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Phytochemical analysis, antipyretic and subacute toxicity study of ethanolic, hydroethanolic, aqueous and chloroform extracts of *Nyctanthes arbor-tristis* L. leaves in mice

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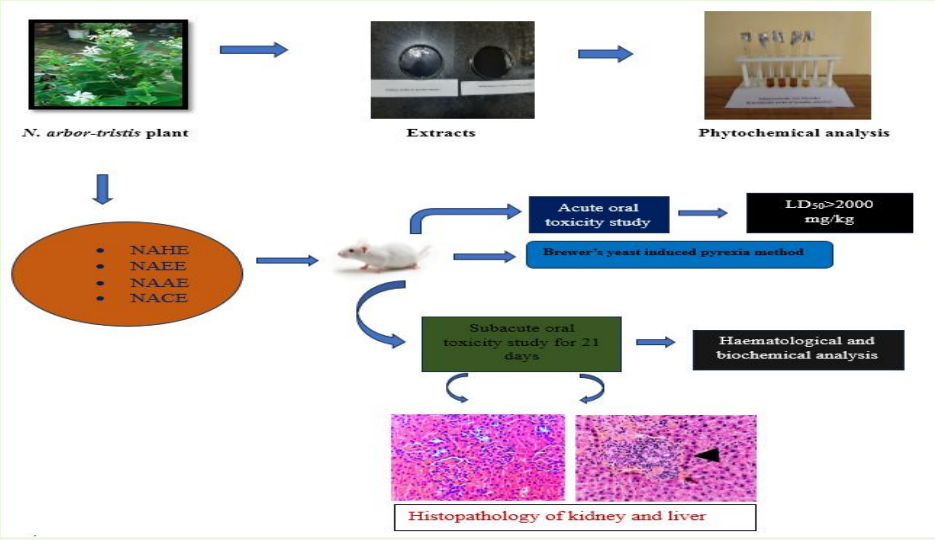
Abstract

Nyctanthes arbor-tristis L. typically recognized as Harsinghar, night time jasmine or Parijat. The present study was an attempt to evaluate phytochemical analysis, antipyretic and subacute toxicity study of *N. arbor-tristis* leaf extract. Paracetamol became used as well known drug for assessment of antipyretic activity. The antipyretic activity of the ethanolic, hydroethanolic, aqueous and chloroform leaf extracts of *N. arbor-tristis* evaluated using brewer's yeast precipitated pyrexia technique. Subacute toxicity examine turned into accomplished as according to Organization for Economic Co-operation and Development guidelines 407. In subacute toxicity study, the examined plant extracts turned into administered orally at doses of 500 mg/kg for 21 days to the four animals groups and their hematological, biochemical and histopathological parameters were evaluated and compared to normal group by sacrificing all group animals. Ethanolic, hydroethanlic, aqueous and chloroform extracts of *N. arbor-tristis* leaf and standard drug paracetamol confirmed tremendous ($p<0.05$) antipyretic activity from 1 h up to 5 h of remark period which became maximum at 5 h put up management of check extracts, which was similar to the usual drug, paracetamol. The present subacute toxicity study found out no such good sized changes within the hemato-biochemical parameters which include haemoglobin, total erythrocytic count, total leucocytic count, packed cell extent, alanine aminotransferase, aspertate amino transferase, total protein and creatinine. Even though, there had been some insignificant modifications on 21st day in haemoglobin content, total leucocytic count, alanine aminotransferase and creatinine ranges. Histopathology research revealed slight degenerative changes in liver and kidney of treated group following administration of tested plant extracts 500 mg/kg.



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1. Introduction

Pyrexia, commonly known as fever, is defined as a rise in body temperature beyond the normal physiological range. It may be caused by infection, tissue injury, inflammation, transplant rejection, or malignancy. These conditions trigger the production of pro-

inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), interferons, and tumour necrosis factor-α (TNF-α). Cytokines enhance the synthesis and binding of prostaglandin E2 (PGE2) within the hypothalamus. PGE2 raises body temperature by increasing cAMP levels, which signals the hypothalamus to boost heat production and minimize heat loss. Body temperature regulation relies on a precise equilibrium between heat production and dissipation, with the hypothalamus governing the thermoregulatory set point that maintains body temperature. During a fever, the body's set point rises, and nonsteroidal anti-inflammatory drugs (NSAIDs) work by lowering it back to normal. However, NSAIDs do not reduce fever caused by non-pyrogenic factors like physical exertion or environmental heat. Medicinal plants have been utilized since antiquity to treat a wide range of ailments. According to the World Health Organization (WHO), more than 80% of the global population depends on medicinal plants for their primary healthcare needs. These plants harbour bioactive molecules that are responsible for their therapeutic benefits. Since ancient times, various parts of plants have been used to ease pain, reduce suffering, and fight diseases. Indeed, most traditional medicines were derived from plants, which remain the primary source of many modern drugs.

Figure 1: ***N. arbortristis*** **plant.**

Different plant parts, such as leaves, flowers, bark, seeds, and roots, can yield a variety of bioactive compounds. The identification of phytochemical constituents has facilitated the creation of new therapeutic agents for treating various conditions, including cancer, alzheimer's disease, and parkinson's disease. Furthermore, plants rich in phytochemicals display diverse biological activities, such as antibacterial, antioxidant, antiviral, antimicrobial, anthelmintic, and antifungal properties. To ensure the proper standardization and quality control of herbal products and formulations, advanced methods are currently used to identify and quantify phytochemical constituents. Bioactive compounds found in plant-based foods offer significant health benefits by helping to prevent and manage various ailments. India, renowned for its rich biodiversity and plentiful medicinal plants, has built up an extensive collection of traditional medicinal knowledge over many generations. Many of these plants remain widely used today. *N. arbor-tristis* is one such medicinal plant belonging to the Oleaceae family. Also known as Harsinghar, Night Jasmine, or Parijat, this shrub or small tree can reach heights of up to 10 meters and is distinguished by its rough bark, which ranges in colour from gray to greenish. This plant grows wild in the sub-Himalayan regions and extends southward to the Godavari area. It is also grown in Indian gardens for its highly fragrant flowers, which serve ornamental purposes. The leaves of *N. arbor-tristis* are widely utilized in Ayurvedic medicine to treat a range of conditions, such as sciatica, chronic fever, rheumatism, and intestinal worm infestations. They are also employed as a laxative, diaphoretic, and diuretic. Furthermore, the leaves are used to treat coughs by administering leaf juice mixed with honey three times a day. Traditionally, a paste made from leaves and honey is used to manage fever, hypertension, and diabetes. The leaves are also employed to treat splenomegaly. Furthermore, *N. arbor-tristis* leaves have been reported to display central nervous system activities, including hypnotic, tranquilizing, and local anaesthetic effects. While traditional healers have long employed *N. arbor-tristis* for diverse therapeutic applications, scientific literature remains lacking in evidence concerning its antipyretic effects and subacute toxicity. Consequently, a systematic scientific study was deemed essential to assess the plant's antipyretic efficacy and subacute toxicity, thus confirming its traditional applications. This study was conducted to evaluate the acute and subacute oral toxicity, along with antipyretic activity of *N.arbor-tristis* leaf extracts in mice.

2. Materials and Methods

2.1 Plant material

Fresh leaves of *N. arbor-tristis* were collected in and around Khanapara campus during the months of July to September for pharmacological experimental purposes. Identification and authentication of plant material was done by Botanical Survey of India (BSI), Eastern Regional Centre, Shillong. The Herbarium Identification Number is BSI/ERC/Tech/plant identification/2012/494. All experimental procedures involving animals were conducted in accordance with the guidelines set by the Committee for the Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC), College of Veterinary Sciences, Assam Agricultural University, Khanapara under approval number 770/GO/Re/S/03/CPCSEA/FVSc/AAU/IAEC/20-21/863 dated 31 July 2021.

2.2 Processing of plant materials

After identification and characterization with the aid of BSI (Botanical Survey of India), leaves were in addition accumulated. The collected leaves were lightly washed with clean water to cast off soil and dirt debris. The leaves were then dried in coloration at room temperature for approximately 7-10 days. To prevent fermentation and microbial spoilage they had been became often. The dried leaves had been then ground or floor into powder by way of laboratory Wiley Mill and saved at room temperature in air tight packing containers after proper labelling till the extract turned into prepared.

2.3 Preparation of ethanolic, hydroethanolic, aqueous and chloroform extracts

The powdered plant material became extracted one after the other use of ethanol, hydroethanol (1:1), distilled water and chloroform following the process defined by means of Prasad (1965). Finely

powdered leaf material was soaked individually in each solvent for 72 h, and this process was repeated three times with intermittent agitation. The resulting combinations had been double-filtered using muslin fabric accompanied via whatman number 1 filter out paper. The filtrates were then concentrated below reduced pressure the usage of a rotary evaporator and finally dried completely on a regulated water bathtub maintained at 50°C. The dried extracts have been stored at 4°C until in addition use for experimental screening. The extraction procedure become finished the usage of standard method with minor modifications.

2.4 Phytochemical analysis of extracts

The ethanolic, hydroethanolic, aqueous, and chloroform extracts had been subjected to qualitative phytochemical analysis to detect the presence of various bioactive materials, including steroids, alkaloids, phenolic compounds, tannins, flavonoids, glycosides, triterpenes, and saponins, following the same old techniques defined through Harborne (1991).

