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Original Research Article

The Positive Effects of the Methanol Leaf Extract of *Andrographis paniculata* on Ethanol-Induced Ulcergenic and Hepatic Damage in Wistar Rats

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Abstract: This study aimed to assess the preventive properties of the methanol extract of *Andrographis paniculata* on the development of ulcers and liver damage in Wistar albino rats. The rats were divided into five groups, each consisting of 10 rats. The groups were given different dosages of *Andrographis paniculata* extract, omeprazole, orally, orally, and ethanol orally. The liver tissues were then examined for lipid peroxidation. The results showed that the extract of *Andrographis paniculata* showed substantial efficacy in preventing liver damage caused by ethanol. The levels of malondialdehyde (MDA) in the positive control group were significantly higher ($p < 0.05$), than those in the normal control group. The administration of *Andrographis paniculata* at doses of 200 mg/kg and 400 mg/kg in groups 3 and 4, respectively, resulted in a statistically significant decrease ($p < 0.05$), in MDA levels. The administration of omeprazole at a dosage of 30 mg/kg also resulted in a notable reduction ($p < 0.05$), in MDA levels. The positive control group exhibited a significant increase ($p < 0.05$), in serum AST, ALT, and ALP levels, while a decrease ($p < 0.05$), in total protein and albumin levels. The administration of Andrographis paniculata at doses of 200 and 400 mg/kg, as well as omeprazole at a dose of 30 mg/kg, led to a significant reduction ($p < 0.05$), in AST, ALT, and ALP levels, and a significant increase ($p < 0.05$), in total protein and albumin levels compared to the positive control group. The findings suggest that the methanol extract of *Andrographis paniculata* may contain antioxidant compounds that exhibit hepatoprotective properties.

Keywords: Positive effects *Andrographis paniculata*, Ulcerogenic, Albino rats, Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Total Protein.

INTRODUCTION

Andrographis paniculata, a significant medicinal plant belonging to the Acanthaceae family, holds substantial importance globally. Widely utilized in traditional herbal medicine across Sub-Saharan Africa, China, and India, *Andrographis paniculata* serves as a valuable botanical resource with diverse therapeutic applications [1]. It is utilised in ethnobotany for the therapeutic management of many conditions such as snake bites, bug bites, diabetes, diarrhoea, fever, and malaria. The plant's adaptability in traditional medicine highlights its potential as a useful asset for the creation of natural cures and pharmaceutical goods [2]. It is a frequently utilised herbal plant in the old Unani and Ayurvedic medicinal systems. The plant has a rich historical background in ancient Indian medical traditions, underscoring its importance and

universal recognition as a useful therapeutic asset [1]. Lately, there has been a growing use of commercially prepared extracts of *Andrographis paniculata* in specific countries. Nevertheless, it is necessary to establish a set of standards for these preparations in order to improve their effectiveness. *Andrographis paniculata*, a plant containing chemicals such as diterpenoids, lactones, flavonoids, and flavonoid glycosides, is traditionally used in Asia and Europe to treat various maladies. Its above-ground and leaves parts are predominantly utilised for therapeutic purposes [3]. *Andrographis paniculata* has been extensively studied and found to possess a diverse range of pharmacological properties. These include its ability to inhibit the growth of cancer cells, alleviate diarrhoea, combat hepatitis, suppress HIV replication, lower blood sugar levels, reduce inflammation, fight against microbial infections, treat malaria, act as an antioxidant, support cardiovascular health, induce cell death in certain cancers, protect the liver, enhance the immune system, and address sexual dysfunctions [4]. It is native to Sub-Saharan Africa, Mainland China, and India. The prevalence of this phenomenon is widespread in tropical and subtropical parts of Asia, particularly in Southeast Asia. Moreover, this plant flourishes in many phytogeographical and edaphic zones throughout Asia [5]. *Andrographis paniculata* is a significant herbal remedy within the Andrographis genus. The number of species in this genus varies in different studies, with estimations ranging from 19 [6], 28, 40, or 44 species. The exact number of species within the Andrographis genus has not been determined. The chromosomal count of *Andrographis paniculata* is 25 in the gametophytic count and 50 in the sporophytic count. Examining genotypic variations is essential for finding germplasm with high production potential. This herbaceous plant is an annual, multi-branched, and erect species that thrives in hedgerows throughout fields, hilly slopes, wasteland, farms, watery habitats, seasides, and roadsides. Furthermore, it has the ability to be cultivated in parks.

