Brassica nigra L., A Potential Hepatoprotective and Anti Tuberculosis Agent

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Abstract:

In clinical background, an anti-tuberculosis medication that may harm the liver. The medicinal plant Brassica nigra L (Brassicaceae) has anticancer and antioxidant properties. Brassica nigra L. hydroalcoholic extracts were screened for antitubercular activity using the NRA and BacT/Alert methods. It was investigated whether the hydroalcoholic extract of Brassica nigra L seeds could protect mice's livers from RIPE-induced hepatotoxicity. A total of eight animals were randomly allocated to six different groups. To induce liver damage, a specific dosage blend of RIPE was prescribed for a duration of fifteen days, Various treatments, including HABN and RIPE combined with Sylmarin, were ran to the treatment sets prior to the introduction of RIPE. The control group got 2% DMSO. Studies were conducted on tissue antioxidant enzymes, DNA damage, serum liver function tests, and histopathological changes. The results of HABN and Standard Sylmarin, which are based on M. tuberculosis's capacity to vary in colour, were disclosed by NRA in the current investigation. The hydroalcoholic extract of Brassica nigra and Standard Sylmarin significantly increased rat liver and body mass ($P<0.1$) compared to animals given RIPE. Furthermore, RIPE not only heightened DNA fragmentation ($P<0.001$) but also reduced lipid levels and raised the serum levels of lactate dehydrogenase (LDH), AST, □-GT. By increasing the ratio of antioxidants to oxidants, HABN treatment prevents oxidative damage caused by RIPE. Biochemical results are grasped by histological investigation. The study demonstrated that HABN's antioxidant.

Keywords: HABN, Anti tuberculosis activity, Assessment of liver function, DNA repair mechanisms, tissue histopathology, antioxidant activity, lipid oxidative degradation.

1. Introduction:

Despite numerous global efforts, tuberculosis (TB) continues to be a potentially life-threatening disease for several years. In 2023, there were expected to be 135000 new cases of multi-drug resistant TB (MDR-TB), along with 1.3 million deaths from TB in 2020, and 10.6 million new TB cases reported. India holds a concerning record in terms of TB, with 63,801 MDR-TB patients and an estimated 2.42 million TB cases in 2022. The DOTS is a vital method for controlling TB under the RNTCP in India. The main

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drugs utilized in DOTS include Isoniazid, Rifampicin, Pyrazinamide, and Ethambutol, all of which are administered at regular intervals for a minimum duration of six to nine months [1]. However, these treatments have a severe side effect called hepatotoxicity, which can happen a few weeks after starting the prescription and persists even after DOTS therapy is finished [2]. Antitubercular drug-induced hepatotoxicity has been investigated in a number of cohorts and settings around the world, and its occurrence has been documented. Due to their historic history, safety, and effectiveness, herbal medicines are the favoured choice for treating all ailments. Review papers on the antitubercular efficacy of therapeutic herbs demonstrate these plants' ability to treat tuberculosis [3]. Various natural active compounds, such as flavonoids, phenolic compounds, glucosinolates, have been identified in the plant Brassica nigra L, which belongs to the Brassicaceae family, based on phytochemical analysis [4,5]. It was applied as a means of relieving internal organ congestion in the treatment of rheumatism. It was used for toothache,snakebite, epilepsy, alopecia, and neuralgia and spasms. Moreover, throat tumours and carcinomas were treated with it. It's claimed that mustard oil promotes hair growth. In addition, mustard is advised in hiccups. It was regarded as antiseptic as well [6]. Pharmacological analysis of Brassica nigra exhibited mutagenic, cytotoxic, and nephroprotective effects. anti-diabetic and immunological effects antioxidant impact. used historically as a vesicant, nerve stimulant, emetic, diuretic, pneumonia, and bronchitis [7]. The relationship between liver damage and the breakdown of isoniazid is evident. Recent research has highlighted a link between the metabolic pathway of isoniazid and hepatotoxicity [8]. According to these studies, isoniazid undergoes metabolism via acetylation primarily through the key hepatic enzyme N-acetyltransferase. People can be classified into slow and fast acetylators, which is determined genetically. In fast acetylators, isoniazid is converted into acetyl isoniazid, which is subsequently excreted as diacetyl hydrazine. Analyses revealed that hydrazine is a particularly notable contributor to liver damage associated with isoniazid. Rifampicin is known to enhance isoniazid hydrazine actions, though the exact mechanisms behind hepatic damage induced by pyrazinamide and rifampicin remain unclear [9]. Experimental substantiationPreparation of Plant Extract: The raw Brassica nigra seeds were gathered from a basic pharmacy in Vijayawada. A delicate balance was used to weigh the 325 g of powdered B. nigra seed samples. They were extracted by maceration for three days straight with sporadic stirring in 80 percent CH2OH (1 by 10 ratio of powdered plant core (grammes) with a solvent (millilitres). The combination was riddled using whatman filter paper no-1 (Whatman, England) after 72 hours. After twice macerating the residue with the equivalent number of hrs, the combination was strained [10]. The mixture of supernatant was dried out in an oven temperature around 400C. To calculate the powdered extract % efficiency weight was measured. After drying the nectar,sealed in a glass container and stored within the freezer until needed again.

