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Phytochemical Analysis, Antioxidant Activity, And Toxicological Evaluation Of *Argyreia Nervosa bojar.* Extracts

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ABSTRACT

Argyreia nervosa bojar., a member of the Convolvulaceae family, is a plant of significant medicinal interest due to its diverse phytochemical profile and therapeutic potential. This study investigates the phytochemical profile and antioxidant potential of different solvent extracts (aqueous, methanol, ethanol, acetone, and ethyl acetate) of *Argyreia nervosa* (AN). The aqueous extract showed the highest concentrations of phenolics (0.99 ± 0.13 mg GAE/100g) and flavonoids (18.89 ± 1.5 mg/100g) compared to other extracts. Antioxidant activity, assessed through DPPH free radical scavenging, was highest in the aqueous extract, with a 78.33% inhibition rate significantly outperforming (p=<0.01) other solvent extracts. Further, the best-performed aqueous extract was evaluated for toxicological effects on Sprague-Dawley rats. Acute and subacute toxicity studies, conducted for oral doses 150, 300, and 600 mg/kg, revealed no mortality or adverse clinical signs in rats, however, mild weight gain was observed. Sub-acute toxicity studies did not significantly alter most hematological and biochemical parameters, except for a dose-dependent increase in serum ALT and AST levels at 600 mg/kg, indicating potential hepatotoxicity at higher doses. These findings not only underscore the medicinal potential of *A. nervosa bojar.* but also confirm its usability in developing new natural therapies.

Keywords: Acute toxicity, biochemical parameters, hematological parameters, and subacute toxicity

INTRODUCTION

Medicinal herbs and plant extracts are increasingly recognized as valuable and effective components of modern medicine (Chaudhari et al., 2020). The World Health Organization (WHO) estimates that

80% of the global population relies on herbal remedies for primary healthcare (WHO, 2019). The Indian subcontinent, in particular, is rich in a diverse range of herbal plants that are integral to traditional Indian medicine practices (Swami et al., 2022). Historically, plant-based treatments have served as the first line of defense for maintaining health and treating diseases (Ballabh et al., 2008; Singh and Kumar, 2019). However, evaluating the toxicological profiles of potential medicinal herbs, extracts, and drugs is essential to establish their safety in animal models and to predict potential health risks in humans due to the inherent adverse effects of some compounds or extracts (Ekor, 2014). Studies have shown that certain herbal extracts, which are rich in phenols and flavonoids, can exhibit a range of biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties (Sunday, 2022). However, the safety of these extracts must be thoroughly evaluated to ensure that any potential adverse effects are identified and addressed.

One of the primary challenges in assessing the safety of herbal extracts is the inherent complexity of their chemical composition. Unlike single-component pharmaceuticals, herbal extracts often contain a wide range of bioactive compounds, each with its own potential for toxicological significance. Furthermore, the relative abundance and bioavailability of these compounds could be influenced by various factors, such as the part of the plant used, the extraction method, and environmental conditions (Ezeonyi et al., 2024).

To address these challenges, researchers have developed a range of strategies for the toxicological assessment of herbal extracts. These strategies often involve a combination of *in vitro* and *in vivo* studies, as well as the use of advanced analytical techniques to characterize the chemical composition of the extracts (Desai et al., 2024).

In vitro studies can provide valuable insights into the potential mechanisms of action and toxicity of herbal extracts by evaluating their effects on enzyme activities, and other biochemical and haematological parameters. Specific biomarkers, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), are commonly used to detect hepatic injury and hepatocellular necrosis (Ephraim et al., 2024). Additionally, hematological parameters are another way to assess the toxicity (Odo et al., 2016).

A. nervosa bojar. plant, a traditional and aesthetic medicinal plant is native to the Indian subcontinent and, was introduced worldwide over some time (Padhi et al., 2013). Most studies on *A. nervosa* have concentrated on its anti-inflammatory, antipyretic, anti-arthritic activity, and other medicinal properties (Alugonda et al., 2014). There is a shortage of research on the effects of *A. nervosa* toxicity on hematology and enzymes (biochemical parameters), highlighting this investigation's significance. This investigation sought to ascertain the toxicity of an aqueous extract of *A. nervosa* as well as its impact on the hematological and certain biochemical parameters (liver enzymes) in Sprague-Dawley rats.

2. Material and Methods

2.1 Collection and preparation of plant material:

The plant parts of *A. nervosa bojar.* species were collected from local vendors, ensuring that the collection process was performed sustainably to avoid harming the future viability of the whole plant. After the plant parts were obtained, they were maintained in the greenhouse of the department to ensure their survival. The species was promptly identified by taxonomists at the time of procurement. Each specimen was preserved in the Herbarium, with roots, leaves, and stem samples carefully dried to remove excess moisture, sealed in zip-lock bags, and stored in a refrigerator until further use. Following collection, all plant components, including leaves, stems, roots, fruits, and flowers, were thoroughly washed to remove dirt and unwanted materials such as grasses, herbs, and other extraneous objects. The samples were then chopped into small pieces, with roots and stems being cut into 3–5 cm segments. After chopping, the samples were left to dry at room temperature in the shade and were turned over at least twice daily to facilitate even drying. Once completely dried, the samples were ground into a fine powder using a machine to prepare them for further analysis.