Andrographis paniculata flourishes in moist and dark habitats, such as forests and wastelands, which facilitate its robust growth [7] encompassing *Andrographis paniculata* has a broad distribution and is extensively cultivated in several places. It thrives prolifically throughout Asia, the Southern and Southeastern Asia parts, Furthermore, *Andrographis paniculata* is located in the southern region of Nigeria. The extensive dispersion and farming of *Andrographis paniculata* underscores its importance as a significant medicinal plant in several geographical regions, especially in Asia, as well as parts of the Americas and Africa [8]. Alcohol has a significant impact on the digestive system, particularly the liver and gastrointestinal tract (GIT). The effects of ethanol on these organs are multifaceted and can lead to various pathological conditions. Alcohol metabolism in the liver produces acetaldehyde, which is highly toxic and can lead to DNA damage and structural alterations in cellular organelles like mitochondria and endoplasmic reticulum [13, 14]. This process also generates reactive oxygen species (ROS), further contributing to liver injury [13].

Alcohol-induced liver damage can manifest as steatosis (fatty liver), inflammation, and ultimately cirrhosis [13]. Ethanol has direct effects on the GIT, including the stomach, small intestine, and colon. Alcohol can disrupt the gut mucosal barrier, leading to cell death, erosions, and loss of epithelium at the villus tips [13]. This disruption of the tight junctions between epithelial cells can increase intestinal permeability, allowing the passage of harmful substances into the body [13]. Alcohol-induced dysbiosis, or an imbalance in the gut microbiome, can further exacerbate these effects. This dysbiosis can lead to reduced concentrations of short-chain fatty acids (SCFAs) and amino acids, as well as an increased proportion of conjugated secondary bile acids [13]. These changes can contribute to nutrient deficiencies and the development of conditions like alcoholic cirrhosis [13, 15]. Furthermore, the breakdown of ethanol in the gastrointestinal tract (GIT) can result in heightened generation of ethanol in the immediate area. This, in turn, may play a role in the development of fatty liver disease associated with obesity [13-15].

Alcohol metabolism in the liver produces acetaldehyde, which is highly toxic and can lead to DNA damage and structural alterations in cellular organelles like mitochondria and endoplasmic reticulum [16]. This process also generates reactive oxygen species (ROS), further contributing to liver injury. Alcohol-induced liver damage can manifest as steatosis (fatty liver), inflammation, and ultimately cirrhosis.

Ethanol has direct effects on the gastrointestinal tract, including the stomach, small intestine, and colon. Alcohol has the ability to disturb the protective lining of the gut, known as the gut mucosal barrier. This disruption can result in programmed cell death (apoptosis), the formation of erosions, and the breakdown of the outer layer of cells (epithelium) at the tips of the finger-like projections called villi. The disruption of the tight connections between epithelial cells can lead to an increase in intestinal permeability, which enables the entry of hazardous substances into the body. However, the search results indicate that the evidence linking alcohol consumption directly to the development of stomach ulcers is weak [16]. While alcohol consumption may worsen the symptoms of existing stomach ulcers, it is not considered a major risk factor for developing them [16]. The main risk factors for stomach ulcers include Helicobacter pylori infection, smoking, and use of nonsteroidal anti-inflammatory drugs [16]. In summary, ethanol has a significant impact on the liver, leading to various forms of liver injury, but its direct role in causing stomach ulcers is not well-established. Alcohol consumption may exacerbate the symptoms of existing stomach ulcers, but it is not considered a primary cause of their development [16].

MATERIAL AND METHODS

Animal and Diet

This study involved fifty healthy male wistar strain rats from the University of Benin's animal house in Nigeria. The rats were kept in standard cages and acclimatized in the Department of Pharmacology at Niger Delta University for two weeks, provided with pelleted chicken feed and water.

Preparation of Plant Extract

Fresh *Andrographicpaniculata* leaves were collected and placed in a clean, dry tray and allowed to shade dry for three weeks. Leaves that were shade dried were ground to powder by the use of an electric blender, the powder was weighed (60kg) and stored in a tightly sealed container. 1 kg of the powdered leave was soaked in 4.6L of methanol for 72hrs for the extraction. Methanol extract was collected, filtered and concentrated under decreased pressure using a rotary vacuum evaporator at 50^{o}C and 40rpm. Extract of methanol was weighed (65.95) and preserved inside a refrigerator at 4^{o}C for further use.

Experimental Design

- Group 1 (normal control) were fed with pelleted chicken feed and normal saline 10 mL/kg.
- Group 2 (Positive control) was administered ethanol (1mL/200g) and allowed free access to pelleted chicken feed and water.
- Group 3 (Test group 1) 200 mg/kgbody weight extract+1mL/200g ethanol were administered orally for 7 days.
- Group 4 (Test group 2) were administered 400mg/kg body weight extract+1mL/200g ethanol 7 days.
- Group5 (Standard group) were administered Omeprazole 30mg/kg body weight 7 days.

Ethics

The ethical council of Niger Delta University Amassoma, Nigeria, has granted approval for all animal protocols in compliance with the National Institutes of Health's Principles of Laboratory Animal Care. The animals were provided with compassionate care in accordance with the National Academy of Sciences' "Guide for the Care and Use of Laboratory Animals (1996)".

Biochemical Assay

Biochemical Parameters

The following biochemical parameters were determined spectrophotometrically using the respective kits and instructions provided in the biochemical kit manual.

Estimations of Biochemical Parameters

Serum Parameters

The study entailed the humane euthanasia of animals and the collection of blood for biochemical examination. The serum was extracted in order to measure liver function parameters using enzymatic kits from Accurex Biomedical Limited Pvt. Ltd., an Indian company, following the instructions provided by the manufacturer. The serum was utilised for the quantification of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatases (ALP).

Determination of Markers of Oxidative stress

The concentration of MDA was quantified using a method developed by Hunter *et al.*, in [6] and later improved by Gutteridge and Wilkins in [7].

Statistical Analysis

The study used GraphPadInStat to analyze the data, assessing its statistical significance using a one-way Analysis of Variance (ANOVA) with the Turkey-Kramer Multiple Comparison Test, with a p-value of less than 0.05.

RESULTS

The results for this study are summarized in table 3.1 table 3.2 below. Table (3.1) shows the mean concentration of ALP, AST, ALP and MDA in wistar rats after pretreatment with *Andrographis paniculata* extract for 7days. Table 3.2 shows the mean concentration of Total Protein and Albumin, wistar rats after pretreatment with *Andrographis paniculata* for 7 days.

Table 3.1: Mean serum ALT, AST, ALP activities and MDA concentration in wistar rats given *Andrographis paniculata* and Ethanol

GROUPS	ALT(IU/L)	AST(IU/L)	ALP(IU/L)	MDA (IU/mg protein)
Group 1: Normal control (Normal Sline)	79.0± 2.9[a]	72.66. ± 4.1[a]	83.88 ± 0.25[a]	1.89 ± 0.16[a]
Group 2: Positive control (Ethanol 1ml/200g)	158.34±13.0[b]	160.04± 5.30[b]	155.76 ± 3.9[b]	6.88. ± 1.83[b]
Group 3: Test 1 (A.P 200mg/kgbody weight+Ethanol 1ml/200g)	145.13± 5.4[c]	137.11± 6.4[c]	129.18 ± 2.9[c]	3.58± 0.16[c]
Group 4: Test 2 (A.P 400mg/kgbody weight +Ethanol 1ml/200g)	125± 10.0[d]	126.65± 6.20[c]	117.32 ± 4.4[d]	2.28 ± 0.91[d]
Group 5: Standard Control (Omeprazole 30mg/kg body weight+ Ethanol 1ml/200kg	128.21± 4.3[d]	127.81± 6.1[c]	115.81± 3.6[d]	2.24± 0.14[d]

The values are shown as the mean ± standard deviation (SD). Values with a different superscript from the control group are statistically significant at a p-value of less than 0.05.

The results in table (3.1) shows that the administration of the Ethanol caused a significant increase ($p < 0.05$) in ALT (158.34± 13), AST (160.04 ± 5.3), ALP (155.76± 3.9) activities in the positive control group compared to the normal control animals. However, treatment with *Andrographis paniculata* at doses of 200mg/kg body weight and 400mg/kg body weight significantly ($P<0.05$) reduced ALT (145.13.0± 5.4 and 125.25± 10.0), AST (137. 11 ± 6.4 and 127.81 ± 6.10), and ALP (129.18± 2.9 and 117.32 ± 4.4) activities compared to the positive control group. Treatment with Omeprazole at dose 30mg/kg also caused a noticeable decrease ($p < 0.05$) in serum ALT (128± 4.3), AST (127.81 ± 6.1) and ALP (115.81± 3.6) compared to the positive control group. The administration of Ethanol in the positive control group caused a remarkable increase ($p < 0.05$) in liver tissue MDA level (6.88. ±1.83) compared to the normal control animals (1.89 ± 0.16). On the other hand, treatment with *Andrographis paniculata* at doses of 200 and 400 mg/kg caused a noticeable decrease ($p < 0.05$) in the liver tissue MDA level (3.58± 0.16 and 2.28 ± 0.91) compared to the positive control group. Also, treatment with Omeprazole at dose 30mg/kg caused a noticeable decrease ($p < 0.05$) in liver tissue MDA level (1.24± 0.14) compared to the positive control group.

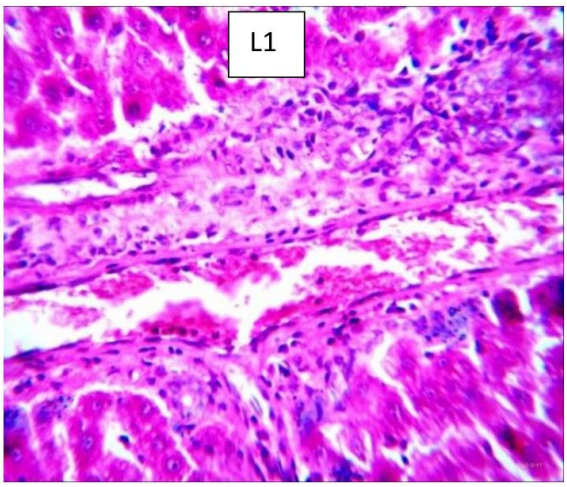
Table 3.2: Mean serum Total Protein and Albumin concentration in wistar rats given *Andrographis paniculata* and Ethanol

TREATMENT	Total Protein (g/dl)	Albumin (g/dl)
Group 1: Normal control (Normal saline)	6.77 ± 0.25 [a]	4.54 ± 0.16[a]
Group 2: Positive Control (Ethanol 1ml/200g)	4.42 ± 0.73[b]	1.92 ± 0.17 [b]
Group 3: Test 1 200mg/kgbody weight extract + Ethanol 1ml/200g	5.73 ± 0.0[c]	4.78 ± 3.0 [a]
Group 4: Test 2 (400mg/kgbody weight extract + Ethanol 1 ml/200g)	6.49 ± 0.62[a]	5.35 ±0.16 [c]
Group 5: Standard Control (Omeprazole 30 mg/kgbody weight + Ethanol 1ml/200g)	6.96 ± 0.51[a]	4.43 ± 0.07[a]

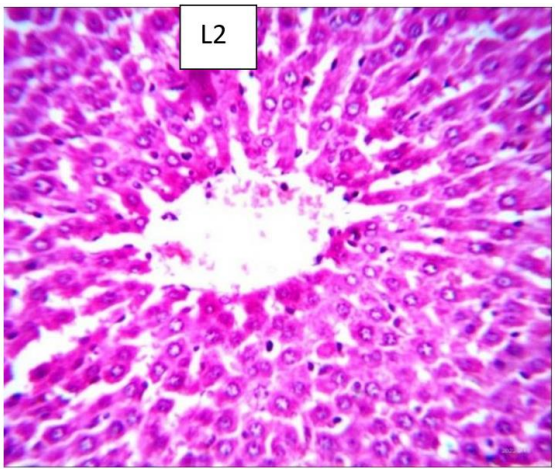
The values are shown as the mean ± standard deviation (SD). Values with a different superscript from the control group are statistically significant at a p-value of less than 0.05.

The result in table (3.2) shows that administration of Ethanol caused a significant decrease ($p < 0.05$) in Total Protein (4.42 ± 0.73) and Albumin (1.92 ± 17) activities in the positive control group compared to the normal control animals. However, treatment with *Andrographis paniculata* at doses of 200mg/kg body weight and 400 mg/kg body weight remarkably elevated Total Protein (4.73± 0.05 and 6.01 ± 0.89) and Albumin (4.78 ± 3.0 and 5.35 ± 0.16) activities respectively compared to the positive control group. Treatment with Omeprazole at dose 30mg/kg body weight also remarkably elevated Total Protein (6.69 ± 0.51) and Albumin (04.43 ± 0.07) activities compared to the positive control group.

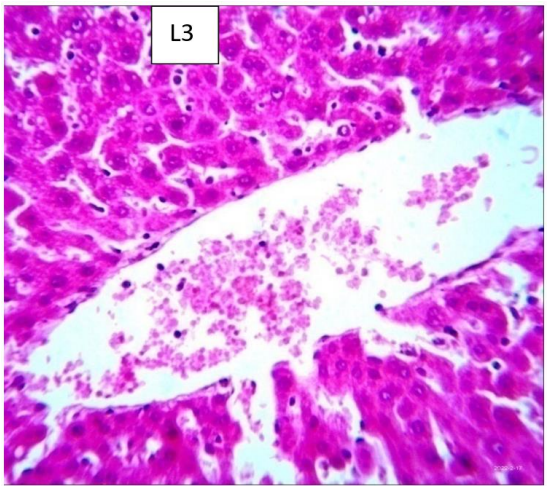
HISTOLOGY
Histology of the Liver



This is a transverse portion of the liver that has been stained with haematoxylin and eosin. x 400 magnification. Sections show normal Liver with normal central vein, sinusoids and hepatocytes consistent with histology of the liver.

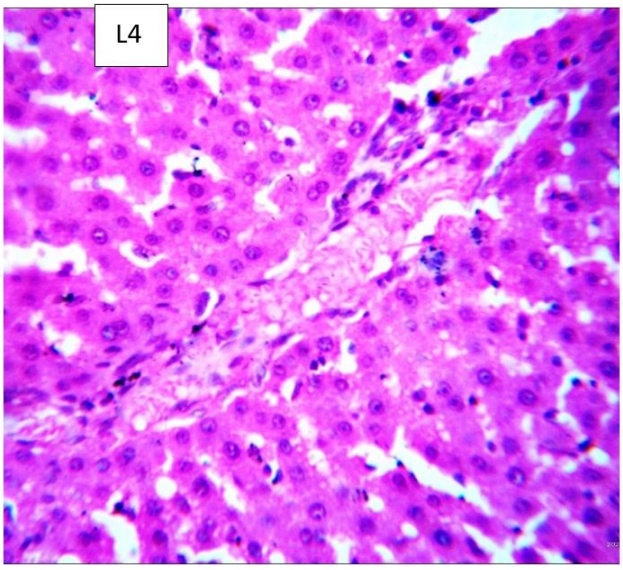


This is a transverse portion of the liver that has been stained with haematoxylin and eosin. x 400magnification. Sections show abnormal histology of the liver with marked congestion, necrosis of the portal tract and vasculitis.



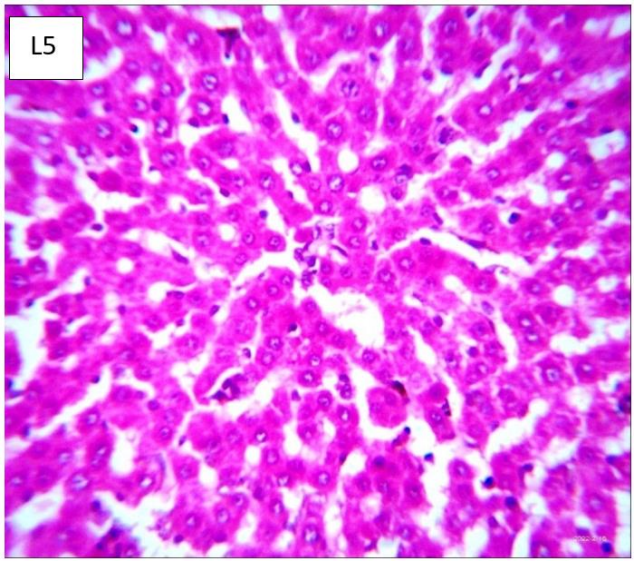
This is a transverse portion of the liver that has been stained with haematoxylin and eosin.

x 400magnification. Section shows congestion and inflammation of blood vessels.



This is a transverse portion of the liver that has been stained with haematoxylin and eosin.

x 400magnification. Sections show normal histology of the liver.



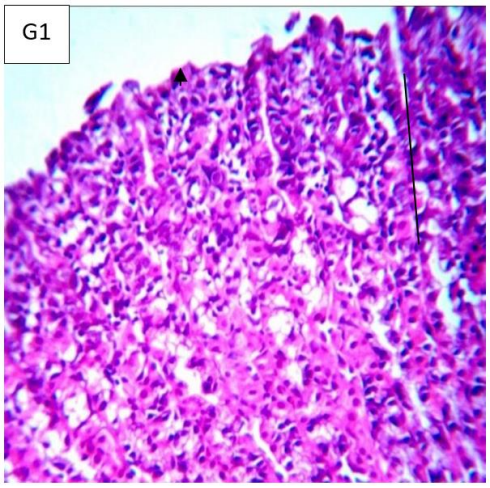
This is a transverse portion of the liver that has been stained with haematoxylin and eosin.

x 400magnification. Sections show normal histology of the liver.

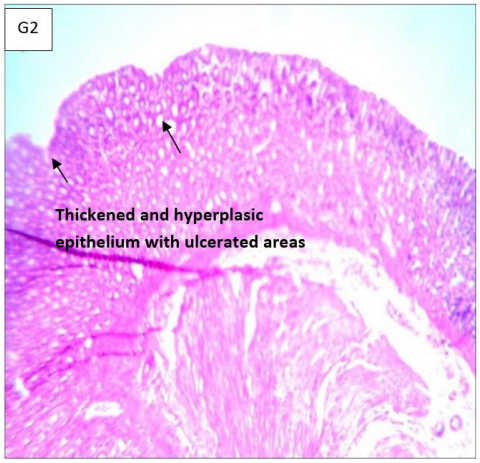
Figure 1: Photomicrograph of liver tissue of an adult wistar rat stained with haematoxylin and Eosin technique.

L1: This is a transverse section of the liver that has been stained with haematoxylin and eosin and magnified 400 times. The sections show a normal liver with a normal central vein, sinusoids, and hepatocytes, which is consistent with the histology of the liver. L2: This is a transverse section of haematoxylin and eosin stained slides, also magnified 400 times. The sections show abnormal histology of the liver, characterised by significant congestion, necrosis of the portal tract, and vasculitis. L3: This is a transverse section of haematoxylin and eosin stained slides, magnified 400 times. The section shows congestion and inflammation of blood vessels. L4: This is a transverse section of haematoxylin and eosin stained slides, magnified 400 times. The sections show normal histology of the liver. L5: This is a transverse section of haematoxylin and eosin stained slides, magnified 400 times. The sections show normal histology of the liver.

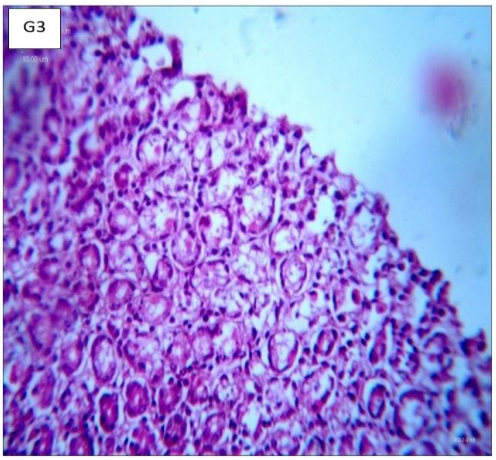
Histology of the Gastrointestinal Tract



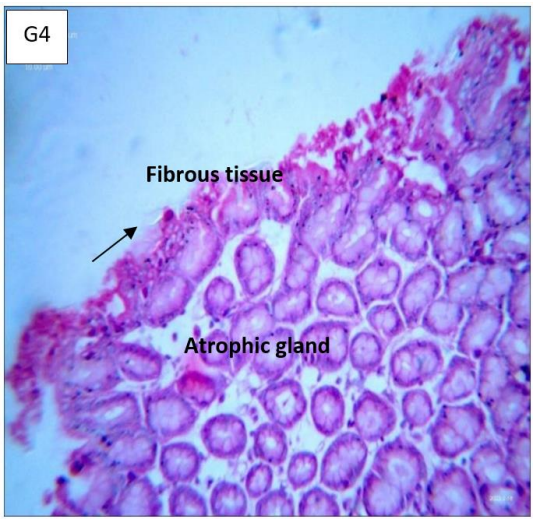
Normal Control): shows transverse section of the gastrointestinal tract with normal gastric pits and mucosa and abundant gastric glands consistent with normal histology of the gastrointestinal tract. X400mag



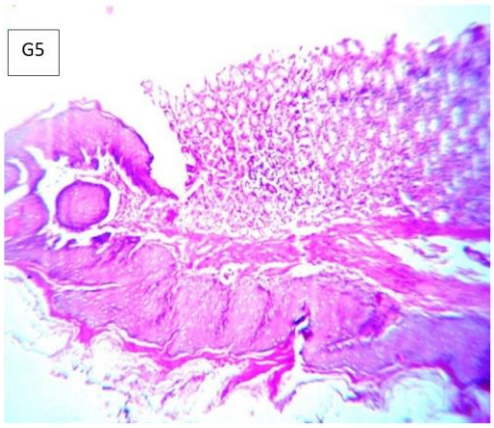
(Positive Control): Transverse section of haematoxylin and eosinstained slides x 400magnification. Sections shows hyperplasic and edematous gastric glands with area of ulceration.



(TEST GROUP I). Transverse section of haematoxylin and eosin stained slides x 400magnification. Section left show healing by fibrous



(TEST GROUP II) shows regenerating epithelium with gastric glands displaying normal columnar epithelium.



Transverse section of haematoxylin and eosin stained slides x 400magnification. Section shows a pyloric ring with surface consisting of nuclear fragments and mild inflammatory cells.

Figure 2: Photomicrograph of gastrointestinal tract tissue of an adult wistar rat stained with haematoxylin and Eosin technique

G1(Normal Control): shows transverse section of the gastrointestinal tract with normal gastric pits and mucosa and abundant gastric glands consistent with normal histology of the gastrointestinal tract. X400mag. G2 (Positive Control): Transverse section of haematoxylin and eosin stained slides x 400magnification. Sections shows hyperplasic and edematous gastric glands with area of ulceration. G3 (TEST GROUP I). Transverse section of haematoxylin and eosin stained slides x 400magnification. Section left show healing by fibrous G4 (TEST GROUP II) shows regenerating epithelium with gastric glands displaying normal columnar epithelium. G5 Transverse section of haematoxylin and eosin stained slides x 400magnification. Section shows a pyloric ring with surface consisting of nuclear fragments and mild inflammatory cells. G6 Transverse section of haematoxylin and eosin stained slides x 400magnification. Sections shows hyperplasic and edematous gastric glands with area of ulceration.

Conclusion: Atrophic gastritis and ulceration of the epithelium.

DISCUSSION

Oxidative stress is a condition where antioxidants and pro-oxidants, like free radicals, are in equilibrium, and elevated levels of pro-oxidants can cause damage to DNA, protein, and lipids [9, 10].

One of the key markers of oxidative stress is malondialdehyde (MDA), which is produced during lipid peroxidation [11]. The study found a significant rise in tissue MDA concentration in the positive control group (group 2) compared to the normal control group (group 1) following the administration of 1 ml/kg of ethanol. These findings suggest that the administration of ethanol caused a decline in the cellular structure of the liver in the animals in group 2.

Nevertheless, administering *Andrographis paniculata* at doses of 200 mg/kg body weight and 400 mg/kg body weight resulted in a noteworthy reduction in liver tissue MDA levels when compared to the positive control group. These findings indicate that *Andrographis paniculata* effectively reduced the rate of lipid peroxidation in the liver and preserved the integrity of liver cell membranes. Similarly, administration of omeprazole at a dosage of 30 mg/kg body weight resulted in a notable reduction in liver tissue MDA level when compared to the positive control group. The study revealed a statistically significant ($p<0.05$) rise in the serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in the positive control group (group 2) as compared to the normal control group (group 1). This signifies a disturbance in the structural organisation of the hepatocytes, leading to the release of certain liver marker enzymes into the bloodstream. The administration of *Andrographis paniculata* before therapy at dosages of 200 mg/kg and 400 mg/kg led to a significant reduction in AST, ALT, and ALP levels.

Similarly, the administration of omeprazole at a dosage of 30 mg/kg body weight led to a significant decrease in blood levels of ALT, AST, and ALP compared to the positive control group (group 2). The delivery of ethanol led to a significant reduction in the levels of serum total protein and albumin, in comparison to the normal control. Animals treated with the *Andrographis paniculata* extract showed an increase in the concentration of total protein and albumin, which was closely correlated with the dosage. This discovery implies that the extract has a beneficial effect on maintaining the structural and functional integrity of the liver.

The decline in serum total protein and albumin levels in the ethanol-intoxicated animals can be attributed to hepatic failure, as the liver is the main generator of most serum proteins, and their production is a dependable sign of proper liver function. The increase in total protein levels found in the treated rats suggests that the plant extracts include important phytochemical compounds that help in the recovery of the damaged liver, which aligns with the results reported by other researchers [12].

CONCLUSION

The study reveals that *Andrographis paniculata* has potent liver protection against ethanol damage. It reduces oxidative stress, maintains liver structural integrity, and restores total protein and albumin levels, indicating its potential as a natural treatment for alcohol-induced liver injury. This suggests *Andrographis paniculata* could be a promising natural remedy for preventing and treating liver-related issues.

REFERENCE

1. Akbar, S. (2011). Andrographis paniculata: a review of pharmacological activities and clinical effects. *Alternative Medicine Review*, *16*(1), 66–77.
2. Burkill, I. H., Birtwistle, W., Foxworthy, F., Scrivenor, J., & Watson, J. (2016). A Dictionary of the Economic Products of the Malay Peninsula. *Ministry of Agriculture and Co-operatives, Kuala Lumpur, Malaysia*.
3. Jarukamjorn, K., & Nemoto, N. (2008). Pharmacological aspects of Andrographis paniculata on health and its major diterpenoid constituent andrographolide. *Journal of Health Science*, *54*(4), 370–381.
4. Akbarsha, M. A., & Murugaian, P. (2010). Aspects of the male reproductive toxicity/male antifertility property of andrographolide in albino rats: effect on the testis and the cauda epididymidal spermatozoa. *Phytotherapy Research*, *14*(6), 432–435.
5. Benoy, G. K., Animesh, D. K., Aninda, M., Priyanka, D. K., & Sandip, H. (2012). An overview on *Andrographis paniculata* (burm. F.) Nees. *International Journal of Research in Ayurveda and Pharmacy*, *3*(6), 752–760.
6. Patidar, A. S., Gontia, A., Upadhyay, G., & Nayak, T. (2011). Biochemical constituents in Kalmegh (AndrographispaniculataNees.) under various row spacing's and nitrogen levels. *World Applied Sciences Journal*, *15*(8), 1095–1099.
7. Niranjan, S. K., Tewari, V., & Lehri, G. (2010). Biological activities of Kalmegh (AndrographispaniculataNees) and its active principles-A review. *Indian Journal of Natural Products and Resources*, *1*(2), 125–135.
8. Mishra, N. S., Sangwan, R. S., & Sangwan, S. (2007). Andrographis paniculata (Kalmegh): a review. *Pharmacognosy Reviews*, *1*(2), 283–298.
9. