = Amount of tyrosine with inhibitor
B = Amount of tyrosine without inhibitor

2.9. Acetylcholinesterase activity

Eldee (2005) method was used to determine the acetylcholinesterase inhibition activity. In this experiment, we followed 96 well plates consisting of 25 uL of 15 mMacetylthiocholine iodide in water, 125 uL of 3 mM5,5′-dithiobis[2-nitrobenzoic acid in Buffer C (50 mM Tris-HCl, pH 8, containing 0.1 M NaCl and 0.02 M MgCl2.6H2O), 50 uL of buffer B (50 mM, pH 8, containing 0.1% bovine serum albumin), and 25 µL of methanolic and aqueous extracts at various concentrations. Later, absorbance was measured spectrophotometrically at 405 nm every 15 minutes. Acetylcholinesterase (0.2 U/ml) was then added to the reaction mixture, and the absorbance was measured five times in a row, every 45 minutes. Galanthamine is used as a positive control.

$$\text{Inhibition } \% = 1 - (A_{sample} / A_{control}) \times 100$$

2.10. Anti-arthritic Activity

The reaction mixture contained 1 mL of the test extracts at varied concentrations and 1 mL of a 5% aqueous solution of bovine serum albumin (BSA). The pH was adjusted by adding a tiny volume of glacial acetic acid. The sample extracts were first incubated at 37 °C for 20 minutes before being heated to 70 °C for 10 minutes. The mixture was allowed to cool for 10 minutes before measuring turbidity at 660 nm. The sample was combined with distilled water to create the blank. Distilled water served as the negative control. Diclofenac sodium served as a positive control. The percentage inhibition was estimated using the following formula.

$$\% \text{ Inhibition } = \frac{\text{Abs (sample)} - \text{Abs (control)}}{\text{Abs Control}} \times 100$$

The concentration of the extract for 50% inhibition (IC_{50}) was determined by the dose-responsive curve.

3. Results

3.1. Alpha-amylase inhibition activity (α-AIA)

In this experiment, we found that Hasawi methanol extract (HME) and Njavara methanol extract (NME) had better α-amylase inhibitory activity than aqueous extract (AE) (Fig 1). Of these two rice varieties, Hasawi rice has shown more activity than Njavara. In the positive control, the IC_{50} value was 55.35 ug/ml. Similarly, HME demonstrated an IC_{50} value of 75.80 ug/ml, which was much lower than HAE, NME, and NAE.

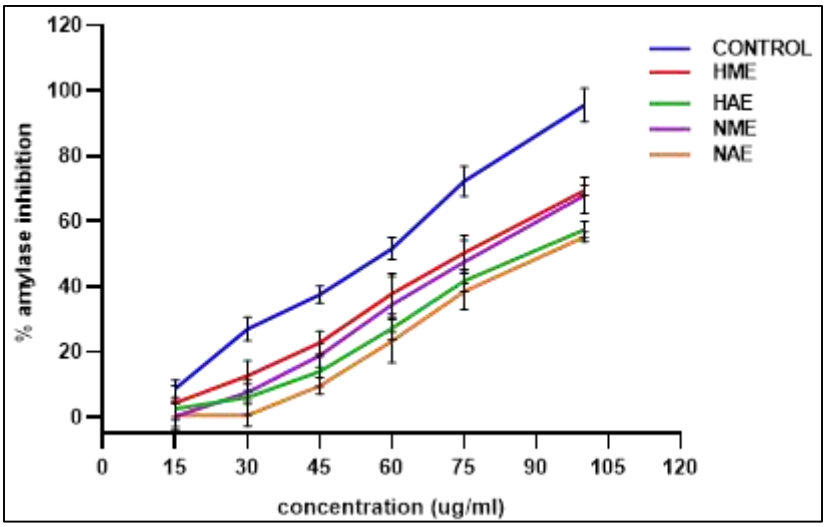

Figure 1 Alpha-amylase inhibition activity (α-AIA) of Hassawi and Njavara rice

3.2. Thrombolytic activity

In this study, Hasawi (HME) and Njavara methanolic (NME) extracts demonstrated high thrombolytic activity when compared to HAE and NAE. But Hasawi methanolic extract displayed more activity than Njavara methanolic extract. Similar trend is also noticed in aqueous extracts (Fig 2).

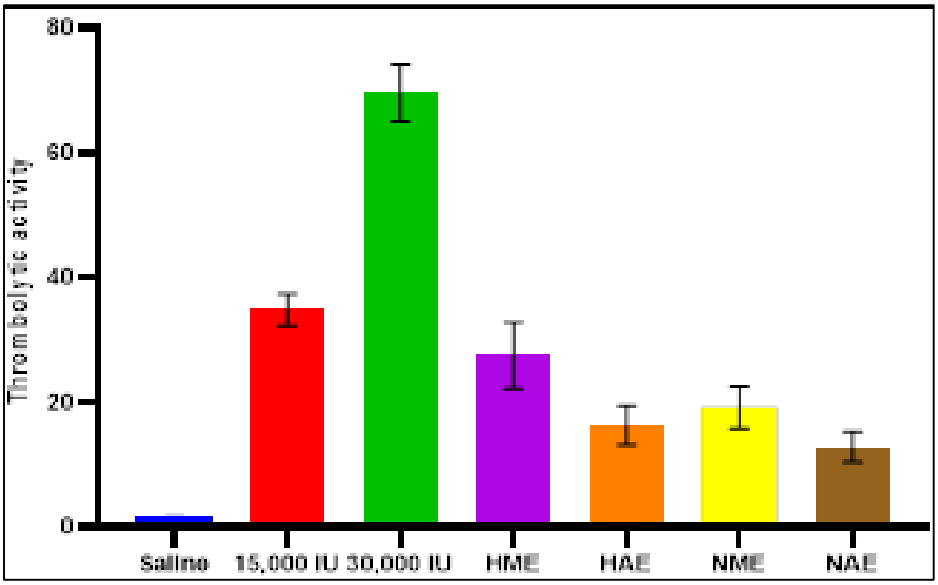

Figure 2 Thrombolytic activity of Hassawi and Njavara rice

3.3. DPPH radical scavenging assay

Results of this experiment indicate that as noticed in the above cases, HME is potent scavenger of DPPH free radicals than NME(Fig 3). Interestingly, aqueous extract of Njavara rice have shown more activity than that of Hasawi, pointing that methanol extract of Hasawi and aqueous extracts are having good antioxidant activity (Fig 3). The free compounds had much higher antioxidant activity than bound compounds. Specifically, the IC_{50} values in HME and HAE revealed 52.5% and 50.3% free radical scavenging activity at 65.34 ug/ml and 104.13 ug/ml concentrations, respectively, compared to NME and NAE.

