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Pharmacological Effect of *Andrographis paniculata* in Ammonium Acetate Induced Hyperammonemia: A Dose-dependent Study

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Abstract

Andrographis paniculata is a commonly used medicinal plant in south Asian countries including India and Sri Lanka. Ammonium acetate was used as a food preservation reagent in food industries. This study is focused on the pharmacological effect of *Andrographis paniculata* on the functioning of the liver, kidney and brain in ammonium acetate induced hyperammonemia rats. The hyperammonemia is induced by intraperitoneal injection of ammonium acetate 100 mg/kg body weight thrice a week for eight consecutive weeks. Hyperammonemia rats are supplemented orally with *Andrographis paniculata* at 50 and 100 mg/kg body weight doses. Hyperammonemic rats showed increased circulating levels of ammonia, uric acid, creatinine, bilirubin, nitric oxide, lipid profile, enzymatic liver marker, sodium/potassium-ATPase and reduced level of urea. *Andrographis paniculata* supplementation prevented the histopathological alteration of a vital organ such as liver, brain and kidney tissue. The pharmacological effects are more pronounced in rats treated with 100 mg/kg b.w of *Andrographis paniculata* and comparable with the standard drug sodium benzoate drug-treated rats.

Keywords: Ammonium Acetate, *Andrographis paniculata*, Antioxidant, Histopathology

1. Introduction

In mammals intake of nitrogenous foods are converted to ammonia before excretion. Ammonia is a common by-product of amino acids, proteins and other derived of nitrogen bases catabolism. An increased level of ammonia is associated with significant neurotoxic effects involving high levels of Reactive Oxygen Species (ROS) production. The increased level of ammonia in the brain leads to several neurological disorders including urea cycle disorders[1], Reye'S syndrome[2] and hepatic encephalopathy[3] associated with liver failure[4,5]. To protect the neurotoxic effect of ammonium acetate, several plant extracts are being tested as they exert less or no side effects and confer protection against ROS induced toxicity.

Andrographis paniculata plant is well known in Tamil Nadu by the Vernacular Name: "Nilavembu". The most commonly *Andrographis paniculata* plant leaves and roots have been traditionally used all over the countries for different medicinal purposes and specifically in Asia and Europe at a larger extent because of the ethnic knowledge and abundance of natural resources. It has many pharmacological activities such as anti hepatoprotective[6], anti immunological potential[7,8], anti-cancer activity[9], anti-inflammation

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activity[10], anti angiogenic[11], anti-hyperglycemic activity[12], anti-malarial activity[13], anti fertility[14] and ect. The lethal dose (LD_{50}) value of *Andrographis paniculata* crude extract is 5000 mg/kg body weight.

Even though *Andrographis paniculata* has many pharmacological effects in the ayurvedic formulation, there is no evidence to support the pharmacological effect of *Andrographis paniculata* ethanolic crude extract in ammonium acetate induced hyperammonemia. Therefore the main objective of the study was to explore the effect of *Andrographis paniculata* on ammonia. Circulating levels of renal markers, Nitric Oxide (NO), lipid profile, activities of liver marker enzymes and sodium/potassium-ATPase were assessed along with and histopathological assessments of liver, brain and kidney tissue in ammonium acetate induced hyperammonemia in albino Wistar rats.

2. Materials and Methods

2.1 Confirmation of the Plant Used

The fresh clean leaves of *Andrographis paniculata* were collected from Coimbatore, India. The taxonomic authentication was confirmed by Botanical Survey of India (BSI), Southern Circle, Coimbatore, India. A specimen of the leaves is deposited and authenticated by Tamil Nadu Agricultural University (BSI/SRC/5/23/2018/Tech/2385).

2.2 Animals

Male albino Wistar rats (180-200 g) were procured from our Central Animal House facility.

A total number of 30 rats are used throughout their experimental period of eight weeks. Rats were housed in Polypropylene cages (47 cm x 34 cm x 20 cm) containing dry husk, renewed every 24 hours. The rats were maintained under a 12:12 hours light/dark cycle at 23±2°C and had water ad libitum and food on a standard pellet diet. The procedures in the present study were explained in detail adhering to CPCSEA guidelines and approval was obtained from IAEC of Karpagam Academy of Higher Education (KAHE/IAEC/2019/15-06/009). All efforts were made to minimize the number of rats used for these studies as well as minimize rats suffering throughout the experiments.

2.3 Chemicals

Ammonium acetate is purchased from Sigma-Aldrich (St. Louis. Mo, USA). Other chemicals were purchased from Merk and Himedia (India) in analytical grade.

2.4 Experimental Induction of Hyperammonemia

Induction of hyperammonemia disease condition in male albino Wistar rats was achieved by the newly made prepared solution of ammonium acetate at a dose of 100 mg/kg body weight (b.w.) through Intraperitoneal route (i.p). Ammonium acetate was administrated in three times a week for eight consecutive weeks throughout the study duration as described[15].