2.5 Experimental animals

Wistar albino mice weighing 30-40 g have been procured from the branch of Veterinary Pharmacology and Toxicology, Assam Agricultural university, Khanapara. The animals have been housed in polypropylene cages and divided into agencies of 6 mice every. Paddy husk changed into used as bedding fabric and was replaced frequently at weekly periods. All animals had been provided with a balanced weight loss plan and smooth drinking water ad libitum and had been maintained underneath well known laboratory situations with a 12:12 h light-dark cycle at an ambient temperature of 22-27°C.

2.6 Acute toxicity study

The acute oral toxicity study carried out in accordance with the OECD guidelines 425 (Organization for Economic Co-operation and Development). Nulliparous and non-pregnant female wistar albino mice weighing 30-40 g have been randomly selected for the examine. The ethanolic, hydroethanolic, aqueous, and chloroform leaf extracts of *N. arbor-tristis* were administered orally to the mice. A limit test was done previous to administration of the test extracts, the animals were fasted overnight with loose get admission to water. Group I served as the vehicle control and received 20% Tween-80, while Groups II-V received ethanolic, hydroethanolic, aqueous, and chloroform leaf extracts of *N. arbor-tristis* at a single oral dose of 2000 mg/kg body weight. The animals had been intently located for behavioural modifications, symptoms of toxicity, and mortality in the course of the primary 72 h after dosing and have been further monitored for a complete length of 14 days to file any behind schedule mortality.

2.7 Evaluation of antipyretic activity

2.7.1 Brewer's yeast induced pyrexia method

The antipyretic activity was evaluated the usage of the technique defined by Loux *et al*. (1972). Prior to brewer's yeast administration, the ordinary body temperature of each mouse became measured rectally using a lubricated scientific thermometer inserted about 4 cm into the rectum for 45 sec. Fever turned into brought on with the aid of subcutaneous injection of a 20% aqueous suspension of brewer's yeast in normal saline underneath the nape of the neck. The animals had been then returned to their respective cages. After 18 h of brewer's yeast injection, mice exhibiting an increase in rectal temperature of 0.5-1.5°C had been decided on and randomly divided into 14 groups for the observe. Pyretic animals received oral management of ethanolic, hydroethanolic, aqueous, and chloroform leaf extracts of *N. arbor-tristis* at doses of 250, 500, and 1000 mg/kg body weight. The standard drug, acetaminophen (paracetamol; French), was administered orally at a dose of 150 mg/kg. The control group received 20% Tween -80. Rectal temperature become recorded at once before and 18 h after brewer's yeast injection (0 h) to affirm the induction of pyrexia. Subsequently, rectal temperature measurements had been taken at 1, 2, 3, 4, 5, and 6 h after oral management of the test extracts or standard drug. The mean rectal temperatures of the treated groups were compared with those of the control group to evaluate the antipyretic efficacy of the extracts.

2.8 Evaluation of subacute toxicity study

The subacute toxicity study of *N. arbor-tristis* leaf extracts turned into carried out in accordance with OECD guiding principle 407, with moderate adjustments. Primarily based at the effects of the intense toxicity have a look at, a dose of 500 mg/kg body weight of the ethanolic, hydroethanolic, aqueous, and chloroform leaf extracts became decided on. Those extracts were administered orally once day by day for 21 consecutive days to four separate group of mice (n = 6). One additional group served as the control and received only the vehicle (Tween-80) for the same duration. All experimental animals were discovered daily for the duration of the 21-day treatment period for the incidence of ordinary scientific signs and symptoms and mortality. Blood samples were gathered from every mouse via the tail vein on days 0, 14, and 21 publish-remedy. Blood was collected into EDTA-coated vials for hematological analysis. For serum guidance, blood samples were centrifuged at 3500 rpm for 15 min, and the separated serum changed into used for the estimation of biochemical parameters, including alanine transaminase (ALT), aspartate transaminase (AST), total protein, and creatinine. Entire blood samples were used for hematological analysis, including hemoglobin (Hb) concentration, total erythrocyte count (TEC), total leukocyte count (TLC), and packed cell volume (PCV).

2.9 Biochemical analysis

The separated serum samples were analysed the usage of a UV-seen spectrophotometer for the estimation of alanine amino-transferase (ALT), aspartate aminotransferase (AST), total protein (TP), and creatinine.

2.10 Haematological assay

Haematological parameter such as haemoglobin, red blood cell, white blood cell, packed cell volume were analysed by manual method using standard light microscope.

2.11 Statistical analysis

Results were expressed as Mean ± SEM. Statistical analysis was performed by MS-excel to calculate mean, standard error of mean (SEM), analysis of variance (ANOVA), and co-efficient of correlation (r) values as per standard method Snedecor and Cochran (2004).

3. Results

Various phytochemical tests were done using ethanolic, hydroethanolic, aqueous and chloroform extracts of *N. arbor-tristis* leaves. They were subjected to phytochemical analysis for the presence of various active principles or phytochemical constituents namely steroids, alkaloids, phenolic compounds, tannins, flavonoids, glycosides, triterpenes, diterpenes and saponins.

3.1 Phytochemical analysis of *N. arbor-tristis* extracts

Ethanolic extract of *N. arbor-tristis* leaves showed presence of steroids, alkaloids, phenolic compounds, glycosides, flavonoids, diterpenes, triterpenes and absence of tannins and saponins. The hydroethanolic extract of *N. arbor-tristis* leaves showed presence of steroids, alkaloids, phenolic compounds, glycosides, flavonoids, tannin, diterpenes, triterpenes and saponins.The aqueous extract of *N. arbor-tristis* leaves showed presence of steroids, alkaloids, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins and absence of tannins.

3.2 Acute toxicity study

The acute toxic effect of ethanolic, hydroethanolic, aqueous and chloroform extracts of *N. arbor-tristis* was determined according to the OECD guidelines 425 (Organization for Economic Co-operation and Development). The general behavioural of the extracts treated animals and control group was observed first for short period (4 h) followed by long period (72 h), did not display any drug related changes in behaviour, breathing, skin effects, water consumption, impairment in food intake and temperature. Up to 14 days of observation period ethanolic, hydroethanolic, aqueous and chloroform extracts of *N. arbor-tristis* leaves at a dose rate of 500 mg/kg did not show any treatment related toxic symptom or mortality. Therefore, the extracts were considered to be safe up to a maximum dose of 2000 mg/kg, and the LD_{50} was considered be >2000 mg/kg..

3.3 Antipyretic activity

In phytochemical analysis ethanolic extract of *N. arbor-tristis* leaves showed presence of steroids, alkaloids, phenolic compounds, glycosides, flavonoids, diterpenes and triterpenes. The hydroethanolic extract of *N. arbor-tristis* leaves showed presence of steroids, alkaloids, phenolic compounds, glycosides, flavonoids, tannins, diterpenes, triterpenes and saponins. The aqueous extract of *N.arbor-tristis* leaves showed presence of steroids, alkaloids, phenolic compounds, flavonoids, glycosides, diterpenes and triterpenes. The antipyretic activity of the ethanolic, hydroethanolic, aqueous and chloroform leaf extracts of *N. arbor-tristis* was evaluated using brewer's yeast induced pyrexia method. The antipyretic activity compared with 1 h to 5 h is displayed in Tables 1, 2,3 and 4. Leaf extracts of NAEE, NAHE, NAAE, NACE and standard drug paracetamol showed significant (p<0.05) antipyretic activity from 1h up to 5 h of observation period which was maximum at 5 h post administration of test extracts. NAEE at 250 and 1000 mg/kg body weight showed non-significant antipyretic activity at 5 h *i.e.,* 36.80 ± 0.21 and 36.72 ± 0.17 whereas at 500 mg/kg body weight it showed 37.18 ± 0.14 which is non-significant with the 250 mg/kg body weight but significantly (p<0.05) different from 1000 mg/kg bodyweight. NAHE at 250, 500 and 1000 mg/kg body weight showed non-significant antipyretic activity at 5 h, *i.e.*, 36.28 ± 0.08, 36.35 ± 0.13 and 36.47 ± 0.16. NAAE and NACE also showed non-significant antipyretic activity at 250, 500 and 1000 mg/kg body weight and at 5 h *i.e.*, 36.35 ± 0.13, 36.70 ± 0.12, 36.32 ± 0.27 and 36.68 ± 0.10, 36.13 ± 0.12, 36.12 ± 0.08.

