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GCMS analysis and Hepatoprotective activity of *Naregamia alata* on Galactosamine - Induced Hepatotoxicity in HepG2 Cells

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ABSTRACT

This study aimed to validate the hepatoprotective activity of extracts from Naregamia alata against Galactosamine-induced hepatotoxicity in HepG2 cells. Cells were exposed to different extracts at the concentrations of (1.56 µg/ml – 12.5 µg/ml) in combination with Galactosamine (0.1%) for 24 hours. Hepatoprotective effects were assessed through various assays, including cell proliferation and oxidative stress mechanism. The anti-proliferative effect was improved in the presence of all the plant extracts at all the tested concentrations. Ethylacetate extract treated HepG2 cells were 70.14% reduction in Galactosamine induced group. Galactosamine led to 198.25% increase in MDA level, which was attenuated by Ethylacetate extract at 138.37% at 6.25 ug/ml. GSH content in cells decreased by 52.25% with Galactosamine, but the plant extract increased GSH levels at 73.25%. Additionally, Galactosamine induced a 34.26 decrease in SOD level, but this necrotic effect was diminished by treatment group 71.85%. In conclusion, extracts from Naregamia alata demonstrated a preventive effect on HepG2 cell injury induced by Galactosamine treatment for 24 hours. The hepatoprotective activity of Ethylacetate extract was also comparable with that of the standard drug silymarin.

Keywords: Naregamia alata; Hepatoprotective activity; Galactosamine; Oxidative stress

1. INTRODUCTION

The liver, responsible for metabolism, detoxification, and excretion, is vulnerable to xenobiotics, making it prone to toxicity and leading to morphological and functional changes [1,2,3]. Because of its central role in various functions, the liver is especially prone to reactive oxygen species (ROS), which can impair the cell's defence mechanisms and initiate oxidative stress. Damage to cells resulting from this process may lead to inflammation and fibrosis, and in severe cases, it can contribute to the development of cancer and necrosis.

Galactosamine is a frequently used substance to induce oxidative stress and liver injury, playing a crucial role in evaluating the therapeutic potential of drugs and dietary antioxidants through experiments involving HepG2 cells [4,5]. Galactosamine is one of the most experimentally used drugs to study hepatotoxic effects in experimental animals due to clinical features resembling acute hepatitis. Biochemically, d-galactosamine affects uridine diphosphate (UDP)-glucose and UDP-galactose, and this in turn leads to loss of intracellular calcium homeostasis and also inhibits the energy metabolism of the hepatocytes. These changes affect cell membranes and organelles and the synthesis of proteins and nucleic acids, thereby cumulatively contributing to the observed hepatic damage.

The use of medicinal plants and products derived from them is prevalent, particularly in developing countries, and is witnessing a notable increase. This trend is largely attributed to the impact of empirical knowledge transmitted across generations and the growing public enthusiasm for natural therapies. Individuals often consume these products, placing their trust in their safety due to their natural origin. Plants contain a variety of compounds, presenting a potential hazard for inducing liver damage. Nevertheless, there is insufficient information about the toxicological and pharmacological profiles of these compounds. Additionally, several cases of intoxication are not adequately reported. Therefore, it is essential to examine the composition of medicinal plants to identify compounds that could possess hepatotoxic potential.

Contrastingly, medicinal plants may contain beneficial compounds that facilitate the discovery of new medicines. Scientific studies have demonstrated that secondary metabolites like polyphenols, anthraquinones, terpenes, and sulforaphane can activate the antioxidant defense system in hepatocytes, with Nrf2 playing a central role. This activation is pivotal in alleviating oxidative stress damage and providing protection to the liver [8].

Naregamia alata Wight & Arn, commonly known as Goanese ipecacuanha, belongs to the family Meliaceae. This undershrub typically attains a height of up to 30 cm and is

extensively distributed throughout South India, being present in all districts up to an elevation of 900 m. It is an endemic plant often found in the sacred grooves of the Western Ghats [9]. It holds significance in folkloric medicine in india, where it is employed for treating various conditions such as rheumatism, itch, malarial and chronic fevers, wounds, anaemia, enlarged spleen, ulcers, vitiated conditions of pitta and vata, halitosis, cough, asthma, splenomegaly, scabies, pruritis, dysentery, dyspepsia, and cataract[10].

The holistic use of the entire *Naregamia alata* plant is rooted in its traditional reputation for high hepato-protective properties, particularly in the treatment of jaundice [11]. The chloroform extract obtained from *Naregamia alata* showcased significant activity against both gram-negative and gram-positive bacteria. Furthermore, the petroleum ether extract derived from the leaves of *Naregamia alata* demonstrated the most potent antifungal activity [12]. Additionally, the ethanolic extract from the leaves and roots, as well as the aqueous extract from the stems of *Naregamia alata*, exhibited noteworthy antibacterial activity. Hence, the present study was undertaken to evaluate the hepatoprotective effects of the plant extract obtained from *Naregamia alata* in a HepG2 cell line subjected to injury induced by Galactosamine.

2. MATERIALS AND METHODS

2.1. Chemicals:

Dulbecco's modified Eagle medium (DMEM) culture medium, trypsin, fetal bovine serum (FBS), and antibiotics/antimycotic solution were acquired from Himedia Laboratories, Mumbai. The plastic wares and other consumables utilized in the study were obtained from Tarsons, Kolkata. Galactosamine, MTT and all specified chemicals and reagents were procured from Sigma Aldrich and SRL Chemicals.

2.2. Collection and preparation of the plants extracts:

Naregamia alata was obtained from western Ghats of Kerala region of south India. These plants underwent thorough examination, identification, and authentication. The whole plant was air-dried and subsequently pulverized into powder. Approximately 500 g of the powdered sample from each medicinal plant was weighed and subjected to successive solvent extraction using Hexane, chloroform, Ethylacetate, Ethanol and water in a soxhlet apparatus. The extraction process was carried out for 3 days for each solvent. The resulting filtrate was evaporated to dryness at 40°C using a rotary evaporator. This extraction procedure was repeated multiple times until a sufficient quantity of extract was obtained. The concentrated extract from each plant was then stored at 4°C until needed for use.

2.3. Phytochemical analysis:

Phytochemical screening was performed using standard procedures. Plant extracts were screened for the following phytoconstituents: alkaloids, carbohydrates, tannins, saponins, terpenoids, flavonoids, tannins, cardiac glycosides [12].

2.4. GC-MS analysis:

The GC-MS analysis of the plant extract was carried out using a Perkin-Elmer Clarus 680 system equipped with a fused silica column (Elite5MS capillary column, 30 m length × 250 µm diameter × 0.25 µm thickness). Helium served as the carrier gas at a constant flow rate of 1 mL/min. Compounds were detected in the GC-MS spectral range of 40 to 600 m/z, employing an ionization energy of 70 eV and a scan time of 0.2 s. The injector, maintained at 250ºC, featured a constant injection volume of 1µL. The column temperature initiated at 50 ºC for 3 minutes, followed by an increase of 10 ºC per minute until reaching 280 ºC. The final temperature was held at 300 ºC for 10 minutes. Identification of the compounds were based on a comparison of retention time, peak area, peak height, and mass spectral patterns with authentic compounds stored in the National Institute of Standards and Technology (NIST) library [13].

2.5. Anti-oxidant activity:

2.5.1. DPPH assay:

The DPPH assay is a straightforward, quick, cost-effective, and widely used method for evaluating antioxidant activity. Despite its association with hydrogen atom transfer, the fundamental chemical reaction in the DPPH assay is identified as an electron transfer (ET) reaction. This differentiation is based on the observation that the transfer of hydrogen from an antioxidant to DPPH is a relatively slow process, considered a marginal reaction path. In contrast, the electron transfer from a deprotonated antioxidant to DPPH is a faster and rate-determining step in this context [14].

The DPPH radical scavenging assay for the test samples (NAH, NAEA, NAC, NAE, NAW) followed a modified method as described by Perumal et al., 2018. In a nutshell, a 0.135 mM DPPH solution was prepared in methanol. Various test samples at different concentrations (5, 10, 20, 40, 80, 160, and 320 µg/ml) and ascorbic acid were mixed with 2.5 ml of the DPPH solution. The reaction mixture was thoroughly vortexed and left at room temperature for 30 minutes. Subsequently, the absorbance of the mixture was measured at 517 nm. The percentage of inhibition was calculated using the formula:

% DPPH scavenging activity = [(OD of control - OD of test) / (OD of control)] ×100

2.5.2. ABTS assay:

The ABTS assay is suitable for evaluating the antioxidant capacity of both hydrophilic and lipophilic samples, given that ABTS is soluble in both water and organic solvents. In the presence of hydrogen-donating antioxidants, the blue/green ABTS undergoes reduction to colorless ABTS at 734 nm, and the extent of antioxidant activity is directly correlated with the reduction in absorbance [15]. The ABTS solution was created by combining 7 mM of ABTS and 2.45 mM of $K_2S_2O_8$ in water, followed by a dark incubation for 12–16 hours at room temperature. Prior to use, the ABTS solution was diluted with a water and ethanol solution to attain an absorbance of 0.7 ± 0.02 at 734 nm using a UV-visible spectrophotometer. In the assay, 2 mL of the ABTS solution was added to 100 μL of test samples at various concentrations. The mixtures were thoroughly blended, incubated at room temperature for 10 minutes, and the absorbance was promptly measured at 734 nm. The percentage inhibition of absorbance was calculated and plotted against the concentration of the standard and sample, and IC50 values were determined using a relevant formula.

The radical scavenging activity was calculated using the equation:

% ABTS scavenging activity = [(OD of control - OD of test) / (OD of control)] ×100

2.6. Preparation of Cell line and Cell viability assay:

Human liver hepatocellular carcinoma (HepG2) cell lines were obtained from the National Centre for Cell Science (NCCS) in Pune, India. These cells were regularly cultured in DMEM supplemented with 10% FBS in a humidified atmosphere with 5% CO_2 at 37°C until they reached confluence. To dissociate the cells, trypsin phosphate versene glucose solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS) was utilized. The stock cultures were kept in 25 cm^2 culture flasks, and all experiments were conducted in 96 microtiter plates. The cells were seeded at a density of 1×10^5 cells/mL, and the culture medium was refreshed twice a week. The cell viability was assessed prior to centrifugation. Subsequently, 50,000 cells/well were seeded in a 96-well plate and incubated for 24 hours at 37°C in a 5% CO_2 incubator. The IC_{50} values for cytotoxicity tests were determined through nonlinear regression analysis (curve fit) based on the sigmoid dose–response curve (variable) and calculated using GraphPad Prism 6 [16].

2.7. Hepatoprotective activity of plant extracts:

The cells were seeded at a density of 1.0×10^5 cells/mL in 96-well flat-bottomed plates and incubated at 37°C in a humidified incubator with 5% CO_2. After 24 hours, when a partial

monolayer had formed, the supernatant was aspirated, and the monolayer was washed once. The final volume in all treated and control wells was maintained at 200 µl. Galactosamine (0.4%), silymarin (100 µg/ml), and varying concentrations (25 µg/mL, 12.5 µg/mL) of plant extracts were added to the specified wells. Silymarin served as a control. Microscopic examination was performed after 60 minutes of Galactosamine intoxication. Cytotoxicity was evaluated by determining the percentage viability of HepG2 cells using the MTT reduction assay. Absorbance was measured with an enzyme-linked immunosorbent assay reader at 540 and 630 nm [17]. Wells containing only medium served as a blank, while untreated wells were utilized as a control in the assay. Further, for protective potential of compounds on GSH level and intracellular ROS generation against Galactosamine, cells were pre-exposed to the plant extract for 24 h then, Galactosamine (0.1%) for 24 h.

2.7.1Determination of glutathione content

Glutathione (GSH) functions as an antioxidant tripeptide in hepatocytes, and its concentrations may decline in the presence of reactive oxygen species (ROS). In a nutshell, cell lysates in cold KCl 1.15% were homogenized with Tris-HCl 25 mM pH 7.4 (2:1, v/v) and then incubated for 60 minutes at 37°C. Subsequently, the mixture was combined with TCA 10% (1:1, v/v) and subjected to centrifugation. A 100 µl aliquot of the supernatant was mixed with 180 µl of EDTA-phosphate buffer and 20 µl of 5,5'-dithio-bis 2-nitrobenzoic acid 5 mM (Ellman reagent). GSH levels were determined spectrophotometrically at 412 nm, inferred from a calibration curve, and adjusted for the protein level of cell lysates [18].

2.7.2.Measurement of malondialdehyde (MDA) content and SOD activity

In 6-well plates, a total of 1x10*6 cells were plated per well and allowed to adhere for 16 hours. Subsequently, the cells were exposed to the test samples for 30 minutes before being subjected to 0.4% Galactosamine for 6 hours. After the treatment, the cells underwent two washes with PBS, were suspended in 0.4 mL of PBS, and then sonicated for 20 seconds. The resulting homogenate was centrifuged at 6000 x g for 10 minutes, and the supernatant was collected for subsequent experiments. The determination of MDA content was conducted using the thiobarbituric acid reactive substances (TBARS) assay. Additionally, homogenate superoxide dismutase (SOD) activity was evaluated using a commercial colorimetric SOD assay kit from Cayman Chemical Company [19].

Table – 1: Phytochemical analysis of *Naregamia alata*

Sample code	Alkaloids	Flavonoids	Saponins	Tannins	Phenols	Cardiac glycosides	Steroids	Terpenoids	Quinones	Proteins
NAH (Hexane)	-	+	-	+	-	+	+	+	+	-
NAEA (Ethylacetate)	-	+	-	+	-	+	+	+	+	-
NAC (chloroform)	-	+	-	+	-	+	+	+	+	-
NAE (Ethanol)	+	+	-	+	+	+	+	+	+	-
NAW (water)	-	+	-	+	+	+	+	+	+	+

3. RESULTS & DISCUSSION

The powdered plant sample of *Naregamia alata* weighing 500 grams, underwent extraction using various solvent.

system. Hexane, Ethylacetate, chloroform, ethanol and water. This method has demonstrated efficiency in extracting active compounds from plant species, particularly the flavonoids, polyphenol, alkaloids and steroids. The serial exhaustive extraction system, ranging from nonpolar to polar solvents, produced five extracts with yield values ranging from Hexane (NAH), Ethylacetate (NAEA), chloroform (NAC), ethanol (NAE) and water (NAW), 7.5g, 3.02g, 0.63g,11.25g and 13.25g respectively. These yields varied depending on the extraction solvent used.

3.1. Phytochemical Analysis

The phytochemical screening of the two plant samples revealed the presence of alkaloids, tannins, terpenoids, flavonoids, and phenols. Phytochemicals that tested positive in the analysis are known for their potential medicinal activities on human health. The presence of flavonoids and terpenoids, both previously reported for their anti-microbial, anti-mutagenic, anti-inflammatory, and anti-allergic properties in various studies, is noteworthy. Therefore, the identification of these beneficial phytochemicals in these plants holds promise for potential health benefits (Table -1).

3.2. GC-MS analysis:

Analysis by GC-MS revealed a chromatogram of the plant extract, as presented in Fig. 3. Table-2 provides details on the functional groups, molecular formula, molecular weight, retention time of the respective compounds. The GC-MS analysis identified 15 major compounds. Major compounds were identified as 1-Hexacosanol, 11- tricosene, N-Heptadecanol-1, Heptadecanoic acid, Heptadecyl ester, 17-pentatriacontene,2-isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate.

Table – 2: GCMS analysis of *Naregamia alata* extract

S.NO	Name of the compound	RT	Molecular weight	Molecular formula
1.	1- HEXACOSANOL	18.49	382	C26H54O
2.	N-TETRACOSANOL-1	19.71	354	C24H50O
3.	11- TRICOSENE	19.72	322	C23H46
4.	1- DOCOSENE	20.96	308	C22H44
5.	2- HEPTADECENAL	20.97	252	C17H32O
6.	N- HEPTADECANOL-1	22.16	256	C17H36O
7.	2- HEPTADECENAL	22.78	252	C17H32O
8.	17- PENTATRIACONTENE	23.28	490	C35H70
9.	CYCLOOCTACOSANE	24.41	392	C28H56
10.	OCTADECANE,1-(ETHENYLOXY)-	25.48	296	C20H40O
11.	SULFUROUS ACID, OCTADECYL 2-PROPYL ESTER	26.49	376	C21H44O3S
12.	HEPTADECANOIC ACID, HEPTADECYL ESTER	26.95	508	C34H68O2
13.	OCTADECANE,1-CHLORO-	27.55	288	C18H37Cl
14.	2-ISOPROPYL-5-METHYLCYCLOHEXYL 3-(1-(4-CHLOROPHENYL)-3-OXOBUTYL)-COUMARIN-4-YL CARBONATE	28.16	524	C30H33O6Cl
15.	HEXADECANE, 1-CHLORO-	28.83	260	C16H33Cl

3.3. Antioxidant Activity Using DPPH Assay:

DPPH is a stable free radical characterized by absorption at 517 nm, and its absorption decreases significantly when exposed to proton radical scavengers. Due to its stability and simplicity, it has been widely used to evaluate antioxidative ability [20]. The assay was conducted in methanol, and the results are expressed as IC_{50}, representing the concentration of a sample scavenging 50% of the DPPH free radicals in a given experimental situation. The IC_{50} value of the *Naregamia alata* extracts (NAH, NAEA, NAC, NAE, NAW) and reference standard ascorbic acid were found to >320 µg/ml, 66.28 µg/ml, >320 µg/ml, 137.42 µg/ml, 178.97 µg/ml and 8.88 µg/ml respectively (Fig-1). Furthermore, the results showed that the DPPH free radical scavenging activity of Ethylacetate, ethanol and water extract was relatively more pronounced than that of hexane and chloroform extract but weaker than that of L-ascorbic acid. The antioxidative property of these extracts may be attributed to the number of flavonoid and phenolic hydroxyl groups.

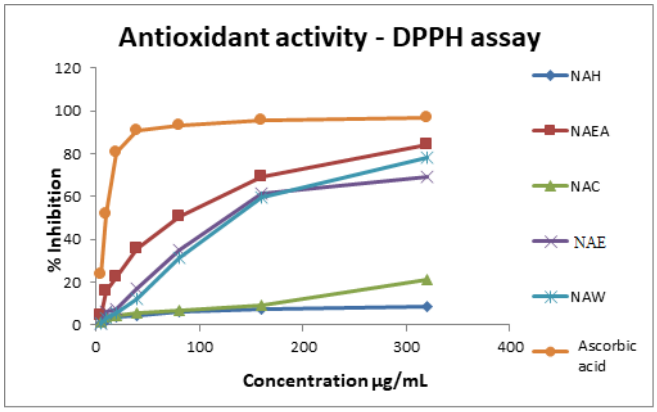


Fig – 1: DPPH radical scavenging assay of *Naregamia alata* extracts

3.4. Antioxidant Activity Using ABTS Assay:

The outcomes illustrating the scavenging activity against free radicals of *Naregamia alata* extracts are presented in Fig - 2. The order of descending ABTS scavenging activity for various extracts was as follows: (NAH, NAEA, NAC, NAE, NAW) and the standard drug (Ascorbic acid) were found to be >320µg/mL, 44.05 µg/mL, >320µg/mL, 163.99 µg/mL, 149.82 µg/mL and 13.07 µg/mL respectively The IC50 value of the ethyl acetate extract was near to that of ascorbic acid and was lower than that of other extracts, indicating robust antioxidant activity in the ethyl acetate extract [21].

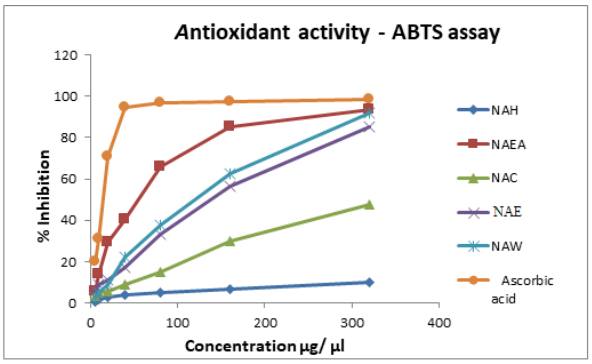


Fig – 2: ABTS radical scavenging assay of *Naregamia alata* extracts

3.5. MTT Assay for HepG2 Cell Line:

The cytotoxicity of compounds was assessed through the MTT assay and examination of morphological changes. The study shows that the *Naregamia alata* extracts did not affect the cell viability of HepG2 cells (Fig - 3) within the 25µg/ml concentrations. In contrast, the plant extract exhibited cytotoxic effects at the higher concentrations of 100 µg/ml (data not shown). So the concentrations were chosen for the cytoprotective activity was 1.25 µg/ml to 12.5 µg/ml with the 60% viability of the cells.

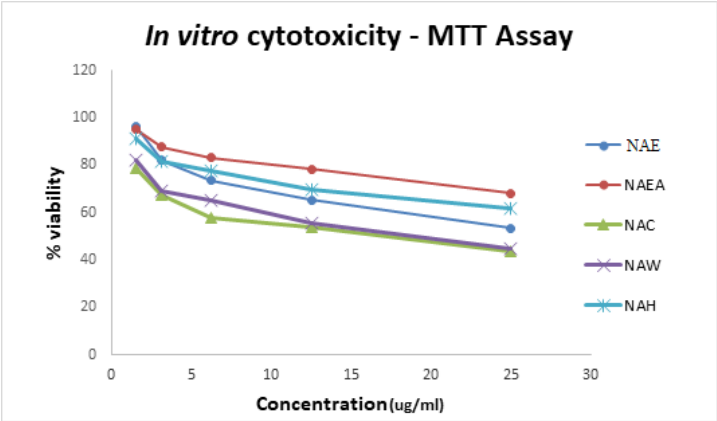
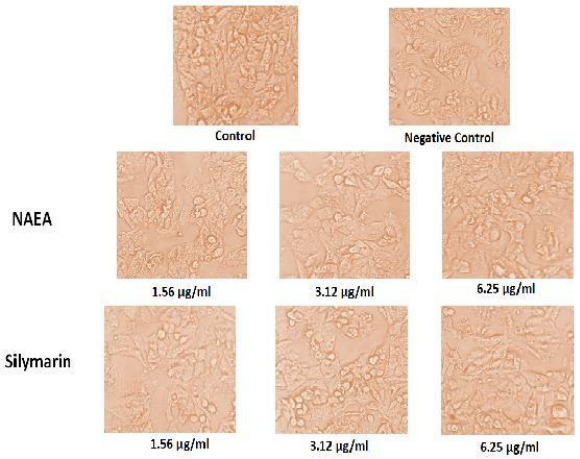


Fig – 3: *In vitro* cytotoxicity of *Naregamia alata* extracts

3.6. Hepatoprotective activity of *Naregamia alata* of Galactosamine-induced HepG2 cell line:

The HepG2 cell line is considered a suitable model for *in vitro* liver toxicity studies due to its retention of many specialized liver functions [22]. In this study, we initially examined the response of HepG2 cells to varying doses of Galactosamine using the MTT assay. The cell viability ratio of the control group was set at 100%. The results revealed that Galactosamine ranging from 0.1 to 1% induced cell death in a dose-dependent manner, with 0.4% Galactosamine mildly affecting cell viability (data not shown).

Subsequent studies involved cell incubation with 0.4% Galactosamine for 6 hours to induce 40–50% cell death. There was no apparent cytotoxic or inhibitory effect on the growth of HepG2 cells with plant extracts based on MTT assay. As shown in Fig. 4 treatment with 0.4% Galactosamine alone resulted in cell death, indicating the sensitivity of HepG2 cells to Galactosamine. However, pre-treatment with plant extract cells from Galactosamine-induced damage, restoring cell survival. At 6.25µg/ml the ethanol extract exhibited a hepatoprotective effect, while the other extracts showed weaker efficacy even at the higher concentrations. Additionally, the hepatoprotective potency of the Ethylacetate extract at 6.25µg/ml was comparable to that of standard drug silymarin (Fig. 5).



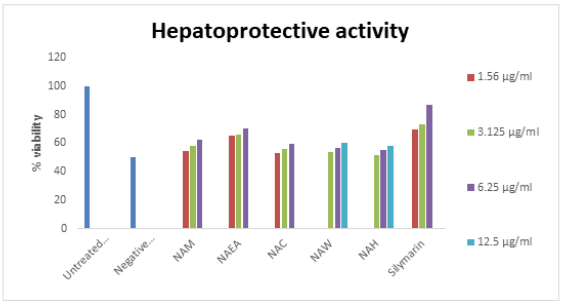


Fig – 4: *In vitro* Hepatoprotective activity of *Naregamia alata* extracts

3.7. Effect of *Naregamia alata* extracts on GSH level:

Glutathione plays a vital role in counteracting oxidative species. Exogenous exposure to Galactosamine is known to elevate intracellular reactive oxygen species generation, leading to cellular oxidative damage in hepatic cells. This damage can be countered by hepatocyte antioxidant defense mechanisms. GSH, a crucial non-enzymatic antioxidant, plays a vital role in the cellular defense system against oxidative stress. Glutathione peroxidase catalyzes the oxidation of GSH to GSSG in the presence of Galactosamine, and glutathione reductase recycles oxidized GSH back to reduced GSH [23]. Following a 24-hour treatment period, plant extract and silymarin exhibited a significant increase in GSH content compared to the normal control. In the negative control, Galactosamine reduced GSH content by 52.25%. The presence of ethyl acetate extract established a preventive effect against oxidative stress by enhancing GSH content by 73.25% in Galactosamine-injured HepG2 cells. Notably, silymarin at 6.25ug/ml significantly

increased the GSH amount by up to 82.19% compared to the negative control (Fig. 6). Therefore, the pre-exposure of HepG2 cells to the plant extract of *Naregamia alata* effectively elevates glutathione levels, enhances antioxidant status, and protects cells against Galactosamine-induced damage.

3.8. Effect of *Naregamia alata* extracts on MDA level:

The toxicity induced by Galactosamine in the HepG2 cell line can be attributed to either a direct solvent effect or the generation of free radicals, leading to the initiation of lipid peroxidation. Lipid peroxidation, a process involving the oxidative alteration of polyunsaturated fatty acids in cell membranes, gives rise to various free radicals. The lipid radical is rapidly quenched by molecular oxygen, forming a peroxyl-fatty acid radical that can generate end products such as MDA and unsaturated aldehydes [24]. To understand the consequences of Galactosamine-induced oxidative damage to cellular macromolecules and explore the protective effects of *Naregamia alata* the formation of MDA was assessed. As depicted in Fig.7, the level of MDA significantly increased to 198.25% in the negative control upon treatment with Galactosamine. In contrast, pre-treatments with Ethylacetate extracts and silymarin inhibited Galactosamine-induced lipid peroxidation in HepG2 cells to 138.37 and 123.48% respectively. These findings suggest that *Naregamia alata* have the potential to suppress intracellular MDA formation induced by Galactosamine, with Ethylacetate extract exhibiting a stronger cytoprotective effect.