2.5 Crude Extract Preparation

The *Andrographis paniculata* leaves were shade dried at room temperature and powered mechanically. 500 gm of the powdered sample was subjected to extraction with ethanol by Soxhlet apparatus. The solvent was collected and removed under reduced pressure in a rotavapor. The leaf extract was stored at room temperature[16].

2.6 Experimental Design

The experiment was performed following regulation specified by the Institutional Animal Ethics Committee of Karpagam Academy of Higher Education (KAHE/IAEC/2019/15-06/009). Healthy male rat (180-200 gm) were used in the current study. Animals were fed on a standard diet with water ad libitum. Experimental male albino Wistar rats with the same weight were split into six groups with six rats each. The extract was administered orally as suspensions with water at inducted doses to untreated and ammonium acetate treated rats three days per week[16].

Group I - Normal rats.

Group II - Rats received intraperitoneal injection of ammonium acetate (100 mg/kg b.w) induced experimental rats.

Group III - Rats received intraperitoneal injection of ammonium acetate (100 mg/kg b.w) + Oral treatment of *Andrographis paniculata ethanolic extract* (50 mg/kg b.w).

Group IV - Rats received intraperitoneal injection of ammonium acetate (100 mg/kg b.w) + Oral treatment of *Andrographis paniculata ethanolic extract* (100 mg/kg b.w).

Group V - Rats received intraperitoneal injection of ammonium acetate (100 mg/kg b.w) + Oral treatment of Standard drug sodium benzoate (50 mg/kg b.w).

2.7 Biochemical Determinations

The biochemical determination of blood ammonia[17], plasma urea[18], plasma uric acid[19], serum creatinine[20] and serum bilirubin[21]using methods.

Activities of enzymatic liver marker like AST[22], ALT[23], ALP[24] and GGT[25] by the methods. To estimate the levels of nitric oxide[26], glutamate[27], glutamine[28] and Na^+/K^+ ATPase[29]. To calculate the TBARS[30], conjugated dienes[31] and lipid hydroperoxides[32] using methods. The antioxidant activities of SOD[33], CAT[34] and reduced glutathione[35] were measured. To determination of total cholesterol[36], phospholipids[37], triglycerides[38] and free fatty acid[39].

2.8 Histopathology

The tissues excised were cleansed with physiological saline and kept in 10% formalin solution used for histopatho-

logical assessments. Histopathology of the vital organs viz. kidney, liver and brain were removed from all the experimental rat's groups were subjected to embedding the tissues in paraffin. Then the experimental tissues were sectioned (2-5 µm) using a microtome to get sections of 2-5 microns. Subsequently, to understand the histopathology of vital organs, the sections were stained with Hematoxylin and Eosin (H and E). The images were captured through Olympus CX41 (40X) light microscope and representative images were displayed in the results.

2.9 Statistical Analysis

One-way Analysis Tool of Variance (ANOVA) was used to assess the statistical significance between different groups using Software Package for the Social Science (SPSS) version 15 followed by Duncan's Multiple Range Test (DMRT). The results are expressed as average ± SD. The significance was assessed at $p \leq 0.05$[40].

3. Results

3.1 Protective Effect of *Andrographis paniculata* Ethanolic Crude Extract on Renal Circulation (Ammonia, Urea, Uric Acid, Creatinine and Bilirubin)

Hyperammonemia-induced rats show significantly increased serum levels of ammonia, uric acid, creatinine, bilirubin and significantly decreased level of urea when compared to the untreated rats. Oral administration of *Andrographis peniculata* ethanolic crude extract (50 mg/kg and 100 mg/kg b.w.) for eight consecutive weeks to hyperammonemic rats resulted in reduced levels of serum ammonia, uric acid, creatinine, bilirubin. However, the serum level of urea is increased significantly when compared to the hyperammonemic rats. The effects of the crude extract at 100 mg/kg b.w. are comparable with the standard drug Sodium Benzoate. Of all the two doses tested, the maximum reduced effect of *Andrographis paniculta* was observed at the dose of 100 mg/kg b.w. (Table 1).

Table 1. Table indicating the difference in the levels of ammonia, urea, uric acid, creatinine and bilirubin in the serum of untreated and treated rats as an index of renal function. "a, b, c, d" indicates statistical significance at $p<0.05$