Table 1: Antipyretic activity of ethanolic leaf extract of *N. arbor-tristis* by brewer's yeast induced pyrexia method

Group	Time (h)						
	18	0	1	2	3	4	5
Control	36.35 ± 0.11	36.85 ± 0.08	37.27 ± 0.12	37.52 ± 0.08	37.87 ± 0.06	38.23 ± 0.11	37.27 ± 0.15
Standard (Paracetamol)	36.97 ± 0.27	37.70 ± 0.19	37.47 ± 0.18	37.28 ± 0.21	36.97 ± 0.20	36.72 ± 0.19	36.35 ± 0.10
NAEE (250 mg/kg)	37.05 ± 0.20	37.90 ± 0.08	37.72 ± 0.09	37.47 ± 0.10	37.15 ± 0.11	37.12 ± 0.33	36.80 ± 0.21
NAEE (500 mg/kg)	38.92 ± 0.14	38.67 ± 0.17	38.32 ± 0.14	38.13 ± 0.11	37.82 ± 0.14	37.38 ± 0.15	37.18 ± 0.14
NAEE (1000 mg/kg)	38.78 ± 0.14	38.63 ± 0.17	38.22 ± 0.19	37.67 ± 0.13	37.40 ± 0.17	37.15 ± 0.11	36.72 ± 0.17

Table 2: Antipyretic activity of hydroethanolic leaf extract of *N. arbor-tristis* by brewer's yeast induced pyrexia method

Group	Time (h)						
	18	0	1	2	3	4	5
Control	36.35 ± 0.11	36.85 ± 0.08	37.27 ± 0.12	37.52 ± 0.08	37.87 ± 0.06	38.23 ± 0.11	37.27 ± 0.15
Standard (Paracetamol)	36.97 ± 0.27	37.70 ± 0.19	37.47 ± 0.18	37.28 ± 0.21	36.97 ± 0.20	36.72 ± 0.19	36.35 ± 0.10
NAHE (250 mg/kg)	37.52 ± 0.14	37.38 ± 0.16	37.02 ± 0.15	36.82 ± 0.14	36.68 ± 0.12	36.43 ± 0.11	36.28 ± 0.08
NAHE (500 mg/kg)	38.25 ± 0.25	38.28 ± 0.15	37.67 ± 0.19	37.37 ± 0.06	37.03 ± 0.11	36.62 ± 0.12	36.35 ± 0.13
NAHE (1000 mg/kg)	38.82 ± 0.26	38.58 ± 0.27	38.17 ± 0.20	37.80 ± 0.06	37.08 ± 0.15	36.90 ± 0.13	36.47 ± 0.16

Table 3: Antipyretic activity of aqueous leaf extract of *N. arbor-tristis* by brewer's yeast induced pyrexia method

Group	Time (h)						
	18	0	1	2	3	4	5
Control	36.35 ± 0.11	36.85 ± 0.08	37.27 ± 0.12	37.52 ± 0.08	37.87 ± 0.06	38.23 ± 0.11	37.27 ± 0.15
Standard (Paracetamol)	36.97 ± 0.27	37.70 ± 0.19	37.47 ± 0.18	37.28 ± 0.21	36.97 ± 0.20	36.72 ± 0.19	36.35 ± 0.10
NAAE (250 mg/kg)	38.72 ± 0.11	38.60 ± 0.13	38.25 ± 0.08	37.87 ± 0.12	37.30 ± 0.16	36.73 ± 0.16	36.35 ± 0.13
NAAE (500 mg/kg)	38.15 ± 0.27	38.12 ± 0.25	37.68 ± 0.19	37.42 ± 0.15	37.30 ± 0.09	36.93 ± 0.11	36.70 ± 0.12
NAAE (1000mg/kg)	38.48 ± 0.18	38.45 ± 0.16	38.23 ± 0.19	37.93 ± 0.12	37.68 ± 0.14	37.30 ± 0.12	36.32 ± 0.27

Table 4: Antipyretic activity of chloroform leaf extract of *N, arbor-tristis* by brewer's yeast induced pyrexia method

Group	Time (h)						
	18	0	1	2	3	4	5
Control	36.35 ± 0.11	36.85 ± 0.08	37.27 ± 0.12	37.52 ± 0.08	37.87 ± 0.06	38.23 ± 0.11	37.27 ± 0.15
Standard (Paracetamol)	36.97 ± 0.27	37.70 ± 0.19	37.47 ± 0.18	37.28 ± 0.21	36.97 ± 0.20	36.72 ± 0.19	36.35 ± 0.10
NACE (250 mg/kg)	39.03 ± 0.15	38.87 ± 0.15	38.62 ± 0.18	38.15 ± 0.15	37.73 ± 0.10	37.07 ± 0.16	36.68 ± 0.10
NACE (500 mg/kg)	38.23 ± 0.23	38.08 ± 0.26	37.52 ±0.13	37.28 ± 0.12	37.02 ± 0.14	36.58 ± 0.13	36.13 ± 0.12
NACE (1000 mg/kg	39.02 ± 0.16	38.92 ± 0.17	38.62 ± 0.21	38.42 ± 0.13	37.47 ± 0.11	36.58 ± 0.15	36.12 ± 0.08

3.4 Subacute toxicity Study

The subacute toxicity study of *N. arbor-tristis* leaf extracts was performed as per the OECD guidelines 407. All the tested group animals treated with plant extracts at a dose of 500 mg/kg daily survived throughout the 21 days.

Table 5: Effect of ethanolic, hydroethanolic, aqueous and chloroform leaf extracts of *N. arbor-tristis* on haemoglobin (g/dl)

Group	Haemoglobin (g/dl)		
	Post treatment (days)		
	0	14	21
Control	11.80 ± 0.81	11.96 ± 0.72	12.3 ± 0.71
NAEE (500 mg/kg)	11.58 ± 0.42	11.73 ± 0.39	6.18 ± 0.46
NAHE (500 mg/kg)	12.00 ± 0.58	12.67 ± 0.56	11.85 ± 0.43
NAAE (500 mg/kg)	12.30 ± 0.74	12.30 ± 0.62	11.00 ± 0.63
NACE (500 mg/kg)	12.75 ± 0.77	13.40 ± 0.75	10.67 ± 0.33

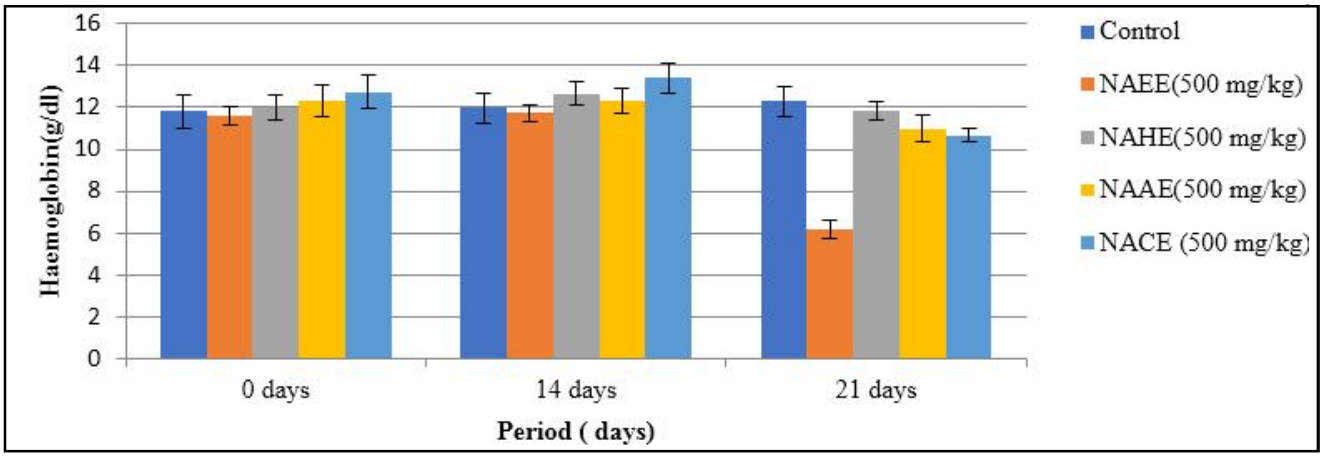

Figure 2: Graph representing effect of ethanolic, hydroethanolic, aqueous and chloroform leaf extract of *N. arbor-tristis* on haemoglobin (g/dl).

3.5 Effect of plant extracts on haematological parameter

3.5.1 Haemoglobin

Mean ± SE of haemoglobin of NAEE, NAHE, NAAE and NACE treated groups on 0,14th and 21st days are presented in the Table 5 and graphical presentation in Figure 2 respectively. The haemoglobin values showed no change in NAHE, NAAE and NACE treated group but NAEE treated group showed decrease in haemoglobin values on 21st days in comparison to NAHE, NAAE, NACE and control.

3.5.2 Total erythrocytic count

Mean ± SE of total erythrocytic count of NAEE, NAHE, NAAE and NACE treated groups on 0, 14th and 21st day are presented in the Table 6 and graphical presentation in Figure 3, respectively. The NACE treated group showed significant (p<0.05) difference on 14th and 21st day in comparison to control group.

3.5.2 Total leucocytic count

Mean ± SE of total leucocytic count of NAEE, NAHE, NAAE and NACE treated group on 0, 14th and 21st day are presented in the Table 7 and graphical presentation in Figure 4, respectively. In the present study, the total leucocytic count showed no change in value in NAEE, NAHE and NACE treated group, but in case of NAAE treated group it was found that there was significant (p<0.05) increase in total leucocytic count on 21 days as compared to all the other treatment groups.