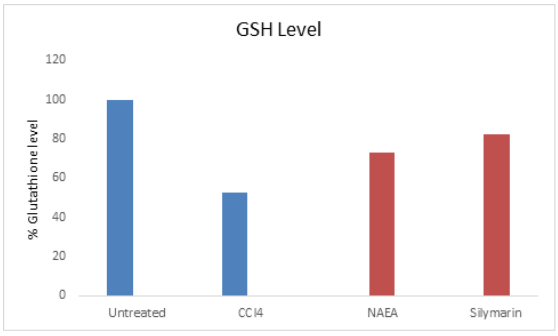


Fig – 6: Hepatoprotective activity of *Naregamia alata* extracts on GSH level induced by Galactosamine in HepG2 cell line

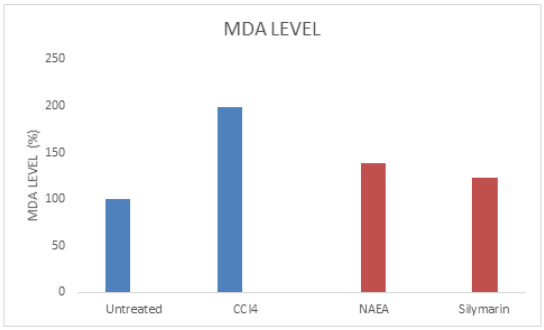


Fig – 7: Hepatoprotective activity of *Naregamia alata* extracts on MDA level induced by Galactosamine in HepG2 cell line

3.9. Effect of *Naregamia alata* extracts on SOD activity

The efficacy of *Naregamia alata* against Galactosamine-induced hepatotoxicity in HepG2 cells was assessed by investigating their impact on antioxidative enzymes. The study focused on SOD which is crucial components of the cellular defense against oxidative stress

[25]. SOD, in particular, plays a pivotal role in catalyzing the dismutation of superoxide radicals into oxidative stress, a vital process in mitigating oxidative stress.

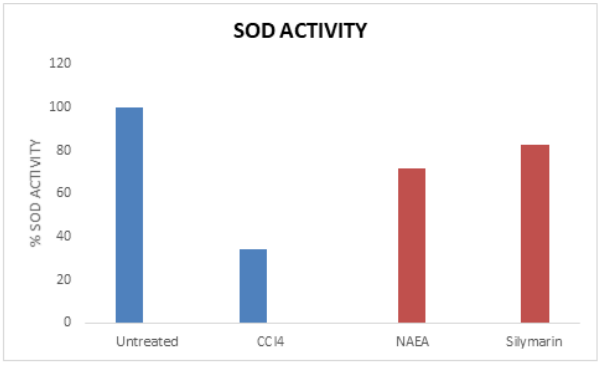


Fig – 8: Hepatoprotective activity of *Naregamia alata* extracts on SOD activity induced by Galactosamine in HepG2 cell line

The hepatoprotective effects of plant extracts may involve the modulation of these endogenous antioxidant enzymes. The results, as depicted in Fig. 8, reveal that exposure of HepG2 cells to Galactosamine significantly decreased SOD activities to 34.26% in the negative control group. However, pre-treatment with Ethylacetate extract and silymarin significantly alleviated this decrease in SOD activities to 71.85 and 82.64 respectively, compared to the untreated group.

4. CONCLUSION

In conclusion, the findings demonstrated the hepatoprotective effects of *Naregamia alata* extracts and notably, the highest effects were observed in Ethylacetate extract treated HepG2 cells injured by Galactosamine. This protective impact could be linked to their antioxidant activity. The results provide motivation for additional investigations into evaluating the liver protective properties of *Naregamia alata* using animal models and clinical trials.

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Author contribution

Rajamathanky Hariharan: Conceptualization, Investigation, Methodology, Resources, Software and Writing - original draft.

Rajasekaran Aiyalu: Conceptualization, Formal analysis, Project administration, Resources, Supervision, Validation, Visualization and Writing - review & editing.

Funding

none to declare

Conflicts of Interests

The authors declared there are no conflicts of interest.

Ethics approval

none to declare

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