2. Preparation of plant hydro-alcoholic extract:

The Botany Department at Acharya Nagarjuna University provided medicinal plant seeds of Brassica nigra, which were identified scientifically after being acquired from a nearby market. To create the hydro-alcoholic extract, 50 grams of purified Brassica nigra seeds were ground and mixed with 80% ethanol in a 1 to 5 ratio then permitted at room temperature for 24hrs, during which it stirred periodically. After this period, the mixture was transferred to a percolator, where the residue was separated using a paper filter. The percolator's tap released two to three drops of the solution every minute. After filtration, the mixture was kept in a water vessel at 40°C for 16 hours, followed by evaporation of the alcohol to achieve a final concentration of 25% [11].

2.1. Extraction and phytochemical analysis: The leaves were subjected to air drying, rinsed with deionised water, and then ground in a blender. The resultant granular residue was extracted using a Soxhlet extractor, starting with C2H5OH followed by a series of solvents in order of increasing polarity (C6H14, C6H6, CHCl3, CH3OH, and H2O). The extracts underwent initial phytochemical analysis, and the findings have been documented in a prior research publication [12]. Evaluation of antitubercular activity: Antimicrobial Intensity Using the previously described microplate Alamar Blue assay, all phytochemicals were tested for their effectiveness against the strains of M.tuberculosis that were previously listed. 200 millilitres of sterile distilled water were put into the wells around the outside of the microplate. Following the addition of 100 ml of working metabolites solutions to the first well of each row, the microplate column was used to carry out a two-fold dilution series. One hundred millilitres of Middlebrook 7H9 broth with supplements was added to the remaining wells. A 100 cc dosage of the Every screening hole and the sober reference reservoirs received evaluate the bacterium. Less than 1% v/v was the final DMSO concentration in the wells. In order to simulate the development of 100,10, and 1 percent of the investigated the diversity of bacteria respectively, 100:100, 10:100, 1:100 attenuated organizers be made concurrently using bacterial abatement. The final values of the metabolites under investigation varied between 200 and 0.097 milligram/mL. Two tests were conducted on each concentration. Every microplate was stored in an airtight plastic bag and incubated for five days at 370C with 5% CO2. During the growth period, 20 milliliters of Trek Diagnostics' Alamar blue solution) and Stable ten percent Tween 80 in 12 milliliters was combined to create a single control growth. Re-incubating the plates at 37º C for a duration of 24 hours. After receiving the same combination of Tween solutions and Alamar blue, all of the wells gestated for another 24 hrs should the well become pink throughout the incubation period. Wells that were distinctly pink were given positive growth scores. The term "minimal inhibitory concentration" refers to the least sample dose at which a colour shift to pink is stopped. There were also additions of common drugs including streptomycin, isoniazid, ethambutol, and rifampicin. At least two trials of each were conducted [13]. Hepatoprotective activity: RIPE induced hepatotoxicity in mice Eight groups of six mice each were randomly divided up among the mice. Vehicle (2% DMSO dissolved in saline, intraperitoneally) was administered to Control(GroupI). For 15 days, Group II was given a mixture of anti-TB at a constant dosage, including pyarazinamide -36mG/Kg, isoniazid-6.5mG/Kg, rifampicin-13.5mG/KG, and ethambutol-24.8mG/KG. The medications were suspended in 0.9% sterile saline solution. Animals in Group III 100 mg/kg, Group IV 200 mg/kg, and Group IV 400 mg/kg were administered HABN dissolved in 2% DMSO intraperitoneally. Before the RIPE challenge, 45 minutes in advance, Group VI was given 100 Mg/kG of 10% DMSO is used to dissolve silymarin. Group VII treated with Silymarin 100Mg/KG, whereas Group VIII treated with HABN (400 Mg/Kg) a single time per every day for fifteen days. Following the final treatment dosage, the Animals has been allowed to starve for 12 hours before being decapitated to end their lives. The liver was removed right away, wiped dry, and washed in 4 °C frozen saline. Little chunk of a hepatic be kept in dil.N2 at -70°C to facilitate biochemical and DNA fragmentation tests. Studies on histopathology were conducted using the remaining part. A heart puncture was used to remove the blood, and At 4 °C, the sera were separated using sedimentation (640 g) without the addition of any additives, and then refrigerated at -200C for biochemical analysis [14]. Biochemical analysis of the lipid & serum profiles. The levels of gamma glutamyl transferase, LDH, ALT, ALP, and ALP are evaluated in the blood. AMP diagnostic kits are used to assess total protein, albumin, lactate