Group	Normal	Ammonium acetate (100 mg/kg b.w.)	Ammonium acetate + *Andrographis paniculate* (50 mg/kg b.w.)	Ammonium acetate + *Andrographis paniculate* (100 mg/kg b.w.)	Ammonium acetate + Sodium benzoate
Blood ammonia	81.6 ± 7.97^{a}	351.83 ± 40^{d}	230 ± 25.37^{c}	139.69 ± 12.56^{b}	134.33 ± 12.97^{b}
Plasma urea	46.6 ± 4.98^{d}	11 ± 0.89^{a}	18.2 ± 1.88^{b}	25.3 ± 1.71c	39.4 ± 2.82^{c}
Plasma Uric acid	7.08 ± 0.58^{a}	10.68 ± 0.89^{d}	9.06 ± 0.75^{c}	8.42 ± 0.63b	8.87 ± 0.63^{b}
Serum Creatinine	2.65 ± 0.18^{a}	5.89 ± 0.47^{d}	4.52 ± 0.36^{c}	3.36 ± 0.24b	3.56 ± 0.25^{b}
Serum Bilirubin	2.34 ± 0.15^{a}	4.76 ± 0.37^{d}	3.99 ± 0.30^{c}	3.06 ± 0.23b	2.89 ± 0.18^{b}

3.2 Effect of *Andrographis paniculata* Ethanolic Crude Extract on Liver Marker Enzymatic Activity

Ammonium acetate induced hyperammonemic rats revealed that there were increased functions of ALT, AST, ALP and GGT in comparison withuntreated rats. Oral administration of *Andrographis peniculata* ethanolic crude extract (50 mg/kg and 100 mg/kg b.w.) in experimental hyperammonemic rats significantly reverted the functions of liver marker enzymes when compared to the hyperammonemic rats (Table 2).

3.3 Effect of *Andrographis paniculata* Ethanolic Crude Extract on NO, Na^{+}/K^{+} ATPase, Glutamate and Glutamine in the Brain

The brain tissue of hyperammmonemic rats indicates the raised levels of NO (Nitrous oxide), Na^{+}/K^{+} ATPase, glutamine and glutamate in when compared to untreated rats. Oral supplementations of *Andrographis peniculata* ethanolic crude extract (50 mg/kg and 100 mg/kgb.w.) in hyperammonemic rats illustrate significantly lowered levels of NO, Na^{+}/K^{+} ATPase, glutamine and glutamate in brain contrast to experimental hyperammonemic rats (Table 4).

Table 2. Table indicating the difference in the specific activities of AST, ALT, ALP and γGTP in the serum of untreated and treated rats as an index of liver function. "a, b, c, d" indicates statistical significance at $p<0.05$

Group	Normal	Ammonium acetate (100 mg/kg b.w.)	Ammonium acetate + *Andrographis paniculate* (50 mg/kg b.w.)	Ammonium acetate + *Andrographis paniculate* (100 mg/kg b.w.)	Ammonium acetate + Sodium benzoate
Aspartate transaminase (IU/l)	60.71 ± 1.98^{a}	111.88 ± 7.96^{d}	105.66 ± 6.66^{c}	76.64 ± 4.42^{b}	70.12 ± 3.21^{b}
Alanine transaminase (IU/l)	27.9 ± 3.0^{a}	62.32 ± 2.66^{d}	55.67 ± 2.31^{c}	29.2 ± 1.32^{b}	22.24 ± 1.98^{b}
Alkaline phosphatase (IU/l)	70.97 ± 3.2^{a}	133.74 ± 8.17^{d}	128.45 ± 7.78^{c}	102.61 ± 6.3^{b}	97.75 ± 5.23^{b}
γGTP (IU/l)	6.39 ± 1.0^{a}	14.99 ± 2.9^{c}	17.54 ± 3.12^{d}	10.19 ± 1.6^{b}	8.45 ± 1.22^{b}

Table 3. Table indicating the difference in the specific activities of SOD, CAT and GPx in the liver of untreated and treated rats as an index of antioxidant function. In addition, the lipid parameters such as triglycerides, total cholesterol, phospholipid and free fatty acids are also assessed and represented between groups. "a, b, c, d" indicates statistical significance at $p<0.05$. U^{A} – One unit of enzyme activity which results in 50% inhibition of nitroblutetrazodium reduction/minute. U^{B} - µmol of hydrogen peroxide utilized/minute