2.2 Extraction Method

The extraction process involved the use of a Soxhlet apparatus to obtain crude extracts from shade-dried, finely powdered parts of medicinal plants using solvents of increasing polarity— Ethylacetate, Acetone, Ethanol, Methanol and water.

Approximately 70–90 grams of the dried plant powder were carefully placed into a clean, dry thimble wrapped in sterile filter paper and loaded into the Soxhlet apparatus. A pre-dried 1000 ml round-bottom flask was attached to the apparatus, and 400 ml of solvent was slowly added through the top mouth of the Soxhlet extractor. A condenser with flowing cold water was then installed, and the flask was heated continuously using a temperature-controlled electric heating mantle. The extraction was performed until the plant material became colorless.

2.3 Quantitative Assay of Phytochemicals

The presence of phenols was confirmed using a ferric chloride test, indicated by a blue or green color (Terfassi et al., 2012). Flavonoids were detected using an alkaline reagent test, resulting in a yellow color that fades with acetic acid addition (Saeed et al., 2012). Antioxidant activity was measured by using DPPH (2, 20 - diphenyl-1-picrylhydrazyl) assay (Mishra et al., 2012).

2.4 Experimental Animals

Eight-week-old Sprague-Dawley rats, weighing between 140 and 160 grams, were acquired from the Animal Resource Unit. Before each dose, the rats were given a week to get used to the lab environment. The rats were kept in a room with air conditioning set at °C and a 12-hour light-dark cycle. Each polycarbonate cage held three rats, who were allowed unlimited access to water and rodent food from Specialty, authorized all protocols for animal experiments.

2.5 Toxicity study

2.5.1 Acute Toxicity Study

Twelve rats total—six males and six females—were split up into four groups. Group 1 was the control group, while aqueous ethanolic, and were administered to the other three groups. The treatment groups received leaf extract by single oral dose at 5000 mg/kg body weight (BW) suspended in 5% Tween-20, whereas the control group got 5% Tween-20. The limit test was conducted using a 5000 mg/Kg BW dosage of OS in compliance with the standards for the Testing of Chemicals (OECD guidelines 425). For each rat, the given volume was changed to 8 mL/Kg BW. At the beginning of the experiment, on day 0, the rats received both the vehicle and the OS extract, and they were observed for 14 days.

2.5.2 Subacute toxicity

The subacute toxicity research was carried out with minor modifications in accordance with the OECD 407 recommendations (2008).

The study involved twelve healthy albino mice. They were placed in 4 different groups of animals. There were twenty individuals in each one comprising of five females and five males. Table 1 shows that all the animals except the control received the differing doses of extract for 28 days through the oral route by gastric lavage done once a day. The experiment commenced by noting physical conditions and animal behaviors. Daily changes on weights of animals as well as the development of abnormalities was monitored.

2.5.3 Animals and their organs weight:

At day zero, which is referred to as just before the inception of the study, body weights of all the animals in all the groups were recorded and later on recorded again at days 1, 2, and 14 for acute toxicity study. Weight measurement was done on days one, seven, twenty-one, and twenty-eight of the subacute study. By the end of the trial, the target animals were slaughtered while the specified organs were removed and weighed for relative organ weight (Variya et al., 2019).

Weight gain (%)= (Wf−Wi/Wi)100

where Wf = final weight; Wi = initial weight.

Relative organ weight (%)=(organ weight/body weight)x100

2.6 Study of biochemical markers in chronic toxicity tests:

Plasma and serum were prepared separately for the analysis of various biochemical markers. The serum was prepared through the coagulation of a whole blood sample in a vacutainer at normal temperatures followed by centrifugation at a speed of 2,000 x g for ten minutes while the plasma was obtained using an anticoagulant-containing vac Protein. An auto-analyzer (Archem BM240, Turkey) was used to assay for biochemical parameters which include: aspartate amino transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, total bilirubin, creatinine, chloride ions, and bicarbonate ions. (Sadauskas et al., 2011).

2.7 Study of hematological parameters in chronic toxicity tests: An automated Hematologic

analyser was used to study parameters like white blood cell (WBC) counts, lymphocyte (LY), monocyte (MO), granulocyte (GR) counts, and their respective percentages. Additionally, red blood cell (RBC) counts, hemoglobin (Hgb) levels, hematocrit (HCT), and other red cell indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were also presented. Platelet (PLT) counts, plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW) were also analyzed (Saravanan et al., 2012).

2.8 Statistical analysis

Each and every value was given as mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to compare the differences. P-values less than 0.05 were regarded as significant.

3. Results

3.1 Determination of phenolic compounds in *Argeria nervosa* bojar. (AN) plant extracts

We noted varied levels of phenol concentrations in methanol, aqueous, acetone, ethylacetate and water extracts for AN whole plant, roots, stem, leaves. The content of phenolic compounds in solvents from a regression equation of a calibration curve, and GAE/100 g was measured varying between 0.25 and 0.99 mg. of phenols. Total phenolic compound content in extracts of the whole plant was highest (0.99 mg GAE/g) among all other extracts. More blue- green colour intensity was observed for aqueous extract of whole plant compared to other solvents evaluated with this reagent. It was followed by ethanol extract. However, the total phenols determined this way cannot be considered absolute (Table 1).