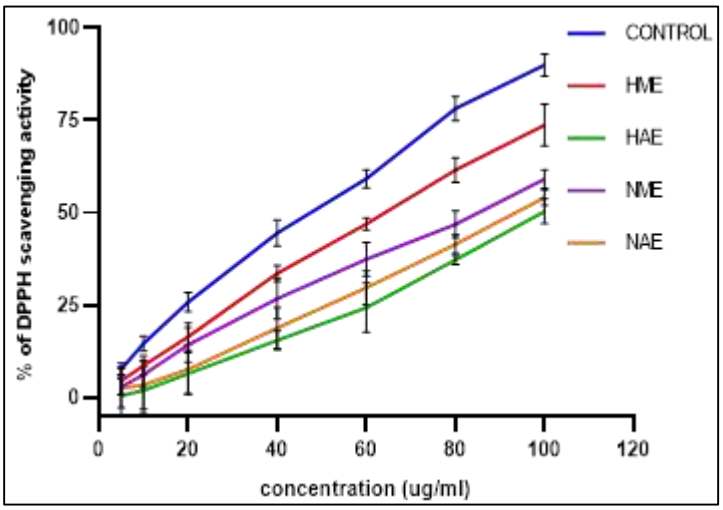

Figure 3 Antioxidant activity of Hassawi and Njavara rice

3.4. Anti-inflammatory activity

In this experiment, with increase in the concentration of the extract, the anti-inflammatory activity increased in all cases. Hasawi methanol extract displayed good activity on par with control at all concentrations tested. Of the two rice varieties, Hasawi is a good anti-inflammatory agent than Njavara rice (Fig 4).

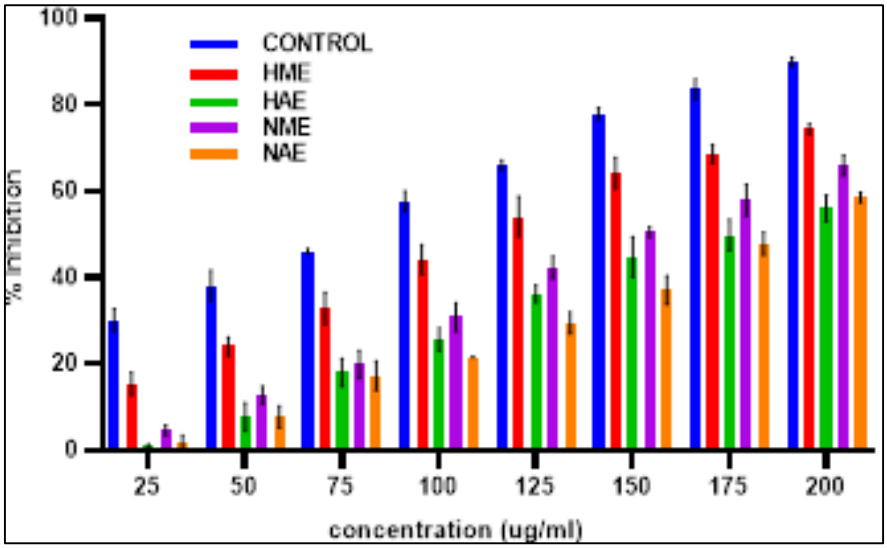

Figure 4 Anti-inflammatory activity of Hassawi and Njavara rice

3.5. Lipid peroxidation:

The IC 50 % value of lipid inhibition rate in HME showed at 64.21 ug/ml concentration which is a 12% higher rate, compared with HAE, NME and NAE. As noted in the previous cases, HME has shown good activity than NME. This point out that methanolic extract has dissolved more compounds in Hasawi than Njavara or Hasawi rice may contain potential compounds than Njavara (Fig 5).