Group	Normal	Ammonium acetate (100 mg/kg b.w.)	Ammonium acetate + *Andrographis paniculate* (50 mg/kg b.w.)	Ammonium acetate + *Andrographis paniculate* (100 mg/kg b.w.)	Ammonium acetate + Sodium benzoate
TBARS (mmol/g tissue)	1.56 ± 0.54^{a}	4.81 ± 1.22^{d}	3.74 ± 1.03^{c}	2.49 ± 0.99^{b}	2.20 ± 0.97^{b}
Lipid hydroperoxide (mmol/g tissue)	105.7 ± 3.85^{a}	144.5 ± 6.06^{d}	137.63 ± 5.86^{c}	125.25 ± 4.14^{b}	128.65 ± 4.21^{b}
Conjugated diene (mmol/100g tissue)	79.65 ± 3.98^{a}	108.03 ± 7.49^{d}	101.55 ± 8.75^{c}	85.49 ± 4.98^{b}	90.65 ± 2.22^{b}
Total cholesterol (mg/100g tissue)	76.8 ± 3.98^{a}	153.59 ± 2.5^{d}	146.64 ± 2.23^{c}	103.67 ± 6.97^{b}	98.33 ± 5.42^{b}
Triglycerides (mg/100g tissue)	57.59 ± 1.88^{a}	103.67 ± 5.97^{d}	96.45 ± 4.65^{c}	76.8 ± 3.98^{b}	72.12 ± 2.76^{b}
Phospholipids (mg/100g tissue)	107.51 ± 1.88^{a}	172.79 ± 8.5^{d}	169.98 ± 7.34^{c}	142.07 ± 6.29^{b}	150.78 ± 5.65^{b}
Free fatty acids (mg/100g tissue)	76.8 ± 3.98^{a}	153.59 ± 7.5^{d}	150.87 ± 6.45^{c}	115.19 ± 8.3^{b}	119.76 ± 7.43^{b}
SOD (UA/g tissue)	6.65 ± 0.55^{d}	3.34 ± 0.3^{a}	4.97 ± 0.39^{b}	5.54 ± 0.41^{c}	5.99 ± 0.43^{c}
Catalase (UB/g tissue)	8.86 ± 0.58^{d}	3.75 ± 0.25^{a}	5.69 ± 0.41^{b}	6.11 ± 0.43^{c}	7.1 ± 0.51^{c}
GPx (µmoles of GSH utilized/g protein)	$43.\ 26 \pm 4.22^{d}$	12.77 ± 1.2^{a}	24.54 ± 2.1^{b}	30.6 ± 2.78^{c}	$35.\ 12 \pm 3.1^{c}$

3.4 Effect of *Andrographis paniculata* Ethanolic Crude Extract in Lipid Peroxidation and Lipid Profile

The hyperammonemic rat shows significantly high volume of lipid peroxidation products such as TBARS (Thiobarbituric Acid Reactive Substance), CD (Conjugated Dienes) and HP (Lipid Hydroperoxides) as compared to untreated rats. Oral administrations of *Andrographis peniculata* ethanolic crude extract (50 mg/kg and 100 mg/kg b.w.) in hyperammonemic rats show significantly low amount of Lipid Peroxidation (LPO) products such as TBARS, CD and HP when compared to ammonium acetate induced hyperammonemic rats in liver, brain and kidney (Tables 3, 4, 5).

Table 4. Table indicating the difference in the specific activities of SOD, CAT and GPx in the brain of untreated and treated rats as an index of antioxidant function. In addition, the lipid parameters such as triglycerides, total cholesterol, phospholipid and free fatty acids are also assessed and represented between groups. "a, b, c, d" indicates statistical significance at $p<0.05$. U^A – One unit of enzyme activity which results in 50% inhibition of nitroblutetrazodium reduction/minute. U^B - µmol of hydrogen peroxide utilized/minute

Group	Normal	Ammonium acetate (100 mg/kg b.w.)	Ammonium acetate + *Andrographis paniculate* (50 mg/kg b.w.)	Ammonium acetate + *Andrographis paniculate* (100 mg/kg b.w.)	Ammonium acetate + Sodium benzoate
Nitric oxide(µmol/mg tissue)	1.58 ± 0.01^a	7.31 ± 0.01^d	4.54 ± 0.65^b	5.14 ± 0.01^c	6.76 ± 0.98^c
Glutamate (mmol/g tissue)	10.32 ± 2.12^a	22.69 ± 4.34^d	13.56 ± 2.41^b	17.11 ± 3.13^c	19.87 ± 3.93^c
Glutamine (mmol/g tissue)	7.93 ± 0.66^a	16.16 ± 1.51^d	9.87 ± 0.64^b	12.54 ± 0.98^c	13.93 ± 1.18^c
TBARS (mmol/g tissue)	55.5 ± 1.32^a	98.95 ± 0.22^d	65.74 ± 4.86^c	54.99 ± 3.87^b	46.65 ± 0.56^b
Lipid hydroperoxide (mmol/g tissue)	123.84 ± 8.63^a	135.85 ± 11.95^d	122.63 ± 5.86^c	105.57 ± 9.46^b	113.65 ± 3.21^b
Conjugated diene (mmol/100g tissue)	61.50 ± 1.32^a	86.95 ± 6.13^d	92.55 ± 8.75^c	76.99 ± 3.87^b	81.65 ± 2.22^b
Total cholesterol (mg/100g tissue)	82.96 ± 3.54^a	145.27 ± 3.39^d	143.64 ± 2.23^c	78.39 ± 4.64^b	81.33 ± 5.42^b
Triglycerides (mg/100g tissue)	73.64 ± 1.88^a	109.19 ± 6.3^d	98.45 ± 4.65^c	87.16 ± 5.75^b	80.12 ± 2.76^b
Phospholipids (mg/100g tissue)	91.0 ± 6.19^a	140.39 ± 6.4^d	148.98 ± 7.34^c	105.55 ± 8.14^b	115.78 ± 5.65^b
Free fatty acids (mg/100g tissue)	51.08 ± 2.77^a	101.0 ± 6.19^d	108.87 ± 6.45^c	76.96 ± 3.54^b	81.76 ± 7.43^b
SOD (UA/g tissue)	12.03 ± 0.83^a	5.3 ± 0.56^d	6.57 ± 1.11^c	10.5 ± 0.67^c	9.77 ± 0.32^b
Catalase (UB/g tissue)	8.31 ± 0.27^c	10.94 ± 0.19^d	14.51 ± 1.09^c	23.55 ± 0.22^b	31.83 ± 2.65^b
GPx (µmoles of GSH utilized/gprotein)	10.89 ± 0.66^c	06.89 ± 0.2^d	8.65 ± 0.51^c	9.12 ± 0.44^b	11.76 ± 0.12^b