Table 1: Phenolic contents in different extracts of *Argeria nervosa* bojar. whole plant extracts

S.N.	Extract	Phenolic contents (mg /100g)
1	Ethylacetate	0.34 ± 0.08
2	Aqueous	0.99 ± 0.13
3	Methanol	0.59 ± 0.05
4	Acetone	0.25 ± 0.04
5	Ethanol	0.67± 0.1 5

3.2 Determination of total flavanoids in *A. nervosa* bojar. whole plant extracts

Flavonoids were estimated in milligrams per gram, as Quercetin equivalents using an *Alkaline reagent test*. The samples had different values ranging between 1.98 and 18.89. The highest amount of flavonoid was extracted in Aqueous from dry whole plant of AN. Maximum total flavonoids in water extract showed a direct correlation with the total amount of phenolics (Table 2). Among all plant parts, whole plant extract performed best. It was established in this study that in terms of concentration of flavonoid, acetone < ethyl acetate < methanol < ethanol <Aqueous. So aqueous was identified as the

most ideal solvent system for the extraction of phenolics and flavonoids from dried whole plant of AN.

Table 2: Flavonoid contents in various extracts of *Argeria nervosa* bojar. Whole plant

S.N.	Extract	Flavonoid contents (mg /100g)
1	Ethyl acetate	5.34 ± 0.7
2	Aqueous	18.89± 1. 5
3	Methanol	10.59 ± 1.5
4	Acetone	9.98 ± 1.4
5	Ethanol	11.67± 1.1 5

3.3 Antioxidant activity of the AN whole plant extracts by DPPH free radicals

The antioxidant activities of the AN extracts prepared in various solvents were ascertained. The absorbance of a blank was also taken down. each case. A comparison of the antioxidant properties of all the extracts. were compared with pyrogallol. Yet, we noted every extract was different in its efficiency. Whole plant extract in aqueous showed maximum antioxidant activity, followed by ethanol extract. Figure 1 states that aqueous extract exhibited maximum inhibitory potential on DPPH radicals at 78.33%. It was about 50% higher than the IC50. The inhibition rate of absorbance relative to the control value was calculated. based on Plant extract concentration versus DPPH reduction.