dehydrogenase, and total bilirubin following the experimental design provided by the manufacturer. Additionally, the quantities of HDL, LDL, TG, and TC are estimated using a standard AMP investigative strip [15-26].

3. Results and Discussions:

3.1. Anti-Mycobacterial Activity:

The result of the Alamar Blue Assay's anti-mycobacterial activity against the prevalent Mycobacterium TB strain H37RV from Table 1 [23-26].

Table 1: Effects of HABN comparable to RIPE on Mycobacteriaum

S.No	Treatments	Mycobacterium tuberculosis H37Rv strain MIC (µg/ml)	Progress of Mycobacteriaum		
			reddish/violet colouration		
			I	II	III
1	Control	--	+	+	+
2	HABN (100 µg/kg)	100	+	+	+
3	HABN M (200 µg/kg)	50	–	–	–
4	HABN M (400 µg/kg)	0.8	–	–	–
5	Isoniazid	0.2	–	–	–
6	Rifampicin	38	–	–	–

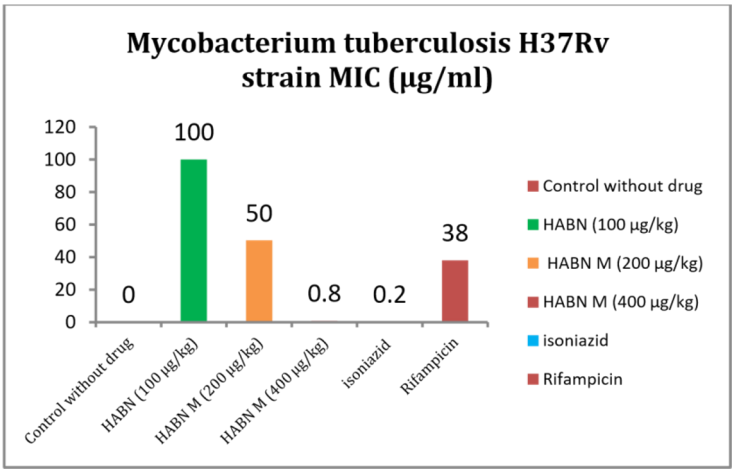

Figure 1: Effects of HABN comparable to RIPE on Mycobacteriaum

The antitubercular activity of hydroalcoholic extracts was assessed using the NRA and BacT/Alert methods. In the current work, NRA is based on Mycobacterium ability to convert NO2 to NO3, with decrease identified by means of particular chemical compositions that cause a color transformation. Serious concentrations employed included 0.2µG/mL-INH, 40µG/mLRifampicin, then various plant expert quantities [27]. Both standard and plant extracts of MIC were lowered equated to positive group, as seen by color variation from Figure 1.