Table 5. Table indicating the difference in the specific activities of SOD, CAT and GPx in the kidney of untreated and treated rats as an index of antioxidant function. In addition, the lipid parameters such as triglycerides, total cholesterol, phospholipid and free fatty acids are also assessed and represented between groups. "a, b, c, d" indicates statistical significance at $p<0.05$. U^A – One unit of enzyme activity which results in 50% inhibition of nitroblutetrazodium reduction/minute. U^B - µmol of hydrogen peroxide utilized/minute.

Group	Normal	Ammonium acetate (100 mg/kg b.w.)	Ammonium acetate + *Andrographis paniculate* (50 mg/kg b.w.)	Ammonium acetate + *Andrographis paniculate* (100 mg/kg b.w.)	Ammonium acetate + Sodium benzoate
TBARS (mmol/g tissue)	1.59 ± 0.06^a	3.94 ± 0.15^c	3.24 ± 0.12^d	2.19 ± 0.18^b	2.65 ± 0.56^b
Lipid hydroperoxide (mmol/g tissue)	78.56 ± 3.87^a	103.56 ± 7.19^c	125.63 ± 5.86^d	94.63 ± 2.45^b	117.65 ± 3.21^b
Conjugated diene (mmol/100g tissue)	54.0 ± 1.21^a	99.49 ± 5.97^c	104.55 ± 8.75^d	86.49 ± 4.64^b	92.65 ± 2.22^b
Total cholesterol (mg/100g tissue)	75.03 ± 3.14^a	175.11 ± 3.83^d	140.64 ± 2.23^c	95.16 ± 4.64^b	100.33 ± 5.42^b
Triglycerides (mg/100g tissue)	47.24 ± 1.88^a	76.48 ± 4.87^d	71.45 ± 4.65^c	65.43 ± 2.32^b	66.12 ± 2.76^b
Phospholipids (mg/100g tissue)	88.48 ± 4.87^a	156.03 ± 7.5^d	142.98 ± 7.34^c	112.86 ± 9.63^b	120.78 ± 5.65^b
Free fatty acids (mg/100g tissue)	54.91 ± 2.8^a	110.83 ± 6.53^d	108.87 ± 6.45^c	75.11 ± 3.2^b	87.76 ± 7.43^b
SOD (UA/g tissue)	9.87 ± 0.42^d	4.89 ± 0.2^a	7.57 ± 1.11^b	8.68 ± 0.23^c	10.77 ± 0.32^c
Catalase (UB/g tissue)	7.03 ± 2.80^d	3.17 ± 0.92^a	4.51 ± 1.09^b	5.11 ± 1.4^c	6.83 ± 2.65^c
GPx (µmoles of GSH utilized/g protein)	6.13 ± 0.66^d	2.88 ± 0.22^a	3.65 ± 0.31^b	4.81 ± 0.54^c	4.76 ± 0.52^c

Hyperammonemic condition rats illustrate the increased amount of total cholesterol, triglycerides, phospholipids and free fatty acids when compared to untreated rats in the liver, brain and kidney (Tables 3, 4, 5). Hyperammonemic rats orally treated with *Andrographis peniculata* ethanolic crude extract (50 mg/kg and 100 mg/kg b.w.) reduced in the total cholesterol, triglycerides, phospholipids and free fatty acids as compared to hyperammonemic rats. In this case, it was found that the observed effect of the crude extract was equivalent to the standard drug sodium benzoate (100 mg/kg b.w).

3.5 Effect of *Andrographis Paniculata* Ethanolic Crude Extract on Enzymatic Activities (SOD, CAT and GPx)

Ammonium acetate induced hyperammonemic rats exhibited decreased activities of the enzymes SOD, CAT and GPx as compared with untreated rats. Oral treatments of *Andrographis peniculata* ethanolic crude extract (50 mg/kg and 100 mg/kg b.w.) showed significantly increased activity of SOD, CAT and GPx when related to hyperammonemic rats in liver, brain and kidney (Tables 3, 4, 5).

3.6 Histopathology Description

Figure 1 represents the characterize the light microscope of untreated and ammonium acetate induced hyperammonemic experimental rats liver stained within hematoxylin and eosin (H and E). Olympus CX41 (40X). Histopathological examination states that intraperitoneal injection of ammonium acetate leads to severe cellular death and infiltration of immune cells in the hepatic tissues. These are associated with typical changes indicating the deterioration of hepatocytes such as enhanced nuclear staining and pyknosin. Hyperammonemic rats orally treated with *Andrographis peniculata* ethanolic crude extract (50 mg/kg and 100 mg/kg b.w.) show mild aberration in hepatocytes infiltration and suggested the congestion of central vein.

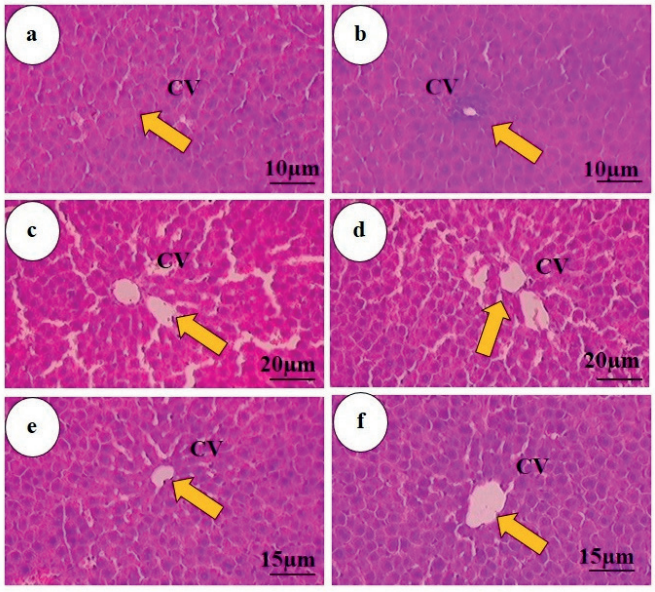


Figure 1. Representative picture depicting the histopathological changes in the liver of normal and experimental rats. The images of the stained sections were captured and represented used Olympus Trinocular microscope at a magnification of 40X. Significant observations in the histoarchitecures in different groups are represented by yellow arrow marks in the figure.
a & b Untreated rats, c. ammonium acetate, d. ammonium acetate + *Andrographis paniculata* (50 mg/kg b.w), e. ammonium acetate + *Andrographis paniculata* (100 mg/kg b.w) and ammonium acetate + Sodium benzoate (50 mg/kg b.w).
Histological examination showed that i.p injection of ammonium acetate caused severe necrosis and infiltration of mononuclear inflammatory cells. In addition, we observed that many hepatocytes were shrunken, pyknotic,and darkly stained small nuclei (indicated by arrow marks). Hyperammonemic rats treated with *Andrographis paniculata* Group a and b yellow arrow shows apparently normal hepatic triads architecture with radically arranged central vein.
Group c and d Yellow shows severe fibrosis, steatosis, tubular epithelial degeneration and fatty infilteration of mononuclear with irregular shaped cells.
Group e and f yellow arrow shows mild steatosis, epithelial degeneration and in filtered mononuclear cells
CV – Central Vein; PV – Portal Vein

Figure 2 represents the light microscope (40X) image of brain section from untreated and ammonium acetate induced hyperammonemic experimental rats with hematoxylin and eosin (H and E). Histopathological evaluation emphasize that i.p. injection of ammonium acetate causes degenerated perineuronal edema the neuron structures. Moreover, many neurons were shrunken with darkly stained small nuclei. When Hyperammonemic rats were co-administred with *Andrographis peniculata* ethanolic crude extract (50 mg/kg and 100 mg/kg b.w.), it does not depict the accumulation of fluid and indicate the absence of perineuronal edema in comparison with hyperammonemic rats.