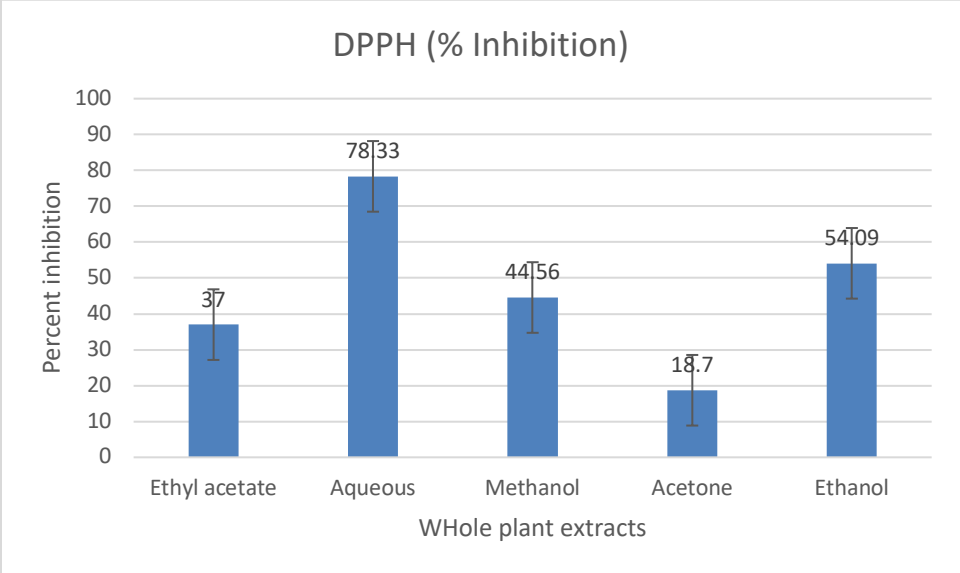


Figure 1: Percent inhibition of AN whole plant extract by DPPH free radicals

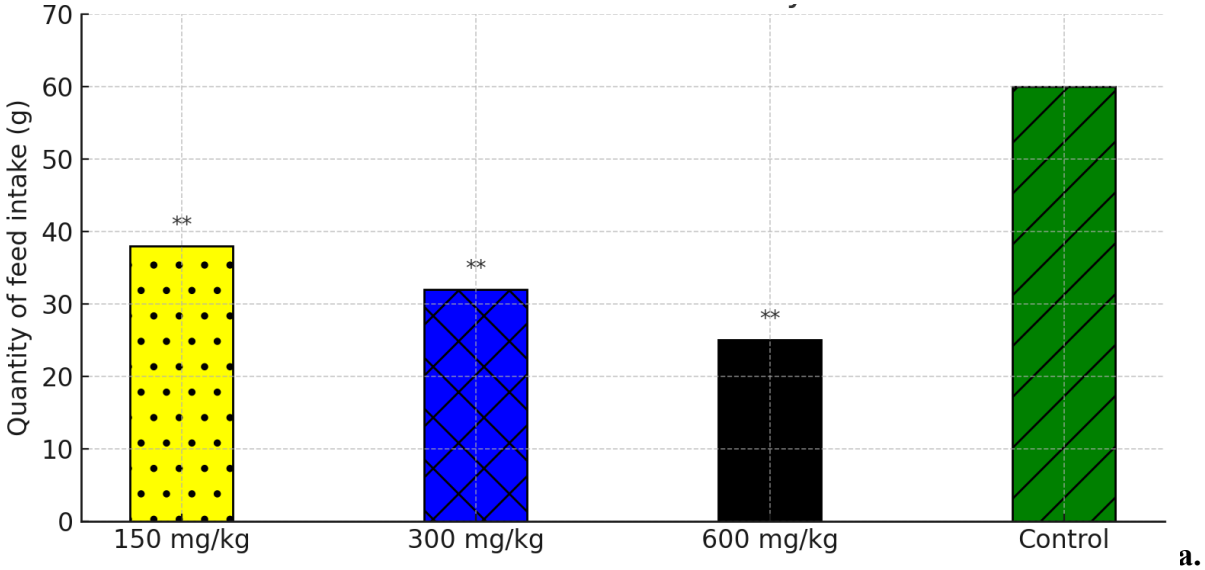
3.4 Acute and subacute toxicity of the weights of animals and selected organs

3.4.1 Acute toxicity studies: Using the Up and down method with oral limit doses of 150, 300 and 600 mg/kg of AN whole plant water extract (ANWPE) gave no death in mice and rats. No fatal effects were observed during the course of both short term and long term monitoring. The animals showed no

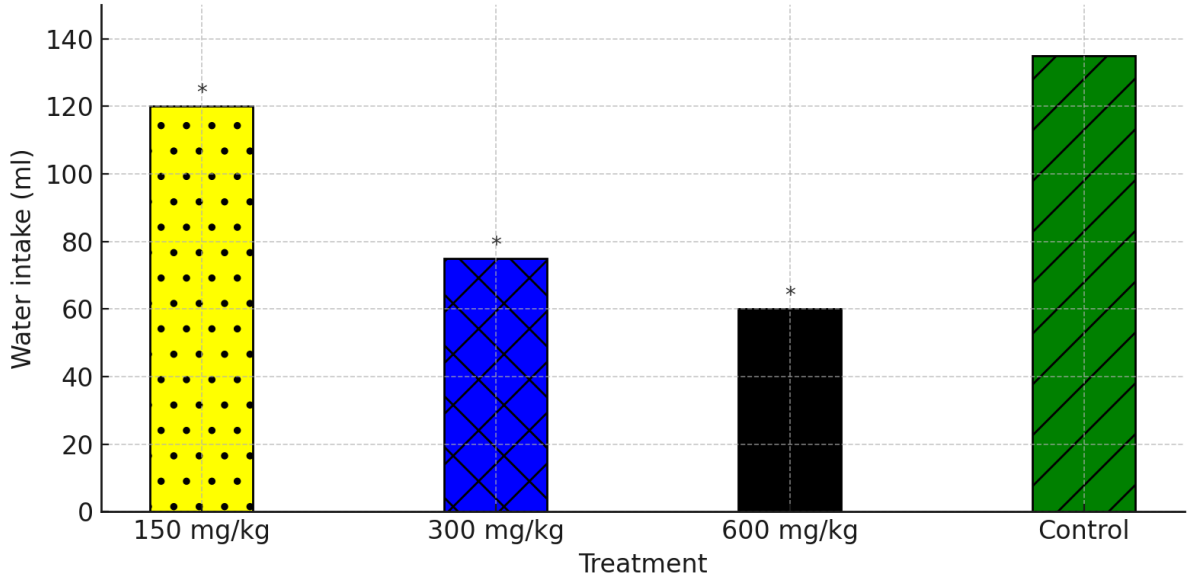
signs of toxicity for the entire 14 days of the study. The suggested amount of extraction therefore may be safe to these doses.

3.4.2 Sub-acute toxicity study with plant extract in rats: The male or female treated rats all lived till day 28 in the three cases where they were administered with 150, 300 or 600 mg/kg doses. Nevertheless, the fur of the treated rats in all doses seemed to be softer compared to those of control. The experiment rats were observed, however, there were no evident toxicity symptoms detected than those with the control. However, water consumption decreased significantly ($P<0.05$) in both 300 and 600 mg/kg groups and food ingested decreased for all doses ($P<0.001$) in comparison to the control (Figure 2).

Figure 2: Sub- Acute toxicity in mice after giving AN whole plant extracts with feed (a) and water (b)



a. Extract with feed



b. Extract with water

3.4.3 Relative weight gain

Results indicated that in both male and female rats, the control group showed a steady increase in body weight (14% and 18% respectively) over the four weeks (Figure 2). The groups administered with varying dosages of the extract (150, 300, and 600 mg/kg) showed weight gains compared to the control, with the highest dose (600 mg/kg) showing the least weight gain in both genders. This indicates that the extract reduced the percentage of weight gain in both male and female rats, maintaining it at a level closer to the control group (Table 3).