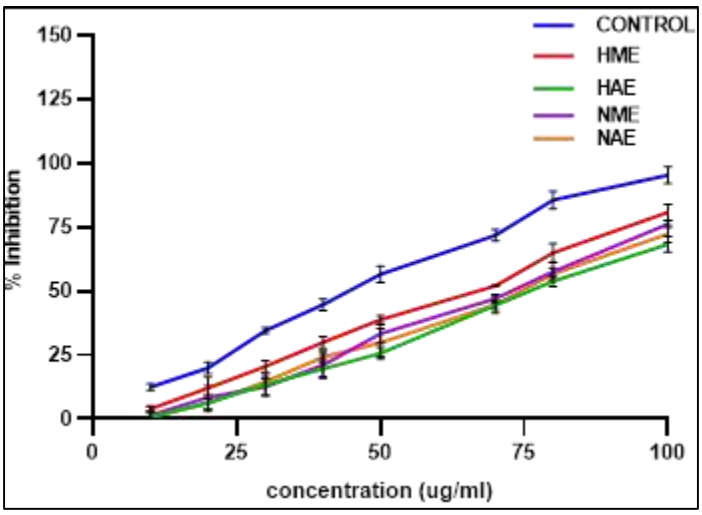

Figure 5 Lipid peroxidation of Hassawi and Njavara rice

3.6. Protease inhibition

In sharp contrast to the earlier experiments, Njavara rice (NME and NAE) displayed good protease inhibition activity than Hasawi rice at higher time intervals (Figure 6). The HME (259 ug/ml), HAE (250 ug/ml), NME (200 ug/ml), and NAE (200 ug/ml) lipid inhibition rates, respectively, had IC 50% values. Figure 6 reveals that NME had a lipid inhibition rate that was marginally higher than that of the other samples.

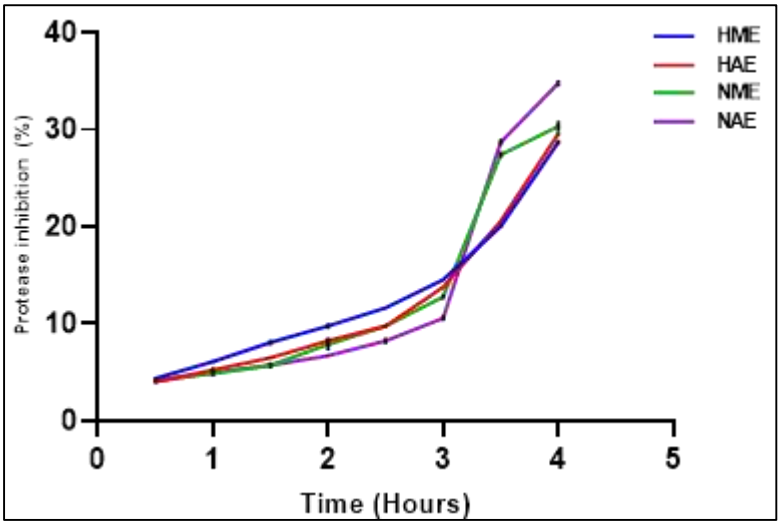

Figure 6 Protease inhibition activity of Hassawi and Njavara rice

3.7. Acetyl cholinesterase activity

In this experiment, HAE and NME showed similar Ach- activity than HME and NAE (Fig 7). Their activities increased with increase in time intervals. In particular, HAE and NME have higher levels of acetylcholine esterase activity than do HME and NAE, despite both rice varieties displaying inhibitory actions. At doses between 25 and 200 ug/ml, inhibition activity varied from 0.50% to 99.60% (Figure 7).

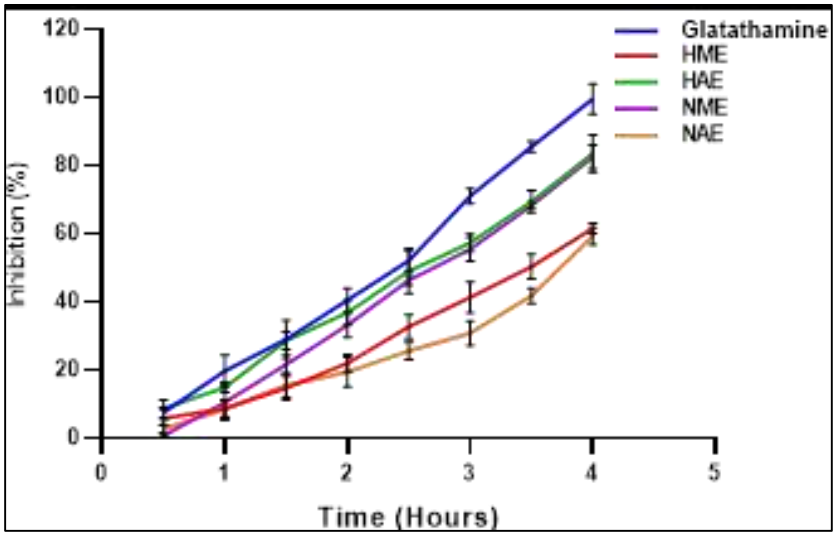

Figure 7 Acetylcholinesterase inhibitory activity of Hassawi and Njavara rice

3.8. Anti-arthritic Activity

In comparison to NAE and HAE, HME and NME have shown stronger anti-arthritic action in this investigation. The standard (positive control) has an IC_{50} value of 45.27 ug/ml. Compared to HAE, NME, and NAE, HME has stronger anti-arthritic action (Figure 8). Methanol extracts show increased acetylcholinesterase, thrombolytic, and antioxidant activity in the current investigation. On the other hand, aqueous extracts have stronger anti-inflammatory, anti-arthritic, lipid peroxidation, protease inhibition, and amylose inhibitory properties.

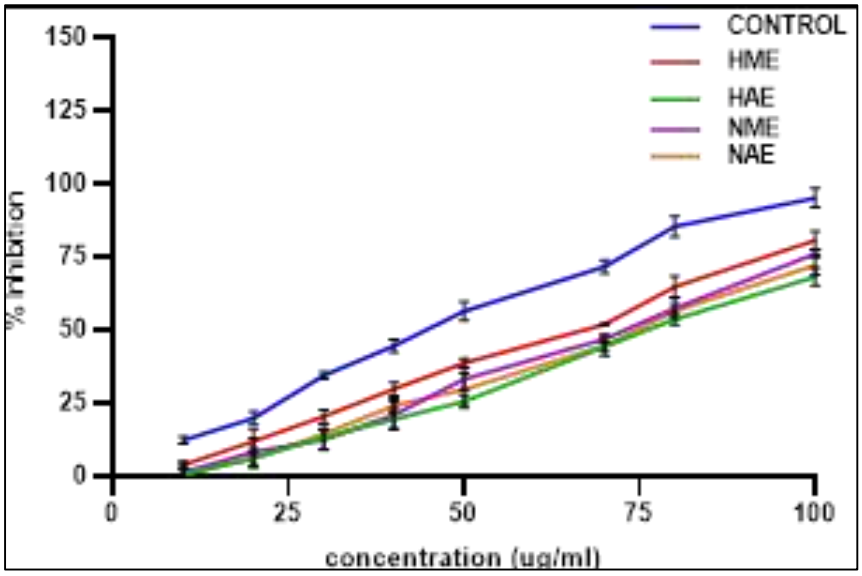

Figure 8 Anti-arthritic activity of Hassawi and Njavara rice

4. Discussion

The present investigation indicates that Hasawi methanol extract (HME) is a potential inhibitor of Alpha-amylase activity (Fig 1). In comparison to Basmati rice, the Hassawi variety includes 54.80% non-starch polysaccharides (NSPs) and selenium Muneera (2020). The total amount of NSPs in pigmented rice cultivars is determined by the rice cultivar, degree of milling, and water solubility Lai (2007). These NSPs may reduce plasma cholesterol levels and aid in the normalization of blood glucose and insulin. The observed results indicate that the presence of tocotrienols in pigmented rice cultivars could be the primary cause of thrombolytic activity. Most traditional rice varieties include tocotrienols and tocopherols, which have anti-thrombotic capabilities against cardiovascular disorders Kamal (1997). Compared to pokkili rice, the black and red Njavara rice bran has more tocopherol (24.86 mg/100 g) and tocotrienol (56.45 mg/100 g) Shylaraj (2017). The results of this study revealed that tocopherols and tocotrienol have a high affinity for thrombin, indicating that they could be used as an anticoagulant.

This resulted from traditional rice grains' antioxidant chemicals' capacity to dissolve polar solvents. According to this finding, Hassawi and Njavara rice have higher concentrations of antioxidants, such as oryzanol (2340 mg/100 g), and they are admired for their ability to reduce and scavenge free radicals. Black rice has been linked to phenolic components such as anthocyanins, proanthocyanins, and tannins more so than white rice, according to past reports (Deng 2013; Gunaratne 2013; Rajendran 2018).

Biotic and abiotic stress leads to the overproduction of reactive oxygen species (ROS), which is one of the highly potent initiations of inflammation in plants Nakajima (2013). The IC50 value of HME in this experiment was found to be 128.28 ug/ml concentration, which is 25% and 50% greater rate than that of NME, HAE, and NAE. To the best of our knowledge, these findings provide information regarding Hassawi rice's anti-inflammatory properties, which have not been discussed in other research. According to the experiment's results on lipid inhibition, the Hassawi and Nvavara rice types include a variety of antioxidants that are involved in antioxidant activity, such as superoxide dismutase (SOD), glutathione reductase (GR), and peroxidases Shalini and Dubey (2003). Tocopherols found in traditional colored rice cultivars shield polyunsaturated fatty acids (PUFA) against lipid peroxidation Shylaraj (2017).

Numerous protease inhibitors, such as papain and cathepsin B, enhance the nutritional value of plants and shield them from insects and pests. The majority of rice's protease inhibitors are still unknown. According to Bijina (2011), several plant species such as *Cicerarietinum*, *Momordicacharantia, Moringa oleifera*, and *Adathodavasica* also contain this type of protease inhibitor. Because of Saudi Arabia's extremely high temperatures, heat inactivation of the enzyme activity in Hasawi rice may be the source of this. We concluded that these protease inhibitors have enormous potential for the development of various drugs that target chymotrypsin, cathepsin and thrombin.

Acetylcholinesterase inhibitors are widely employed as pesticides for plants and as treatments for neurological illnesses in people. We found that the acetylcholine esterase inhibitory activity exhibited by both rice cultivars was highly linked

with flavonoid, phenolic, alkaloids, and insect tissues. According to earlier research, *U.maritima* bulb extract significantly inhibited the activity of acetylcholine esterase in *S. oryzae*. Maazoun (2017). The Liliaceae family also showed similar kinds of results Mukherjee (2007). Based on these results, the experiment demonstrated that toxicity in the rice types Hasawi and Njavara may interfere with the ability of phenolic and alkaloids to inhibit pests. Methanol extracts shown increased acetylcholinesterase, thrombolytic, and antioxidant activity in the current investigation. On the other hand, aqueous extracts have stronger anti-inflammatory, anti-arthritic, lipid peroxidation, protease inhibition, and amylose inhibitory properties. There is a link between the total phenolic and flavonoid content and the phytoconstituents found in traditional rice types and their biochemical activity. In addition to enhancing food quality, these actions will shield the plant from harmful disease infections and drought stress.

5. Conclusion

According to this study, the pharmacological activity of Hassawi and Njavara methanol and aqueous extracts is 15% higher than that of white rice. In conclusion, both types of rice exhibit lipid peroxidation, acetylcholinesterase, thrombolytic, anti-arthritic, antioxidant, and anti-inflammatory properties. Methanol extracts showed increased acetylcholinesterase, thrombolytic, and antioxidant activity in the current investigation. On the other hand, aqueous extracts have stronger anti-inflammatory, anti-arthritic, lipid peroxidation, protease inhibition, and amylose inhibitory properties. Of these two varieties, Hasawi showed good activities concerning amylose inhibition, thrombolytic activity, antioxidant activity, and anti-inflammatory and lipid peroxidation. However good protease inhibition and acetylcholinesterase activities were noted in Njavara than Hasawi. These points to a specific phytochemical composition that these two rice varieties contain. There is a link between the total phenolic and flavonoid content and the phytoconstituents found in traditional rice types and their biochemical activity. In addition to enhancing food quality, these actions will shield the plant from harmful disease infections and drought stress. The obtained data indicated that the rice types, Hasawi and Njavara were a promising source of phytoconstituents that are not fully explored.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors read and approved the final manuscript and declare that there is no conflict of interest.

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