Table 6: Effect of ethanolic, hydroethanolic, aqueous and chloroform leaf extracts of *N. arbor-tristis* on total erthrocytic count (10^6 cumm)

Group	Total erythrocytic count (10^6 cumm)		
	Post treatment (days)		
	0	14	21
Control	10.00 ± 0.52	10.16 ± 0.40	10.83 ± 0.60
NAEE (500 mg/kg)	10.50 ± 0.76	11.67 ± 0.88	11.60 ± 0.71
NAHE (500 mg/kg)	10.50 ± 0.43	10.50 ± 0.67	11.07 ± 0.25
NAAE (500 mg/kg)	11.00 ± 0.86	11.08 ± 0.99	12.55 ± 0.55
NACE (500 mg/kg)	11.83 ± 0.60	12.33 ± 0.61	12.72 ± 0.60

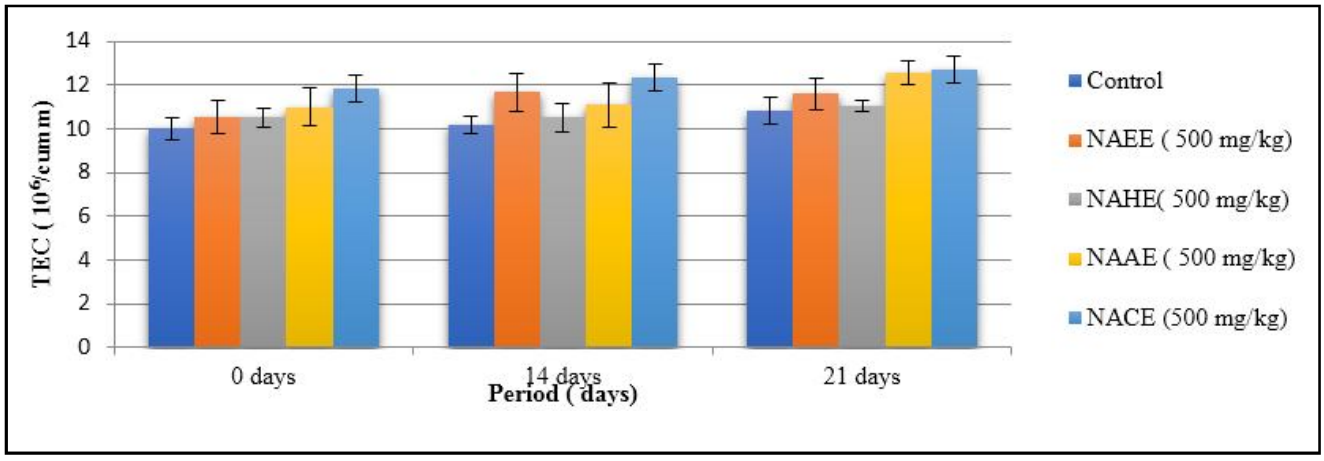

Figure 3: Graph representing effect of ethanolic, hydroethanolic, aqueous and chloroform leaf extract of *N. arbor-tristis* on total erythrocytic count(10^6/cumm).

Table 7: Effect of ethanolic, hydroethanolic, aqueous and chloroform leaf extracts of *N. arbor-tristis* on total leucocytic count (10^3cumm)

Group	Total leucocytic count (10^3cumm)		
	Post treatment (days)		
	0	14	21
Control	11.68 ± 0.71	11.68 ± 0.86	11.93 ± 0.65
NAEE (500 mg/kg)	11.85 ± 0.56	12.02 ± 0.44	11.98 ± 0.64
NAHE (500 mg/kg)	11.87 ± 0.76	11.88 ± 0.87	11.32 ± 0.50
NAAE (500 mg/kg)	11.83 ± 0.58	12.72 ± 0.66	14.38 ± 0.51
NACE (500 mg/kg)	12.17 ± 0.59	12.50 ± 0.78	13.38 ± 0.59

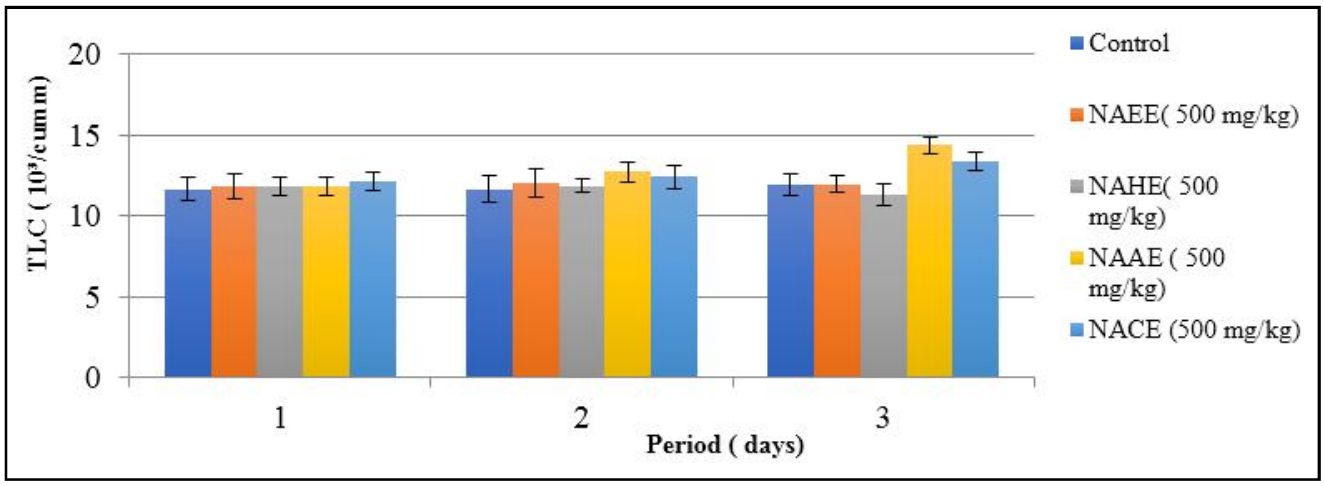

Figure 4: Graph representing effect of ethanolic, hydroethanolic, aqueous and chloroform leaf extract of *N. arbor-tristis* on total leucocytic count (10^3/cumm).

3.5.4 Packed cell volume (PCV)

Mean ± SE of packed cell volume of NAEE, NAHE, NAAE and NACE treated group on 0, 14th and 21st day are presented in the Table 8 and graphical presentation in Figure 5 respectively. NAHE, NAAE and NACE treated group showed no change in packed cell volume. NAEE treated group showed the significant (p<0.05) reduction in packed cell volume on 14th day, but this volume become normal on 21 days.

Table 8: Effect of ethanolic, hydroethanolic, aqueous and chloroform leaf extracts of *N. arbor-tristis* on packed cell volume

Group	Packed cell volume (PCV)(%)		
	Post treatment (days)		
	0	14	21
Control	42.33 ± 0.80	43 ± 1.03	42.66 ± 1.08
NAEE(500 mg/kg)	43.00 ± 0.63	39.67 ± 0.67	43.33 ± 0.49
NAHE (500 mg/kg)	42.83 ± 0.79	42.67 ± 0.71	42.83 ± 0.65
NAAE(500 mg/kg)	43.83 ± 0.95	40.17 ± 0.65	44.33 ± 0.99
NACE(500 mg/kg)	41.50 ± 0.67	41.50 ± 0.62	41.83 ± 0.65

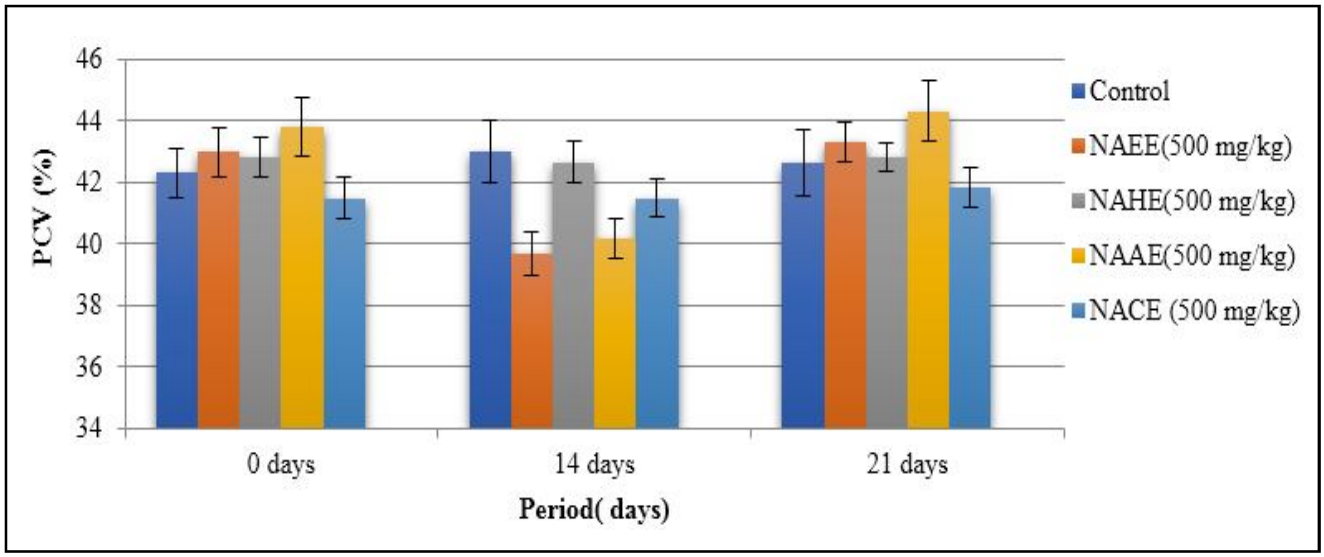

Figure 5: Graph representing effect of ethanolic, hydroethanolic, aqueous and chloroform leaf extract of *N. arbor-tristis* on packed cell volume (%).