Table 3: Effect of sub-acute administration of AN extract on the relative weight of organs (%)

Organ	**Relative weight of organs (%)**			
	Control	**Extract dose**		
		150mg/kg	**300mg/kg**	**600mg/kg**
Liver	3.66±0.32	4.24±0.38	3.91±0.11	4.01±0.23
Heart	0.35±0.01	0.44±0.02	0.36±0.01	0.32±0.02
Lungs	0.64±0.07	0.89±0.09	0.63±0.01	0.73±0.07
Kidney	0.72±0.03	0.60±0.07	0.74±0.03	0.72±0.06
Spleen	0.45±0.02	0.49±0.05	0.66±0.03	0.53±0.11

Note: Values are presented as Mean ± S.E.M, n = 6;

Significant about control at *$p < 0.05$, One-way ANOVA.

3.5 Study of Liver and Kidney Function Indices and biochemical enzyme activities

In the enzyme activity study, rats treated with 150 mg/kg and 300 mg/kg doses of *A. nervosa* extract showed a significant decrease in kidney alkaline phosphatase (ALP) activity compared to the control group (39.69 ± 0.34), with values dropping to 26.99 ± 1.20 and 29.61 ± 0.67, respectively. The highest dose (600 mg/kg) resulted in a further decrease to 17.12 ± 0.86, indicating a dose-dependent reduction in kidney ALP. Similarly, the kidney aspartate aminotransferase (AST) activity significantly increased in the 150 mg/kg and 300 mg/kg groups (16.28 ± 1.34 and 16.91 ± 0.04, respectively) but decreased in the 600 mg/kg group (11.34 ± 0.36), suggesting a potential hepatoprotective effect at higher doses. Serum ALP and AST levels also increased with treatment, with the 150 mg/kg and 300 mg/kg doses significantly elevating these markers compared to control (7.84 ± 0.02, 4.60 ± 0.15 for 150 mg/kg and 7.37 ± 0.16, 4.41 ± 0.16 for 300 mg/kg), while the 600 mg/kg dose showed a lesser increase. Liver ALP and AST activities decreased across all treated groups, with the most significant reduction observed at the 600 mg/kg dose (1.13 ± 0.04 and 1.23 ± 0.03), indicating potential protective effects on liver function (Table 4).

For the liver and kidney function indices, the administration of *A. nervosa* extract significantly affected serum biochemical parameters. Serum total protein levels increased markedly in rats receiving 2.5 mg/kg (299.25 ± 1.49) and 12.5 mg/kg (451.82 ± 0.94) doses compared to control (248.43 ± 4.73). The serum total bilirubin level showed a dose-dependent response; it decreased significantly at 2.5 mg/kg (1.39 ± 0.02), while at higher doses (12.5 mg/kg and 25 mg/kg), it showed a moderate increase (1.93 ± 0.04 and 1.91 ± 0.04, respectively), indicating a complex interaction with hepatic function. Serum creatinine levels were significantly reduced in all treatment groups, suggesting improved renal function, particularly at 12.5 mg/kg (639.45 ± 5.60) compared to control (751.85 ± 5.29). Serum chloride and bicarbonate ions were variably affected; serum chloride levels decreased significantly with increasing doses, with the lowest level observed at 25 mg/kg (126.42 ± 1.99), while serum bicarbonate remained relatively unchanged across all groups, indicating a maintained acid-base balance (Table 5).

Table 4: Effects of *A. nervosa* extract on enzyme activities of liver, kidney, and serum of rats

Group/Doses	Kidney ALP	Kidney AST	Serum ALP	Serum AST	Liver ALP	Liver AST
Control	39.69 ± 0.34	8.19 ± 1.03	5.28 ± 0.03	2.37 ± 0.18	3.14 ± 0.14	0.93 ± 0.07
150 mg/kg body weight	26.99 ± 1.20	16.28 ± 1.34	7.84 ± 0.02	4.60 ± 0.15	1.84 ± 0.08	1.56 ± 0.08
300 mg/kg body weight	29.61 ± 0.67	16.91 ± 0.04	7.37 ± 0.16	4.41 ± 0.16	1.63 ± 0.02	1.58 ± 0.09

Group/Doses	Kidney ALP	Kidney AST	Serum ALP	Serum AST	Liver ALP	Liver AST
600 mg/kg body weight	17.12 ± 0.86	11.34 ± 0.36	7.62 ± 0.11	3.95 ± 0.09	1.13 ± 0.04	1.23 ± 0.03

Note: Values are presented as Mean ± S.E.M, n = 6;

Significant about control at *$p < 0.05$, One-way ANOVA.