3.2. Effects of HABN comparable to the Silymarin on liver and Body weights: The influence of HABN on liver weight was comparable to that of Silymarin. Likewise, treatment with anti-TB medications directed to a substantial reduction in final body-mass. HABN treatment notably mitigated the body weight loss caused by RIPE. In contrast, the groups treated with both Silymarin and HABN experienced a greater weight gain from Table 2.

Table 2: Effects of HABN comparable to the Silymarin on liver and Body weights

Treatments	Liver-weight (Gm)	Initial body-mass (Gm)	Final body-mass (Gm)
+ve treament	63.31 ± 1.23	24.23 ± 0.51	32.7 ± 0.45
RIPE	4.12 ± 0.68*	24.27 ± 0.49	26.24± 0.37***
RIPE+HABN(100mG/kg)	4.92 ± 0.74	24.54 ± 0.45	28.43 ± 0.54***
RIPE+HABN(200mG/kg)	5.71 ± 0.97+	24.98 ± 0.23	30.79 ± 0.25***
RIPE+HABN(400mG/kg)	5.65 ± 0.13+	25.31 ± 0.56	32.19 ± 0.56+++

RIPE+Silymarin (100 mG/kg)	5.45 ± 1.56+	25.29± 0.23	33.57 ± 0.34+++
Silymarin 100 mg/kg alone	6.56 ± 2.45	25.35 ± 0.34	34.12 ± 0.68+++
HABN (400 mg/kg) alone	6.43 ± 1.12+	24.45± 0.46	34.56 ± 0.67+++

The results (n = 6) are accessible as mean ±SEM. Following a One-Way study of modification, Signs *, **, and *** indicate significant for the resistor set at $P < 0.05$, $P < 0.001$, and $P < 0.0001$, respectively. A significant difference between the RIPE + HABN (200 mg/kg) and RIPE + HABN (400 mg/kg) groups at $p < 0.0001$, whereas +, +++ signals significance from the RIPE group at $p < 0.05$ and $p < 0.0001$.

3.3. Lipid-profile: Lipid-profile modified by the lipid peroxidation influenced by Hepatic toxins Polyunsaturated fatty-acids. Trigycerides LDL and Total Cholesterol all markedly elevated ($P<0.0001$) as a result of RIPE treatment while lowering HDL levels.Alterations in Lipid Profile and Protective Effects of HABN
Hepatic toxins instigate lipid peroxidation, leading to disturbances in lipid metabolism through modifications of polyunsaturated fatty acids. Following the administration of RIPE, levels of triglycerides, LDL, and total cholesterol exhibited substantial increases (*$P < 0.0001$), whereas HDL levels revealed a significant decline in contrast to the control group ($P < 0.001$). These observations underscore the harmful consequences of RIPE on lipid equilibrium.
Conversely, the simultaneous administration of HABN alongside RIPE facilitated the restoration of the disrupted lipid profile, exhibiting a dose-dependent effect. The subjects receiving a higher dosage of HABN showcased notable protective outcomes against RIPE-induced dyslipidemia, akin to the advantages seen with Silymarin treatment at a concentration of 100 mg/kg. Additionally, animals treated solely with HABN at 400 mg/kg body weight displayed no significant deviations in lipid parameters when compared to the control group from Table 3.

Table 3: Lipid-profile

Treatments	**TC mG/dL**	**Triglycerides (U/L)**	**HDL mG/dL**	**LDL mG/dL**
Control	64.54 ± 2.47	53.24 ± 1.34	42.24 ± 1.98	27.1 ± 2.79
RIPE	89.10 ± 1.25****	97.23 ± 3.35****	23.78 ± 2.41 ****	62.18 ± 1.79****
HABN(100 mg/kg) + RIPE	92.14 ± 2.34	86.67 ±1.23	29.45 ± 2.45	57.34 ± 2.34

HABN(200 mg/kg) + RIPE	83.43 ±1.89***	77.78 ± 4.78****+++	32.45 ± 1.23##	46.78 ± 2.08****++++
HABN(400 mg/kg) + RIPE	74.53 ± 2.15++++	68.47± 2.34****+++	36.65 ± 2.34++++	36.54 ± 2.45++++
Silymarin 100 mg/kg + RIPE	71.19 ± 1.78 ++++	62.97 ± 2.07++++	39.23 ± 2.08++++	32.67 ± 1.11++++
Silymarin 100 mg/kg	58.13 ± 2.67++++	58.23 ± 1.13++++	43.17± 2.09++++	27.13± 1.09++++
HABN (400 mg/kg)	62.23 ± 1.13++++	61.22 ± 3.12++++	41.34 ± 0.23++++	30.11 ± 2.33++++

Results are presented as mean ± SD (n=6). The RIPE treatment comprised ethambutol (24.8 mg/kg), pyrazinamide (36.0 mg/kg), isoniazid (6.75 mg/kg), and rifampicin (13.5 mg/kg). HABN refers to the hydroalcoholic extract of Brassica nigra. The determination of statistical significance employed one-way ANOVA, succeeded by Tukey's multiple comparison test. Asterisks (, *, *) denote the levels of significance for the RIPE group (*$P < 0.005$, $P < 0.001$, $P < 0.0001$), the control group ($P < 0.005$, $P < 0.001$, $P < 0.0001$), as well as comparisons between the HABN (400 mg/kg) + RIPE and HABN (200 mg/kg) + RIPE groups at $P < 0.0001$.

3.4. The impact of HABN on liver-function-tests (LFTs): Following the administration of RIPE, there was a significant increase in serum levels of AST, ALT, ALP, γ-GT, and LDH when compared to the control group, suggesting hepatotoxic effects. In contrast, the concurrent administration of HABN alongside RIPE effectively alleviated liver damage, which was indicated by a marked reduction in these biochemical markers. Notably, the hepatoprotective properties of HABN exhibited a dose-dependent relationship, wherein higher concentrations yielded enhanced protective outcomes.
Interestingly, within the groups treated solely with either HABN or Silymarin, there was no significant change in liver enzyme levels relative to the control, implying that neither compound independently generated hepatic stress (see Table 4).

Table 4: The effect of HABN on liver function tests (LFTs)

Treatment	AST (U/I))	ALT (U/L	ALP (U/P)	Γ-GT1(U/l))	LDH (U/L))
Control	100.34 ± 2.78	86.46 ± 2.35	148.57 ± 2.45	1.34 ±0.45	45.65 ±1.52
RIPE	198.98 ± 2.89****	231.2 ± 5.13****	296.34 ± 3.67****	4.87 ±0.56****	157.53 ± 1.18****
HABN(100 mg/kg) + RIPE	190.23±3 .34++++	187.23±4. 23	282.34±2. 58	3.61±0.23	131.12±
HABN(200 mg/kg) + RIPE	172.34 ± 2.47++++	171.34.9 ± 2.56++++	233.14 ± 2.56++++	2.96± 0.34*+	111.52 ±2.36 ++++
HABN(400 mg/kg)	143.67 ± 3.67++++	132.01 ± 2.45*+++ +	175.64 ± 3.98*+++ +	2.48 ± 0.19++++	87.12 ±2.01+++ +
Silymarin 100 mg/kg	100.28 ± 2.78++++	73.12 ± 3.45++++	135.23 ±4.35+++ +	1.18 ± 0.22++++	41.23 ± 2.13++++
HABN (400 mg/kg) alone	108.34 ± 4.32++++	89.12± +++	146.34 ±6.76+++ +	1.53 ±0.45+++ +	44.54 ± 1.85++++

All data is expressed as mean ± SD (n=6). The RIPE treatment protocol comprised ethambutol (24.8 mg/kg), pyrazinamide (36.0 mg/kg), isoniazid (6.75 mg/kg), and rifampicin (13.5 mg/kg). For clarity, HABN denotes the hydroalcoholic extract derived from Brassica nigra, while Silymarin was administered at a dosage of 100 mg/kg. The analysis of statistical significance was performed using a one-way ANOVA, succeeded by Tukey's multiple comparison test. The significance levels were indicated as $P < 0.05$, $P < 0.01$, and $P < 0.0001$ for the RIPE-treated group, with the control group presenting values at $P < 0.05$, $P < 0.01$, and $P < 0.0001$, and the HABN (400 mg/kg) + RIPE cohort compared to the HABN (200 mg/kg) + RIPE group at $P < 0.0001$.

4. Influence of HABN on antioxidant enzymes:

The effects of HABN on the activity of antioxidant enzymes in liver tissues, particularly following the administration of RIPE, are encapsulated in Table 4. A statistically significant reduction in phase I antioxidant enzyme activity, specifically in catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD), was documented in the group treated with RIPE when juxtaposed with the control group ($P < 0.05$). In contrast, the supplementation of HABN led to a marked enhancement in the activity levels of these enzymes in relation to the RIPE-treated group, thereby suggesting a protective function of HABN in mitigating oxidative damage induced by RIPE.

Furthermore, when Silymarin was administered at a dose of 100 mg/kg alongside RIPE, a notable restoration of hepatic CAT, POD, and SOD levels was observed compared to the RIPE-only group. The hepatoprotective effects manifested with HABN at 400 mg/kg were found to be comparable ($P > 0.05$) to those of the Silymarin-treated group at 100 mg/kg with respect to CAT and SOD activity. Nevertheless, the protective efficacy demonstrated by the Silymarin 100 mg/kg combined with RIPE was statistically superior ($P < 0.05$) to that observed in the HABN + RIPE co-treatment group. In the evaluation of POD and SOD activities, mice receiving 100 mg/kg of Silymarin exhibited a significant increase ($P < 0.05$), although no substantial change in CAT levels was noted ($P > 0.05$) compared to the control. Conversely, the administration of HABN at 400 mg/kg resulted in a significant increase ($P < 0.05$) in both CAT and SOD activity; however, POD levels remained largely unaffected ($P > 0.05$) when compared to the control group.

Table 5: The effect of HABN on antioxidant enzymes

Treatments	CAT (U/min)	POD (U/min)	SOD (U/mg protein)	GSH (nM/g tissue)	GST (µM/mg protein)	GPx (nM/min/mg protein)	GSR (nM/min/mg protein)	γ-GT (nM/min/mg protein)
Control	1.67± 0.64	2.14 ± 0.32	11.45±0.15	41.58 ± 2.45	7.45± 0.23	122.45 ± 3.23	211.78 ± 2.35	92.2 ± 2.34
RIPE	0.68 ± 0.23****	1.22 ± 0.11 ****	3.98± 0.34 ****	29.52 ± 1.46****	2.87 ± 0.32 ****	65.43± 1.89 ****	159.67 ± 3.56****	61.34 ± 2.44*
HABN (100 mg/k	1.43 ± 0.34	1.45 ± 0.21	4.69 ± 0.2	32.14 ± 0.58	3.98 ± 0.46	83.56 ± 1.76	165.23 ± 2.45	69.63 ±4.23