3.6 Effect of plant extracts on biochemical parameters

3.6.1 Biochemical parameter

3.6.1.1 Alanine transaminase activity (ALT U/L)

Mean ± SE of alanine transaminase activity of NAEE, NAHE, NAAE and NACE treated groups on 0, 14th and 21st day are presented in the Table 9 and graphical presentation in Figure 6, respectively. There was no change in alanine transaminase activity in NAHE treated group. But NAEE, NAAE and NACE group showed significant ($p<0.05$) increase in ALT activity on 21st day.

3.6.1.2 Aspartate amino transferase activity (AST U/L)

Mean ± SE of aspartate aminotransferase activity of NAEE, NAHE, NAAE and NACE treated groups on 0, 14th and 21st days are presented in the Table 10 and graphical presentation in Figure 7, respectively. Aspartate aminotransferase activity of mice treated with NAEE, NAHE, NAAE and NACE showed no significant change between days and between groups.

Table 9: Effect of ethanolic, hydroethanolic, aqueous and chloroform leaf extracts of *N. arbor-tristis* on alanine transaminase activity (U/L)

Group	Post treatment (days)		
	0	14	21
Control	21.03 ± 0.45	21.53 ± 0.42	21.36 ± 0.32
NAEE (500 mg/kg)	21.70 ± 0.49	21.73 ± 0.31	48.17 ± 1.17
NAHE (500 mg/kg)	21.87 ± 0.39	22.23 ± 0.45	22.53 ± 0.60
NAAE (500 mg/kg)	21.53 ± 0.33	21.88 ± 0.28	31.67 ± 0.49
NACE (500 mg/kg)	21.37 ± 0.61	22.20 ± 0.46	61.83 ± 1.19

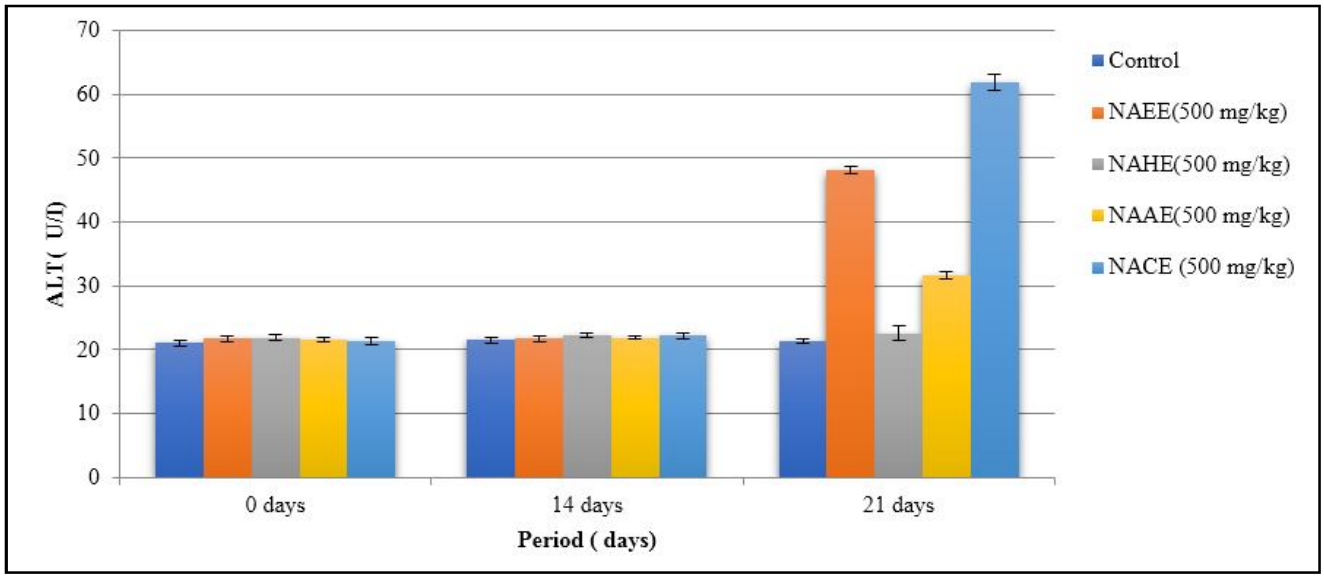

Figure 6: Graph representing effect of ethanolic, hydroethanolic, aqueous and chloroform leaf extract of *N. arbor-tristis* on alanine transaminase activity (U/L).

Table 10: Effect of ethanolic, hydroethanolic, aqueous and chloroform leaf extracts of *N. arbor-tristis* on aspartate amino transferase activity (U/L)

Group	Aspartate amino transferase (U/L)		
	Post treatment (days)		
	0	14	21
Control	53.50 ± 0.99	53.83 ± 0.87	53.83 ± 0.90
NAEEE (500 mg/kg)	52.83 ± 1.05	52.83 ± 0.98	52.50 ± 0.62
NAHE (500 mg/kg)	52.17 ± 1.14	52.50 ± 1.06	51.67 ± 0.84
NAAE (500 mg/kg)	52.33 ± 0.80	52.83 ± 0.79	52.33 ± 0.80
NACE (500 mg/kg)	53.83 ± 0.70	54.00 ± 0.63	52.67 ± 0.80

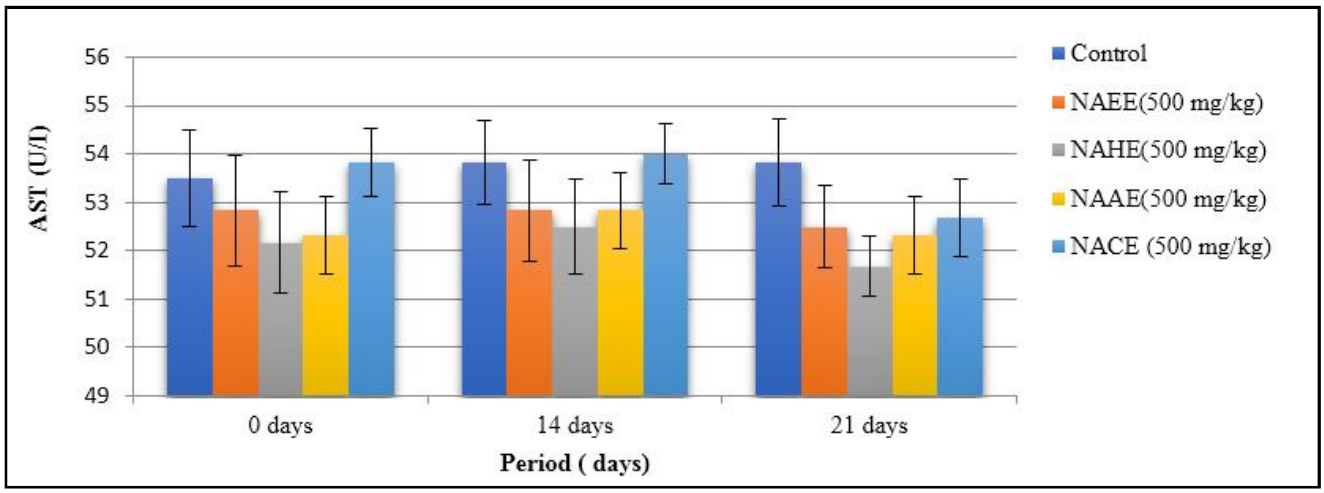

Figure 7: Graph representing effect of ethanolic, hydroethanolic, aqueous and chloroform leaf extract of *N. arbor-tristis* on aspartate amino transferase activity (U/L).

3.6.1.3 Total protein (g/dl)

Mean ± SE of total protein activity of NAEE, NAHE, NAAE and NACE treated group on 0, 14th and 21st day are presented in the Table 11 and graphical presentation in Figure 8, respectively**.** In the present study, total protein concentration of NAEE, NAHE, NAAE and NACE treated groups showed no change up to 21st days post treatment. But there was significant difference (p<0.05) of NAAE treated group with that of NAEE and NACE on 14th day and 21st day.

3.6.1.4 Creatinine (g/dl)

Mean ± SE of creatinine activity of NAEE, NAHE, NAAE and NACE treated groups on 0, 14th and 21st day are presented in the Table 12 and graphical presentation in Figure 9, respectively In the present study, NAHE and NAAE treated group showed no change in creatinine activity, but NAEE and NACE treated group showed significant (p<0.05) changes in creatinine activity on 21st day post treatment. NACE treated group on 21st day showed significant (p<0.05) increase in creatinine comparison to all the treatment groups.

Table 11: Effect of ethanolic, hydroethanolic, aqueous and chloroform leaf extracts of *N. arbor-tristis* on total protein (gm/dl)

Group	Total protein(gm/dl)		
	Post treatment (days)		
	0	14	21
Control	3.57 ± 0.16	3.58 ± 0.16	3.73 ± 0.13
NAEE (500 mg/kg)	3.72 ± 0.31	4.00 ± 0.24	3.77 ± 0.13
NAHE (500 mg/kg)	3.72 ± 0.17	3.95 ± 0.12	3.85 ± 0.12
NAAE (500 mg/kg)	3.38 ± 0.27	3.42 ± 0.29	3.72 ± 0.11
NACE (500 mg/kg)	3.92 ± 0.10	4.10 ± 0.21	4.17 ± 0.29

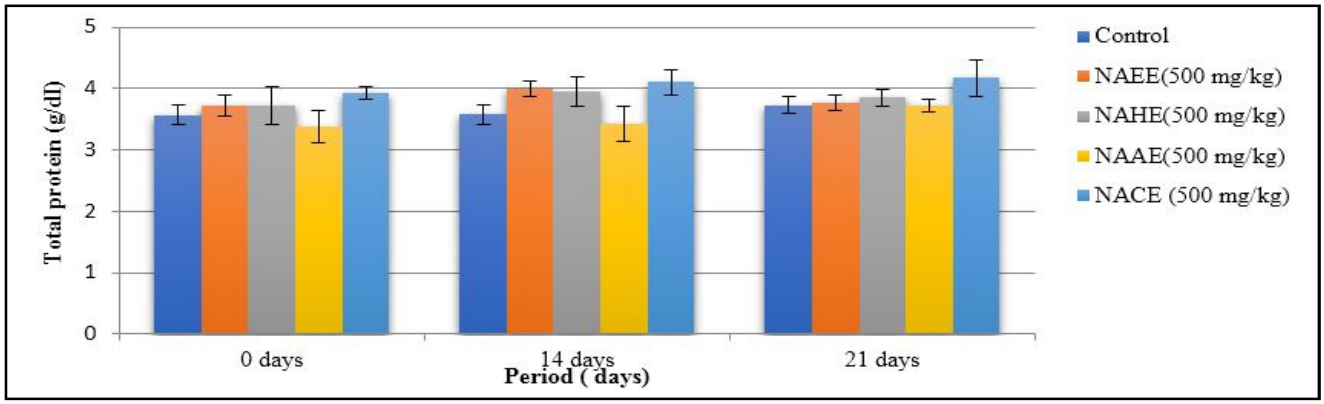

Figure 8: Graph representing effect of ethanolic, hydroethanolic, aqueous and chloroform leaf extract of *N. arbor-tristis* on total protein (g/dl).

Table 12: Effect of ethanolic, hydroethanolic, aqueous and chloroform leaf extracts of *N. arbor-tristis* on creatinine (mg/dl)

Group	Creatinine (mg/dl)		
	Post treatment (days)		
	0	14	21
Control	0.33 ± 0.06	0.4 ± 0.05	0.42 ± 0.03
NAEE (500 mg/kg)	0.32 ± 0.04	0.28 ± 0.03	0.55 ± 0.08
NAHE (500 mg/kg)	0.28 ± 0.03	0.33 ± 0.03	0.35 ± 0.06
NAAE (500 mg/kg)	0.23 ± 0.02	0.28 ± 0.03	0.42 ± 0.06
NACE (500 mg/kg)	0.32 ± 0.05	0.35 ± 0.04	0.73 ± 0.05

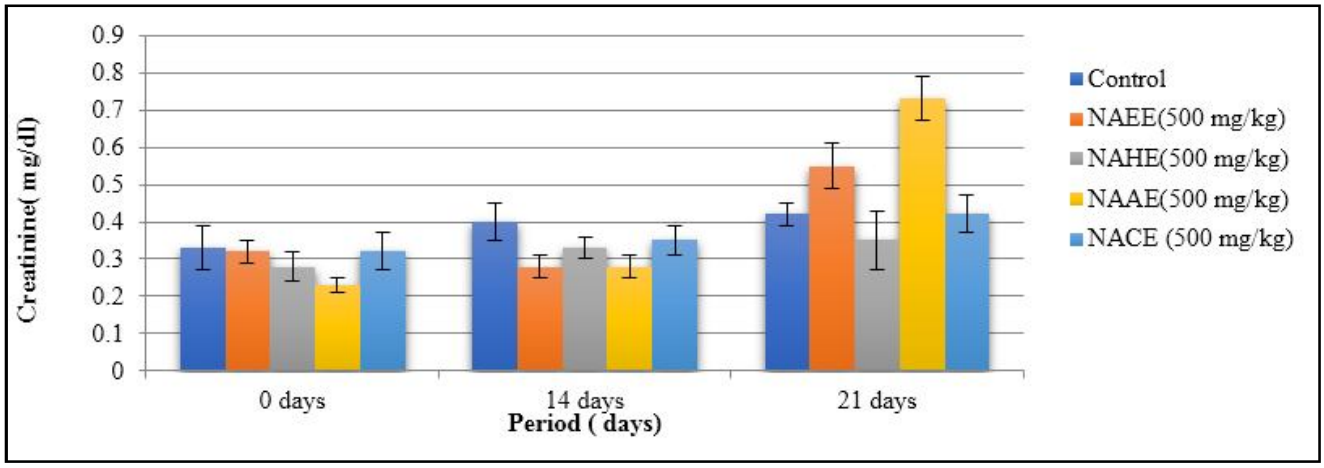

Figure 9: Graph representing effect of ethanolic, hydroethanolic, aqueous and chloroform leaf extract of *N. arbor-tristis* on creatinine (mg/dl).

3.7 Effect of plant extracts on histopathology parameters

3.7.1 Liver

Liver from control mice did not reveal any pathological alterations Figure 10. In NAEE treated group, hepatic parenchyma showed binucleated hepatocyte and intravenous serous fluid accumulation Figure 11. In NAHE treated group, hepatic parenchyma showed focal minimal necrosis Figure 12. Focal minimal aggregation of mononuclear cells and erythrocytes with few regenerating hepatocytes could be seen in the hepatic parenchyma of NAAE treated group Figure 13. NACE treated group showed central vein filled with erythrocytes Figure 14.

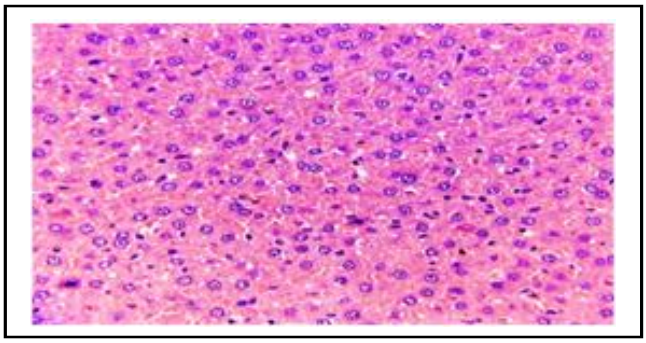

Figure 10: Photomicrograph of liver in control group showing normal arrangement of hepatocytes (H & E, 400X).

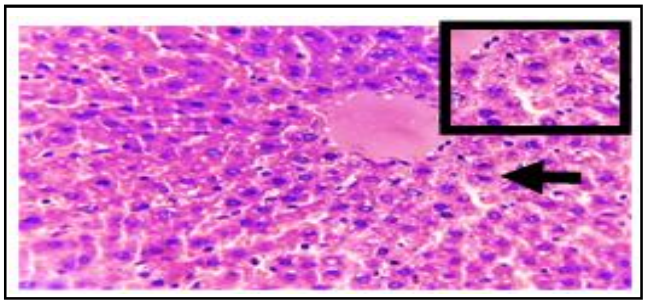

Figure 11: Photomicrograph of liver showing binucleated hepatocytes (arrow) and intravenous serous fluid accumulation, in NAEE treated group(H&E,400X).

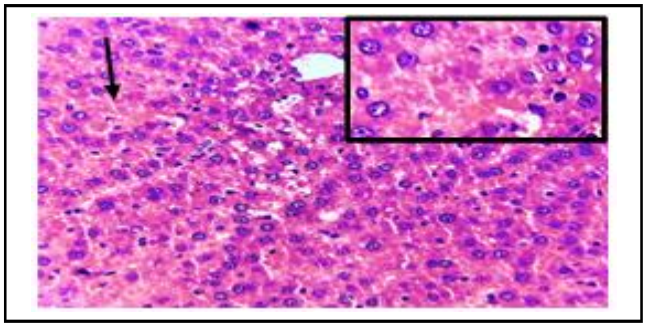

Figure 12: Photomicrograph of liver showing focal minimal necrosis (arrow),in NAHE treated group (H&E, 400X).

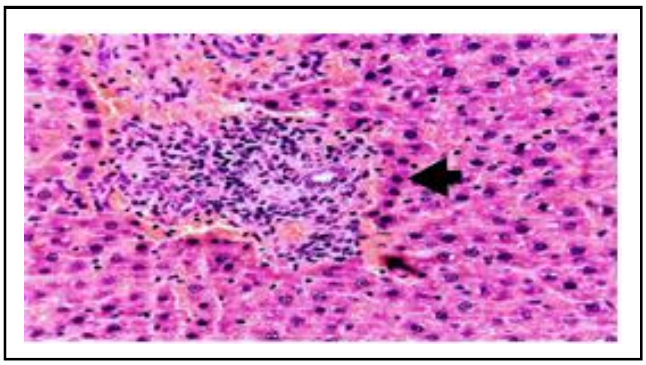

Figure 13: Photomicrograph of liver showing focal minimal aggregation of mononucleat cells and erythrocytes with few regenerating hepatocytes(arrow), in NAAE treated group (H&E,400X).

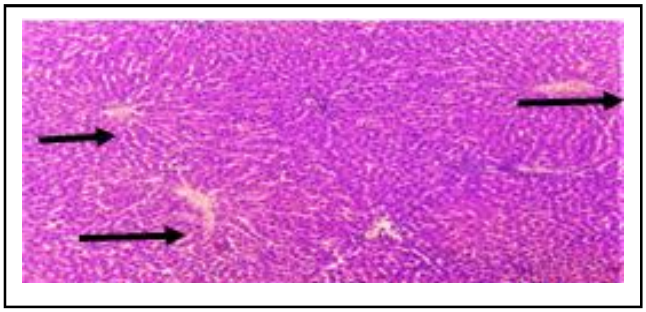

Figure 14: Photomicrograph of liver showing central vein filled with erythrocytes (arrows), in NACE treated group(H &E, 400X).

3.7.2 Kidney

Kidney of control mice did not reveal any pathological alterations Figure 15. However, complete loss of glomerular tuft and focal minimal sloughing of necrotised tubular epithelium could be seen in the renal parenchyma of NAEE treated groups Figure 16. NAHE treated groups showed focal minimal glomerular tuft shrinkage Figure 17. Kidney of NAAE treated groups showed focal minimal renal tubular necrosis Figure 18. Kidney of NACE treated mice did not reveal any pathological change Figure 19.

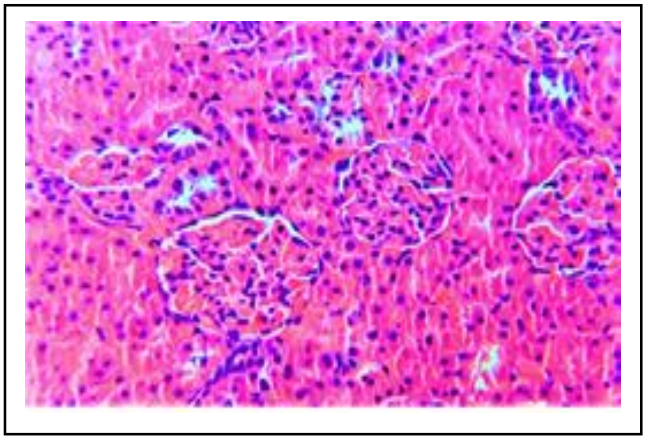

Figure 15: Photomicrograph of kidney in control group showing normal arrangement (H&E, 400X).

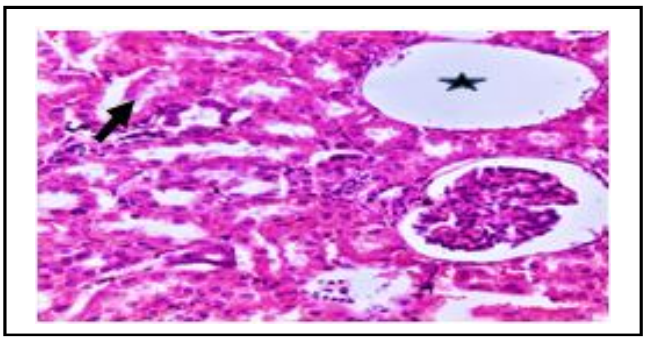

Figure 16: Photomicrograph of kidney showing complete loss of glomerular tuft (star) and focal minimal sloughing of necrotised tubular epithelium (arrow), in NAEE treated group (H&E, 400X).

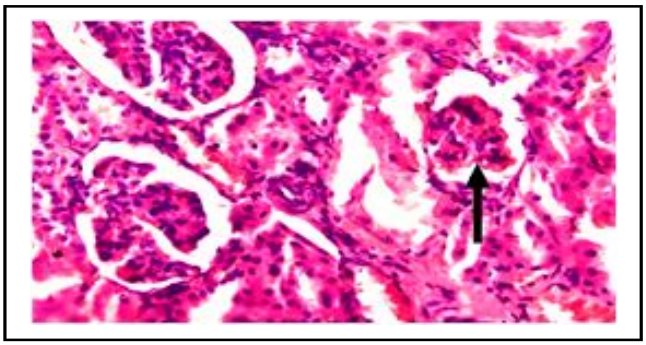

Figure 17: Photomicrograph of kidney showing focal minimal glomerular tuft shrinkage, in NAHE treated group (H & E, 400X).

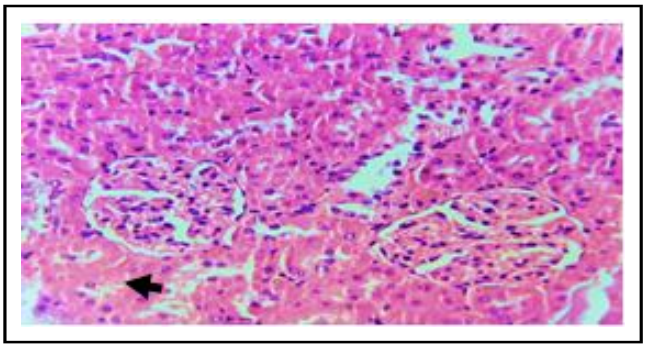

Figure 18: Photomicrograph of kidney showing focal minimal renal necrosis (arrow), in NAAE treated group (H&E, 400X).

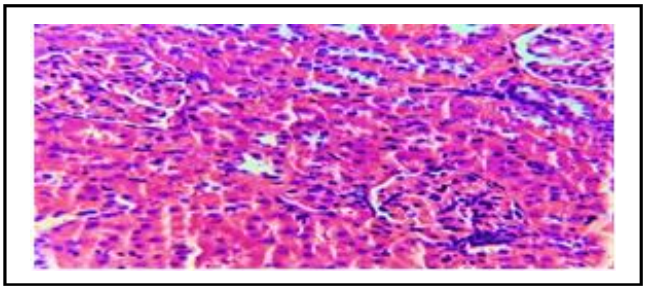

Figure 19: Photomicrograph of kidney showing normal architecture, in NACE treated group (H & E, X40).

4. Discussion

Flowers are acknowledged to synthesise many chemical substances that posses as many biological sports. A number of the compounds show off particular and precise residences which have lengthy been exploited in animal and human clinical structures by using influencing metabolic pathways, natural plant compounds continue to be the important source of drug treatments for management of animal and human health. A variety of parts belonging to one of a kind chemical instructions such as terpenes, steroids, glycosides, flavonoids and alkaloids had been observed to be wonderful from the leaves of *N. arbor-trisitis*. Phytochemical analysis of ethanolic extract showed the presence of traditional plant elements together with alkaloids, steroids, tannins, flavonoids, reducing sugars, saponins, and terpenoids and hydroethanolic extract confirmed presence of steroids, glycosides, phenols, tannins, flavonoids, diterpenes, triterpenes and saponins.

The aqueous extract confirmed presence of steroid, glycoside, flavonoids, diterpenes, triterpenes and saponins and absence of tannins and chloroform extract confirmed presence of flavonoids, diterpenes and triterpenes and absence of steroids, alkaloids, glycosides, tannins and saponins. Phytochemical results in the present study indicate that the plants contain various chemical compounds including retonoids, sesquiterpene lactones, glycosides, anthracenes and tannins. Some of these compounds especially sesquiterpene lactones and retonoids have been reported elsewhere to have biological activities against inflammatory conditions, helminthiasis and many more conditions. Phytochemical consequences within the gift examine suggest that the plants comprise diverse chemicals which include retonoids, sesquiterpene lactones, glycosides, anthracenes and tannins.

Some of those compounds especially sesquiterpene lactones and retonoids were said elsewhere to have organic activities in opposition to inflammatory situations, helminthiasis and many greater conditions. Fever can be a result of contamination or one of the sequelae of tissue damage, irritation, graft rejection, or different disorder states. Antipyretics are drugs, which reduce the accelerated body temperature. Regulation of body temperature requires a delicate balance among the manufacturing and loss of warmth. Hypothalamus regulates the set factor at which body temperature is maintained. In fever this set factor is extended and tablets like paracetamol do no longer influence body temperature whilst it's miles expanded by means of elements such as exercise or will increase in ambient temperature. The ethanolic, hydroethanolic, aqueous and chloroform extracts of *N. arbor-tristis* at all doses showed significant (p<0.05) increase in antipyretic activity up to 5 h of observation period which was similar to the standard drug paracetamol.

Antipyretic activity of leaf extract can be due to presence of steroid, glycoside and flavonoids. Singha *et al*.(2014) pronounced that petroleum ether and methanol extracts of the bark of a *N. arbor-tristis* exhibited antipyretic activity against yeast induced pyrexia model in mice at the dose rate of 200 mg/kg. In standard, protection research of herbal drug treatments were conducted by appearing acute and subacute toxicity assessments in laboratory animals (e.g. rodents and subhuman primates) (Fennell *et al*., 2004). On this take a look at, we tested acute and subacute oral toxicity of *N. arbor-tristis* leaf extract in mice. Acute and subacute poisonous effects are fundamentally one of-kind from every different with respect to the amount of take a look at reagent used and time intervening earlier than consequences are discovered.

While, acute effects are commonly determined soon after a single exposure and subacute results are usually monitored through an extended time period in the course of which repeated exposure happens check agent. Acute oral toxicity take a look at was performed in line with OECD test pointers 425 in female mice for all of the four type of leaf extracts (ethanolic, hydroethanolic, aqueous and chloroform) at 2000 mg/kg body weight, as single oral dose. There has been no mortality, no exchange in behaviour and body weight inside the 14 days commentary duration. Khan *et al*.(2017), in their take a look at on *N. arbor-tristis* found that ethanolic and methanolic extract at the doses of 50-3000 mg/kg orally become now not poisonous in mice and did no longer show any signs of abnormality and the LD_{50} became found to be extra than 3000 mg/kg. From the existing look at, it may be concluded that *N. arbor-tristis* leaf extract on the doses employed inside the present take a look at is secure for oral management without any poisonous signs and symptoms. Subacute toxicity study turned into executed in the present have a look at following oral administration of *N. arbor-tristis* extracts (ethanolic, hydroethanolic, aqueous and chloroform) at 500 mg/kg body weight, as per OECD guidelines 407. Subacute toxicity study did no longer display any behavioural alteration, gross abnormality and mortality up to 21 days.

Hematopoietic parameter is one of the most sensitive goal for poisonous compounds and important index of physiological and pathological reputation. Also blood profile usually gives vital information on the response of body to injury or stress (Mukinda *et al*.,2020). The haemoglobin values confirmed no alternate in NAHE, NAAE and NACE dealt with group but NAEE treated group showed substantial (p<0.05) decrease in haemoglobin cost on 21 days publish treatment in assessment to govern. Comparable findings were also found by way of Salokhe *et al*. (2020) wherein hydroethanolic extract of *Rosmarinus officinalis* leaves at 300, 500 and 1000 mg/kg body weight confirmed no reduction of haemoglobin stage up to 28 days of examine duration. In the present study 21days remedy of *N. arbor-tristis* leaf extracts (ethanolic, hydroethanolic, aqueous and chloroform) on the dose of 500 mg/kg confirmed no big reduction in general erythrocytic be counted. Similar findings were also found through Salokhe *et al*. (2020) in which hydroethanolic extract of *Rosmarinus officinalis* leaves at 300, 500 and 1000 mg/kg confirmed no exchange in general erythrocytic count number up to 28 days of observe period. Nath and Yadav (2014) also suggested that extract from leaves of *Hibiscus rosa-sinensis* at 400 mg/kg doses for 14 days did no longer alter general erythrocytic rely. However, at 800 mg/kg body weight confirmed slight boom of RBC counts.

However, Pawar *et al*. (2016) suggested that ethanolic extract of *N. arbor-tristis* calyx at 500 and 750 mg/kg body weight showed widespread (0.05) inhibition of RBC manufacturing. Salokhe *et al*. (2020) and Nath and Yadav (2014) findings had been in settlement with the outcomes obtained from the existing take a look at in which all of the extract at 500 mg/kg body weight showed no reduction of general erythrocytic be counted. In the present study the entire leucocytic matter showed no change in value in NAEE, NAHE and NACE treated group, however in case of NAAE treated group it

became observed that there has been considerable ($p<0.05$) boom in overall leucocytic anticipate twenty first day compared to all the different remedy businesses.

Similar findings changed into stated by Salokhe *et al*. (2020) in which hydroethanolic extracts of *Rosmarinus officinalis* leaves at 300, 500 and a 1000 mg/kg body weight did now not reason any giant exchange in the tiers of WBC remember. NAHE, NAAE and NACE dealt with groups confirmed no trade in packed cell extent. NAEE treated group showed tremendous ($p<0.05$) reduction in packed cellular extent on 14th day, but this quantity have become regular on 21st day. Similar findings changed into said through Salokhe *et al*. (2020) wherein hydroethanolic extracts of *R. officinalis* leaves at 300, 500 and 1000 mg/kg body weight did now not motive any giant alternate in the tiers of packed cell volume. Those observations had been in agreement with the result received from present study. The have a look at of biochemical parameters are a hallmark of toxicity, on the crucial organs. In order to finish the toxicity profile of various extracts, it's miles crucial to understand its effect on the liver, because it's miles the foremost organ concerned in drug biotransformation.

Stage of AST, ALT and total protein turned into evaluated to estimate the liver toxicity by means of capsules or some other hepatotoxic agents. ALT is extra precise to liver and therefore a higher parameter for detecting liver damage. Inside the present study there has been no alternate in alanine transaminase activity in NAHE and NAAE treated groups. But, NAEE and NACE treated groups showed increase in ALT activity on 21st day.

Consequently, it shows possibility of liver injury by using treatment with *N. arbor-tristis* ethanolic and chloroform leaf extracts. Pawar *et al*. (2016) stated that ethanolic extract of *N. arbor-tristis* calyx at one of a kind doses (200, 500 and 750 mg/kg) confirmed dose established boom in ALT activity. Similar findings were additionally found by using Nath and Yadav (2014) that it is a associated study where methanolic extract of leaves of *Hibiscus rosa-sinensis* at 800 mg/kg body weight confirmed great growth of ALT activity. AST is likewise associated with diseases of other organs which includes coronary heart muscular tissues besides liver. Inside the present study, aspartate aminotransferase activity of mice dealt with NAEE, NAHE, NAAE and NACE showed no full-size change. Comparable findings become said through Pawar *et al*.(2016) who determined ethanolic extract of *N. arbor- tristis* calyx at distinct doses (200, 500 and 750 mg/kg) showed no change in AST activity. Within the present 21 days subacute toxicity study of *N. arbor-tristis* leaf extract (ethanolic, hydroethanolic, aqueous and chloroform) on the dose rate of 500 mg/kg showed no change in total protein. Comparable findings become additionally pronounced by Pawar *et al*.(2016) who observed no alternate in overall protein in animals administered with ethanolic extract of *N. arbor-tristis*. Creatinine is considered as an vital marker of kidney characteristic Mukinda *et al*. (2020). In the present study, NAHE and NACE dealt with institution confirmed no exchange in creatinine activity. However, NAEE and NACE dealt with group confirmed slight increase in creatinine activity on 21st day post treatment however in the everyday range. Pawar *et al*. (2016) also stated that ethanolic extract of *N. arbor-tristis* calyx at different doses (200, 500 and 750 mg/kg) confirmed no change in creatinine level that is just like findings of present study. The histopathological outcomes of liver and kidney discovered various levels of histopathological lesions in treated groups. These outcomes advise that management of ethanolic, hydroetha-nolic, aqueous and chloroform extracts of *N. arbor-tristis* at 500 mg/kg body weight for 21st days can also cause minor damage of liver and kidney in experimental animals, which might be because of the presence of energetic ingredients if the extract is fed for the lengthen length and residues of solvents in extracts. These histological modifications have been in settlement with preceding reviews of *N. arbor-tristis* ethanolic extract of calyx at 250, 500, 750 mg/kg body weight research in mice. Pawar *et al*. (2016) who observed numerous levels of histopathological lesions in animals liver that is congestion, haemorrhages, perivascular MNC infiltration, degeneration, bile duct hyperplasia. Kidney also showed congestion, haemorrhages, tubular degeneration, glomerular atrophy. Nath and Yadav (2010) in related have a look at mentioned that methanolic extract from leaves of *H. rosa-sinensis* at 800 mg/kg body weight showed sizable harm to the liver (dilated sinusoids, apoptotic nuclei and inflammatory infiltrate inner sinusoidal capillaries) and kidneys (disorganization of tubules and glomeruli, enlarged interstitial spaces) of treated animals. In the present study among all of the four extracts (*i.e.,* ethanolic, hydroethanolic, aqueous and chloroform) of *N. arbor-tristis* hydroethanolic extract turned into observed to be secure @ 500mg/ kg body weight.

5. Conclusion

Among all the four extracts (*i.e.* ethanolic, hydroethanolic, aqueous and chloroform extracts) of *N. arbor-tristis* beneath look at hydroethanolic extract @ 1000 mg/kg body weight confirmed higher antipyretic activity in evaluation to different three extracts within the present study. The prevailing take a look at indicated that leaves of *N. arbor-tristis* can be used as an alternative to antipyretic drug. However, in addition studies are important to isolate energetic elements liable for therapeutic impact and dose dedication. Within the present study among all the four extracts (*i.e.,* ethanolic, hydroethanolic, aqueous and chloroform) of *N. arbor-tristis* hydroethanolic extract became determined to be safe @ 500 mg/kg body weight.

Acknowledgements

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Abbreviations

NAEE: *Nyctanthes arbor-tristis* ethanolic extract; NAHE: *Nyctanthes arbor-tristis* hydroethanolic extract; NAAE: *Nyctanthes arbor-tristis* aqueous extract; NACE: *Nyctanthes arbor-tristis* chloroform extract; OECD: Organization for Economic Co-operation and Development.

Conflict of interest

The author declared no conflict of interest relevant to this article.

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