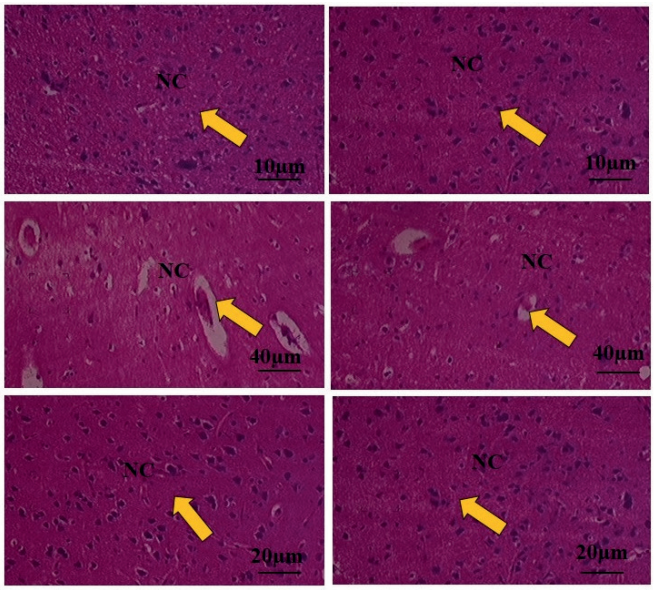


Figure 2. Representative pictures depicting the histopathological changes in the brain of normal and experimental rats. The images of the stained sections were captured and represented used Olympus Trinocular microscope at a magnification of 40X. Significant observations in the histoarchitecures in different groups are represented by yellow arrow marks in the figure.
a and b Untreated rats, c. ammonium acetate and d. ammonium acetate + *Andrographis paniculate* (50 mg/kg. b.w), ammonium acetate + *Andrographis paniculate* (100 mg/kg. b.w) and ammonium acetate + Sodium benzoate (50 mg/kg. b.w). Hyperammonemic rats exhibits degenerated perineuronal edemathe neuron structures. Moreover, many neurons were shrunken, pyknotic,and darkly stained small nuclei. Hyperammonemic rats treated with *Andrographis*

paniculate did not show any neuronal edema or neuronal alterations.
Group a and b yellow arrow shows apparently normal cerebral architecture.
Group c and d yellow arrow shows severe microcystic degeneration and perineuronal edema.
Group e and f yellow arrow shows minimal necrotic degeneration and perineuronal edema.
NC– Neuronal cell

Figure 3 represents the light microscope (40X) of untreated and ammonium acetate induced hyperammonemic experimental rats brain stained within hematoxylin and eosin (H and E). Ammonium acetate induced hyperammonemic rats shows tubular epithelial degeneration by mononuclear cell infiltrated edema and necrosis structures. Hyperammonemic rats that received oral supplementation of *Andrographis peniculata* ethanolic crude extract (50 mg/kg and 100 mg/kg b.w.) did not indicate tissue damage or neuronal alterations, cells albeit mild epithelial degeneracy with mild necrosis. These changes appear to indicate the non toxic nature of the plant extract.

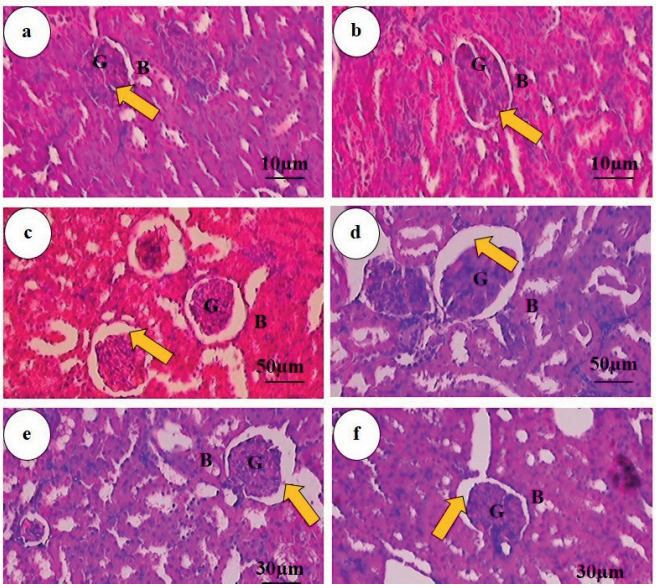


Figure 3. Representative pictures depicting the histopathological changes in the kidney of normal and experimental rats. The images of the stained sections were captured and represented used Olympus Trinocular microscope at a magnification of 40X. Significant observations in the histoarchitecures in different groups are represented by yellow arrow marks in the figure.
a and b Untreated rats, c. ammonium acetate and d. ammonium acetate + *Andrographis paniculate* (50 mg/kg. b.w), ammonium acetate + *Andrographis paniculate* (100 mg/kg. b.w) and ammonium acetate + Sodium benzoate (50 mg/kg. b.w). Hyperammonemic rats showed tubular epithelial degeneration with mononuclear cell infiltrated edema and necrosis structures. Hyperammonemic rats treated with naringin did not show any tissue damage and it shows mild degeneration of epithelial cells with mild necrosis.
Group a and b yellow arrow shows apparently normal histological architecture of kidney tissue.
Group c yellow arrow shows severe tubular necrosis and glomeruli surrounded by tightly packed cells.
Group d yellow arrow shows very less tubular necrosis and glomeruli surrounded by tightly packed cells.
G – Glomeruli; B – Bowmanns capsule.

4. Discussion

The increased levels of ammonia in the blood cause harmful effect and decreased level of urea leads to urea cycle disorder which causes lack of one or more of the urea cycle enzymes such as Carbamyl Phosphate Synthetase I (CPS I), N-acetylglutamate synthetase, Ornithine Transcarbamylase (OTC), Argininosuccinate Synthetase (ASS), Argininosuccinate Lyase (ASL) and arginase[41]. These enzymes can remove ammonia from bloodstream[42]. In a healthy individual, urea is eliminated in the urine through the kidneys. Disorder in the urea cycle could result in the accumulation of ammonia leading to hyperammonemia and coma irreversible brain damage[43,44]. Therefore present study findings on the ability of the orally administered ethanolic crude extract *Andrographis paniculata* in decreasing the serum levels of ammonia, uric acid, creatinine and bilirubin and increased amount of urea formation in experimental hyperammonemic rats.
Liver damage might be due to the increased activities of aspartate transaminase and alanine transaminase that are abundant in liver. When rats fed with monosodium glutamate it causes increased activities of plasmaalanine transaminase, aspartate transaminase, alanine transaminase and gamma glutamyl transpeptidase which poses a major threat for liver damage[45].
The release of liver marker enzymes into the blood and its cytoplasmic location during liver damage as indicated by elevated serum levels indicates structural damage. During physiological process, ammonium ions are produced when amino acids are metabolized in the liver[46,47]. There ions are hazardous if not eliminated through the reactions of urea cycle. Free glutamates could be obtained when there is a disease in sodium moiety that are present in the monosodium glutamate[48,49]. In the present study consequently, hyperammonemic rats treated with oral supplementations of ethanolic crude extract *Andrographis paniculata* decreased levels of liver marker enzymes like alanine transaminase, aspartate transaminase, alanine transaminase and gamma glutamyl transpeptidase (ALT, AST, ALP and GGT).

The physiological processes of all the organs and tissues are influenced by nitric oxide which are short lived highly reactive free radical. Free radicals regulates crucial physiological processes such as neurotransmission and memory formation, regulation of blood pressure, liver regeneration and mediates the immune system through improved bactericidal and tumoricidal activities[50]. In present study indicate orally administration of ethanolic crude extract *Andrographis paniculata* decreased the level of nitric oxide in experimental hyperammonemic rats.

The amino acid undergoes deamination to form ammonium ions that are malignant and harmful if not eliminated through the urea cycle. The amino acid metabolism and their derivatives take place to a certain extent in the liver. Free glutamates could be obtained via dissociation of sodium moiety that is present in monosodium glutamate. Thus the excess ammonium ion due to the intake of glutamate or monosodium glutamate causes liver damage and further release of transiminase enzyme that results in high levels of ammonium ions in plasma[51,52]. The increase in the activities of serum transaminases is consistent with earlier observations in male albino rats. Detailed that oxidative stress causes an increase in serum transaminase due to alterations in the membrane permeability and integrity, thus altering leakage of intracellular enzymes. The results showed that increased levels of glutamate and glutamine in orally treated of ethanolic crude extract *Andrographis paniculata* in hyperammonemic rats indicates the restoration of these enzymes and membrane permeability.

Thus high levels of ammonium intoxication lead to excess function of N-methyl D-aspartate receptors which results inneuronal degeneration and death[53]. This could be due to the elevated level of Ca^{2+} ions in the postsynaptic neuron. When Ca^{2+} binds with calmodulin, it activates Nitric Oxide Synthase (NOS) and increases Nitric Oxide (NO) formation which leads to a neurotoxic process. Thus triggering the NMDA receptors could lead to the highest production of superoxide (O_2) radicals that areinduced by hydroxyl radicals generated by NO and O_2. The free radical scavenging properties of the *Andrographis paniculata* ethanolic crude extract is evident in treated as the levels of antioxidant enzymes are restored and lipid peroxidation. The reduction in the levels of lipid peroxidation products could be taken as a measure of effective scavenging of free radicals[54]. Therefore, it can be that *Andrographis paniculata* ethanolic crude extract inhibits excess activation of NMDA receptors and NMDA receptor and receptor-mediated neurotoxicity thus it gives neuroprotection. This neuroprotective activity of *Andrographis paniculata* ethanolic crude extract proves the way for effective treatment of acute neuronal damage and disability.

5. Conclusion

Andrographis paniculata is commonly well-known as 'King of Bitters' under the family of *Acanthaceae*. The *Andrographis paniculata* plant leaves and roots have been usually used all over the countries for a different medicinal purpose like the common cold; relieve the body of heat (fever) and to displace toxins from the body[55]. In this present study overall conclusion advises that the *A. paniculata* has the potential of the excellent anti-hyperammonemic agent. The sodium benzoate was compared with 100 mg/kg of ethanolic crude extract, it was found to be more or less similar. The ammonium acetate induced experimental hyperammonemia orally treated with the ethanolic crude extract in different concentrations like 50 and 100 mg/kg b.w. in the rats with consequences of changes in renal circulation and histopathological components 100 mg/kg b.w act as an effective dose of hyperammonemic function in future studies.

6. Conflict of Interest

No conflict of interest for the authors was found.

7. References

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