g) + RIPE								
HABN (200 mg/kg) + RIPE	1.87 ± 0.22+++++	1.47 ± 0.15******+++	5.87 ± 0.24******+++	33.98 ± 1.54**	5.20 ±0.65*****	98.78 ± 4.56**	181.56 ± 5.62*****	76.78 ± 2.45******* + #
HABN (400 mg/kg) + RIPE	2.12 ± 0.21+++++	1.78± 0.13*+++++	8.12 ± 0.45 ******++++	36.42 ± 2.12+++	6.12 ± 0.56******+++	112.58 ± 2.78******++++	199.56± 4.12*+++++	86.54 ± 3.56++++
Silymarin 100 mg/kg + RIPE	2.35± /.12++++	2.13 ± 0.19++++	8.86 ± 0.12******+++	38.14 ± 2.05*+	6.98 ± 0.43++++	114.34 ± 3.34***	206.23 ± 3.56******++	91.23 ± 1.45*
Silymarin 100 mg/kg	1.88 ± 0.16++++	2.42 ± 1.06++++	11.96 ±0.28******++++	42.78 ± 2.48+++++	6.43 ± 0.35++++	131.98 ± 2.34++++	219.34 ± 4.79++++	95.13 ± 3.14a
HABN (400 mg/kg)	2.21 ± 0.14**++++	1.98± 0.8++++	10.24 ± 0.42 **+++++	41.9 ± 1.68+++++	5.67 ± 0.48**+++++	126.56 ± 4.34++++	207.45 ± 3.78*+++++	89.12 ± 2.56a++++

All data are presented as mean ± SD (n=6). Statistical significance was assessed utilizing one-way ANOVA followed by Tukey's multiple comparison tests. The significance thresholds for the RIPE group were established at $P < 0.05$, $P < 0.01$, and $P < 0.0001$. Evaluations of comparative differences with the control group were similarly determined at $P < 0.05$, $P < 0.01$, and $P < 0.0001$; moreover, distinctions between the HABN (400 mg/kg) + RIPE and HABN (200 mg/kg) + RIPE groups were regarded as significant at $P < 0.0001$ (see Table 5).

4.1. The impact of hydroalcholic of BN extract hepatic protein levels and oxidative-stress-markers: The efficacy of HABN in preventing oxidative stress and changes in protein levels induced by RIPE is illustrated in Table 6. Treatment with RIPE led to a decline in the amount of dissolved protein content in the hepatic specimens of mice equated to the resistor set. Mice that received a combination of +Headings, HABN, and RIPE exhibited higher soluble protein concentrations in their tissues than those treated solely with anti-tuberculosis medications. Administering Silymarin and HABN individually to mice did not significantly elevate protein levels relative to the control group; however, it highlighted that HABN has a protective effect

on soluble protein concentrations that is dependent on the dosage. When undergoing RIPE therapy, the levels of HABN and Silymarin in the liver tissues were higher than the resistor set's, suggesting a notable increase in hepatic oxidative stress. In a manner that correlates with the dose, the combination of HABN treatment and RIPE mitigated the harmful effects of the anti-TB medication. HABN demonstrates a comparable capacity for improvement as the cohort receiving the standard Silymarin treatment.

Table 6: The protecting properties of HABN on Hepatic and oxidative-stress-indicators

Treatment	Proteini-(µG/mG tissue)	TBARS-(nM of MDA/mg protein)	H2O2-(nM/min /mg tissue)
Control	1.56±1.14	7.06±0,20	6.45±0,78
RIPE	0.64 ± 0.36****	18.87 ± 1.78****	8.56 ± 0.34****
HABN (100 mg/kg) + RIPE	0.87 ± 0.12	15.67 ± 1.56	7.68 ± 1.56
HABN (200 mg/Kg) + RIPE	1.15 ± 0.15++	11.45± 0.34****++#	6.28 ± 0.78**+++
HABN (400 mg/kg) + RIPE	1.48 ± 0.12++++	9.89 ± 1.24*++++	5.12 ± 0.12++++
Silymarin 100mg/kg + RIPE	1.95 ± 0.15+++	8.43 ± 1.45c++++	4.54 ± 0.45++++
Silymarin 100mg/kg	1.61 ± 0.16++++	7.07 ± 1.09e++++	4.07 ± 0.33++++

5. Discussion:

The phytochemical composition of Brassica nigra has been characterized primarily by the presence of flavonoids, steroidal alkaloids, triterpenes, and glycosides. These compounds are recognized for their potent antioxidant activities, leading to the hypothesis that extracts derived from Brassica nigra may provide hepatoprotective benefits against the hepatotoxicity linked to anti-tuberculosis (anti-TB) pharmaceuticals. An evaluation was conducted to assess the impact of widely utilized anti-TB agents—specifically pyrazinamide, isoniazid, ethambutol, and rifampicin—on hepatic function in a murine model. The findings indicated pronounced hepatic injury,

evidenced by significant alterations in liver function biomarkers and increased serum levels of AST, γ-GT, ALP, ALT, and LDH.

Furthermore, the co-administration of Brassica nigra extract (HABN) demonstrated a notable amelioration of hepatotoxic effects induced by anti-TB medications, exhibiting a dose-dependent reduction in serum concentrations of LDL, cholesterol, triglycerides, AST, γ-GT, ALP, ALT, and LDH. These outcomes suggest that HABN provides a protective influence on hepatic function, likely attributable to its diverse bioactive components. Prior research has indicated that flavonoids such as luteolin and quercetin are not only beneficial in enhancing lipid metabolism but also in diminishing the production of inflammatory cytokines. Additionally, treatment with HABN at a dosage of 400 mg/kg alone did not result in significant alterations in hepatic enzyme levels, thereby affirming its safety for potential therapeutic applications.

In a parallel investigation, the administration of Silymarin at a dosage of 100 mg/kg in conjunction with anti-TB agents resulted in a significant reduction of serum levels of AST, γ-GT, ALP, ALT, and LDH, thereby safeguarding hepatic integrity. However, the isolated administration of Silymarin did not yield substantial differences in ALT, ALP, γ-GT, and LDH levels when compared to the control group, although a minor increase in AST levels was noted, suggesting that the absence of Silymarin might have contributed to this effect.

The mechanism underlying the hepatotoxicity associated with anti-TB drugs is primarily attributed to oxidative stress stemming from excessive generation of free radicals. An observable accumulation of anti-TB medications within hepatic tissues, coupled with a reduction in hepatic GSH and antioxidant enzyme levels, points to a disruption in metabolic balance that favors oxidative stress. The diminished levels of critical antioxidant enzymes, including CAT, SOD, and GPx, were insufficient to counteract the overproduction of lipid hydroperoxides, which are significant mediators of oxidative stress-induced hepatic damage.

Cellular antioxidants, such as GST, CAT, POD, GR, SOD, and GPx, are essential in defending against oxidative injury, and this protective mechanism has been the subject of considerable research. In the context of the GSH reaction system, GSH is regenerated through the activity of GSR, thus preserving its vital functions. Moreover, GSH acts as a cofactor for GST, an enzyme that is ubiquitous in both the cytosol and the endoplasmic reticulum. GST is critical for the conjugation of GSH with electrophilic substances, facilitating the detoxification of xenobiotics and preventing the formation of harmful metabolites.

The phenomenon of lipid peroxidation, which significantly contributes to hepatic injury, has been associated with the activation of liver stellate cells, resulting in increased collagen synthesis and potentially elevated levels of fibrogenic cytokines. It is well established that oxidative stress and subsequent tissue injury can activate the complement system, leading to the augmented production of C3b, which may exacerbate liver damage further. Considering these observations, it is plausible that the phytochemicals derived from Brassica nigra possess inherent antioxidant properties that alleviate liver damage by mitigating oxidative stress induced by anti-TB drug administration. Nonetheless, additional research is warranted to meticulously elucidate the specific active compounds responsible for these hepatoprotective effects.

6. Conclusion:

The current study's findings showed that the anti-TB medication caused oxidative stress and negatively impacted DNA, lipids, and protein, all of which had an adverse effect on the liver. In addition to controlling histological changes, HABN showed a potent anti- tubercular activity against Mycobacterium tuberculosis with hepatoprotective activity by reducing oxidative DNA damage and increasing antioxidant enzymatic and nonenzymatic levels. Studies using serological testing to measure liver function also demonstrated how HABN protected against deteriorations brought on by anti-TB drugs. The presence of different antioxidant phytochemicals may be responsible for these protective benefits. To facilitate the translation of innovative results into practical applications, human tissue and samples should be used in future experiments examining the processes behind the pathogenesis of anti-TB agents. Finding of potent Novel Natural Multi drug therapy for tuberculosis with controlled adverse effects is the major task in the pharma innovations.

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