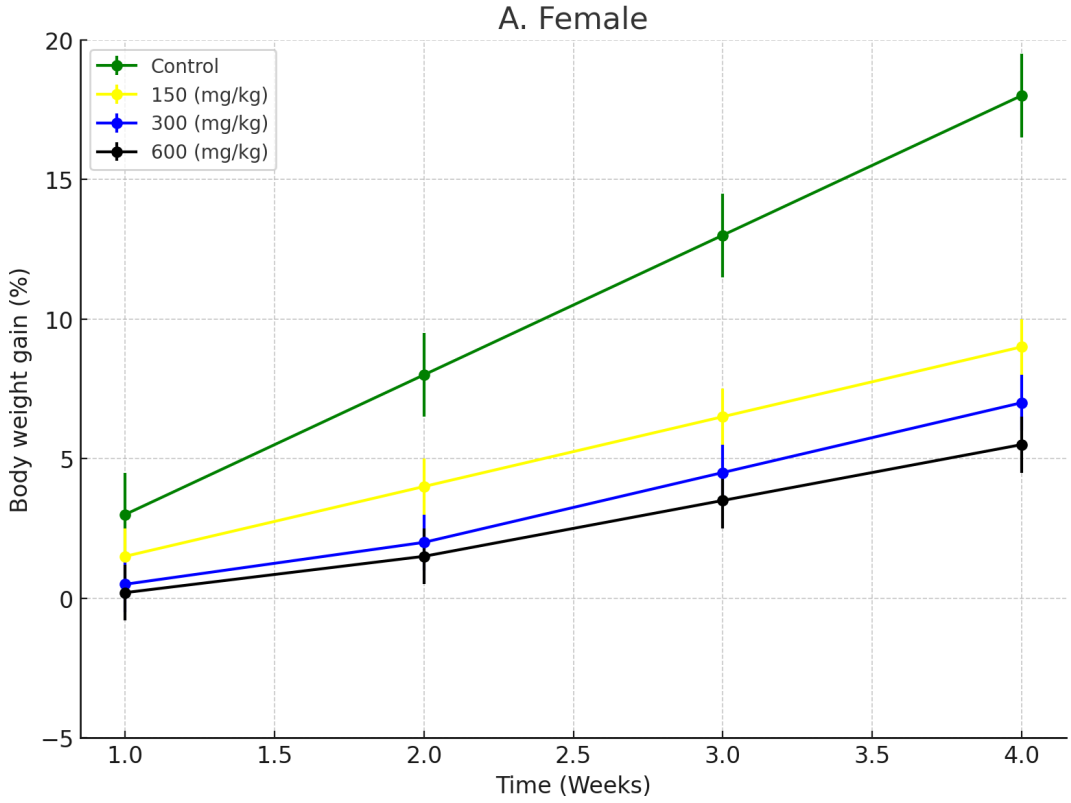
Table 5: Effects of *A. nervosa* extract on Liver and Kidney Function Indices in Rats

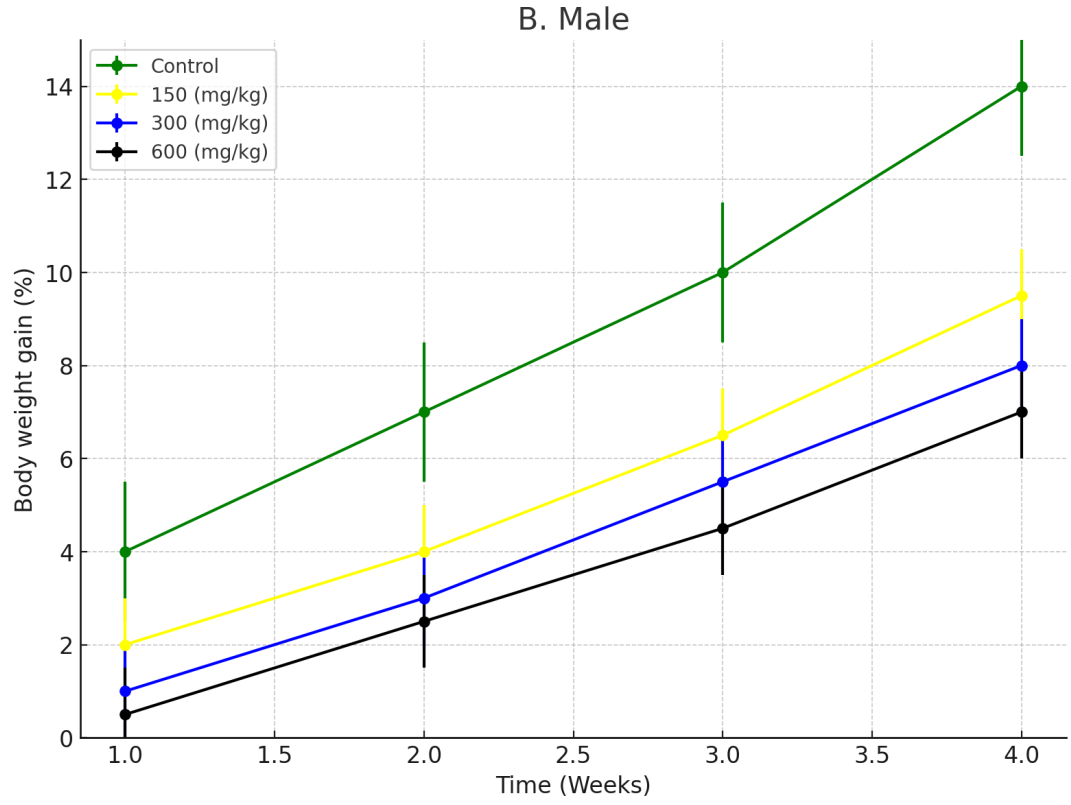
Groups/Doses (mg/kg body weight)	Serum Total Protein (mg/ml)	Serum Total Bilirubin (µmol/L)	Serum Creatinine (mmol/L)	Serum Chloride Ions (mmol/L)	Serum Bicarbonate Ions (mmol/L)
Control	248.43 ± 4.73	2.13 ± 0.01	751.85 ± 5.29	483.84 ± 4.77	40.25 ± 2.67
150mg/kg body weight	299.25 ± 1.49	1.39 ± 0.02	664.69 ± 6.06	368.34 ± 2.16	41.74 ± 0.07
300 mg/kg body weight	451.82 ± 0.94	1.93 ± 0.04	639.45 ± 5.60	341.99 ± 9.26	40.77 ± 0.06
600 mg/kg body weight	306.25 ± 2.18	1.91 ± 0.04	689.56 ± 6.85	126.42 ± 1.99	41.06 ± 0.21

Note: Values are presented as Mean ± S.E.M, n = 6;

Significant about control at *$p < 0.05$, One-way ANOVA.

Figure 3: Effect of AN extract on weight gain of females (A) and males (B)


A. Female
Control
150 (mg/kg)
300 (mg/kg)
600 (mg/kg)
Body weight gain (%)
Time (Weeks)
20
15
10
5
0
−5
1.0
1.5
2.0
2.5
3.0
3.5
4.0


B. Male
Control
150 (mg/kg)
300 (mg/kg)
600 (mg/kg)
Body weight gain (%)
Time (Weeks)
14
12
10
8
6
4
2
0
1.0
1.5
2.0
2.5
3.0
3.5
4.0

When compared with the control, there was a statistically significant ($p<0.05$) rise in urea and decrease of bicarbonate in males rats at 300 mg/kg. Serum level of AST increased in a dose dependent manner compared to the controls. Compared with the control, there was a significant increase in it at values of 150 and 600 mg/kg. Significantly increased serum ALT of 600mg/kg compared with controls. The other parameters did not differ considerably except in the control group (Table 6).

3.7 The haematological parameters:

In the female and male rats showed a significant ($p<0.05$) increase in lymphocytes at 300 and 600 mg/kg and mean platelet volume at 600mg/kg dose of extract compared.

The other hematological parameters did not show any significant difference with the control. The mean relative weight of the ovaries differed significantly from the control ($p < 0.05$) for each dosage. Still, the rise was not dose-related (Table 6).

Table 6: Effect of aqueous extract of *A. nervosa* on biochemical parameters of female rats

Parameter	600 mg/kg	300 mg/kg	150 mg/kg	Control
WBC (x10³/µL)	10.24 ± 2.67	8.50 ± 1.60	8.16 ± 1.92	9.12 ± 0.95
LY (x10³/µL)	4.38 ± 0.68	3.58 ± 1.00*	2.16 ± 0.39*	4.28 ± 1.07*
MO (x10³/µL)	1.00 ± 0.36	1.50 ± 0.35	1.06 ± 0.37	1.34 ± 0.14
GR (x10³/µL)	4.30 ± 2.23	3.42 ± 1.43	4.94 ± 1.77*	3.68 ± 0.45
LY (%)	54.14 ± 7.90*	45.10 ± 11.68	39.06 ± 13.82	44.78 ± 7.54
MO (%)	8.88 ± 0.87*	13.34 ± 1.84	11.10 ± 2.30	12.24 ± 1.66
GR (%)	36.12 ± 7.20*	40.26 ± 11.11	49.80 ± 11.98*	42.20 ± 6.29
RBC (x10⁶/µL)	5.91 ± 0.24	6.21 ± 0.38	6.66 ± 0.62	6.02 ± 0.25
Hg (g/dL)	12.92 ± 0.56	13.20 ± 0.90	14.86 ± 1.40	13.18 ± 0.63
HCT (%)	40.24 ± 2.08	43.46 ± 2.61	45.54 ± 3.49	41.10 ± 1.56
MCV (fL)	68.22 ± 1.16	69.54 ± 0.48	68.92 ± 1.72	68.12 ± 0.83
MCH (pg)	21.22 ± 1.17	21.68 ± 0.25	22.32 ± 0.47	22.20 ± 0.18
MCHC (g/dL)	31.36 ± 0.46	31.34 ± 0.23	32.42 ± 0.64	31.62 ± 0.35
RDW (%)	15.42 ± 0.91	14.18 ± 0.28	15.38 ± 0.56	16.58 ± 0.62
PLT (x10³/µL)	514.20 ± 36.70	531.33 ± 47.07	467.60 ± 43.13	504.00 ± 3.20
PCT (%)	0.44 ± 0.03	0.48 ± 0.04	0.41 ± 0.04	0.41 ± 0.07
MPV (fL)	9.24 ± 0.10*	9.00 ± 0.15	7.74 ± 0.17	7.11 ± 0.17
PDW (fL)	14.46 ± 0.54	14.46 ± 0.68	13.46 ± 0.46	13.16 ± 0.65

Note: Values are presented as Mean ± S.E.M, n = 6;

Significant about control at *$p < 0.05$, One-way ANOVA.

Table 7: Effect of aqueous extract of *A. nervosa* on biochemical parameters of male rats

Parameter	600 mg/kg	300 mg/kg	150 mg/kg	Control
WBC (x10³/µL)	12.50 ± 3.29	8.60 ± 2.40	6.76 ± 0.87*	12.60 ± 3.29
LY (x10³/µL)	9.25 ± 1.87*	7.38 ± 1.96	5.14 ± 0.78*	9.16 ± 2.90*
MO (x10³/µL)	0.44 ± 0.11	0.18 ± 0.11	0.10 ± 0.03	0.56 ± 0.17
GR (x10³/µL)	2.93 ± 0.32	3.55 ± 0.52	3.10 ± 0.21	3.16 ± 0.90
LY (%)	71.20 ± 3.80	86.36 ± 2.11	75.96 ± 4.84	74.40 ± 5.16
MO (%)	4.62 ± 1.71	2.24 ± 0.05	1.50 ± 0.32	5.34 ± 1.72
GR (%)	58.23 ± 4.15	41.23 ± 7.20	38.50 ± 4.66	60.40 ± 3.33
RBC (x10⁶/µL)	7.11 ± 0.36	7.7 ± 0.11	7.71 ± 0.21	7.24 ± 0.38
Hgb (g/dL)	13.28 ± 0.49	14.58 ± 0.35	14.50 ± 0.13	13.20 ± 0.57
HCT (%)	38.30 ± 1.48	42.38 ± 1.57	40.64 ± 0.51	38.14 ± 2.91
MCV (fL)	53.33 ± 1.31	53.74 ± 1.52	52.88 ± 1.04	51.28 ± 1.24
MCH (pg)	18.33 ± 0.53	19.04 ± 0.50	18.76 ± 0.30	18.32 ± 0.72
MCHC (g/dL)	34.40 ± 0.10	35.44 ± 0.11	35.62 ± 0.35	34.18 ± 0.14
RDW (%)	17.30 ± 0.45	17.36 ± 0.18	16.86 ± 0.68	18.18 ± 0.29
PLT (x10³/µL)	694.50 ± 71.41	598 ± 54.72	737.20 ± 58.02	671.80 ± 41.29
PCT (%)	0.44 ± 0.13	0.44 ± 0.04	0.50 ± 0.04	0.41 ± 0.07
MPV (fL)	7.84 ± 0.69*	7.16 ± 0.07*	3.04 ± 1.64	4.71 ± 1.79
PDW (fL)	22.43 ± 7.22	17.52 ± 1.89	16.18 ± 0.90	20.60 ± 4.50

Note: Values are presented as Mean ± S.E.M, n = 6;

Significant about control at *$p < 0.05$, One-way ANOVA.

4. Discussion

According to Majouli et al. (2017), natural plant extracts utilized in ethnopharmacology's traditional remedies can also be effectively employed in pharmacological models, offering alternative treatments for a range of illnesse, However, the toxicological assessment of herbal extracts is crucial for determining their safety in animal models, which can provide insights into potential human health risks. The Present study on *A. nervosa* bojar. provides significant insights into the phenolic and flavonoid content, antioxidant activity, and the potential toxicity of plant extracts in rats. Plants contain

phenolic compounds, a chemically varied class of secondary metabolites with a variety of health advantages, including antioxidant properties (Csepregi and Hideg, 2018). The findings of present study indicated that extracts from *A. nervosa*, particularly the aqueous extract, possess high concentrations of phenols and flavonoids, which are compounds known for their potent antioxidant properties. The antioxidant activity, evaluated using DPPH free radical scavenging assays, showed that the aqueous extract demonstrated the highest antioxidant capacity among all solvent extracts, which aligns with its high phenolic and flavonoid content. This strong antioxidant activity suggests a potential protective effect against oxidative stress, a condition that can cause cellular damage and contribute to various diseases (Lipiński et al., 2017). However, the toxicity evaluation of these extracts is crucial to determine their safety for therapeutic use. Acute and subacute toxicity studies conducted on Sprague-Dawley rats revealed that the aqueous extract exhibited minimal toxicity and was well tolerated at various dosages. In trials on acute oral toxicity, the ethanolic extract of *A. nervosa* demonstrated low toxicity, which made it a viable option for protecting the nephron against gentamicin-induced nephrotoxicity (Gadiparthi et al., 2019). Despite the presence of high phenolic and flavonoid content, which could theoretically contribute to toxicity through pro-oxidant effects at high concentrations, the study found no significant adverse effects in terms of organ weights, hematological parameters, or liver and kidney function tests. Interestingly, while the antioxidant activity of phenols and flavonoids could mitigate oxidative stress and thus reduce potential toxicity, their abundance did not correlate with any increase in toxicity markers in the animal models used. This lack of a direct correlation between high antioxidant content and toxicity supports the safe use of *A. nervosa* extracts at certain concentrations, although further studies are necessary to fully elucidate the mechanisms involved and to establish comprehensive safety profiles for human applications. Studies have shown that certain herbal extracts, such as those from *Ardisia elliptica* and *Plectranthus neochilus*, exhibit no acute toxicity even at high doses, indicating their safety for consumption (Ephraim et al., 2024; Pei et al., 2024). The elevation of alanine aminotransferase (ALT), Alkaline phosphatase (ALP), and aspartate aminotransferase (AST) is a common indicator of liver damage (Hrytsyk and Hanna, 2023). Several studies have shown that extracts like *Nauclea latifolia* and Acalypha torta do not significantly alter these enzyme levels, suggesting a lack of hepatotoxicity. ALT, AST, and ALP levels can increase due to various factors, including the presence of flavonoids in herbal extracts. However, a dose-dependent rise in ALP does not necessarily indicate liver damage, as other tissues can influence it.

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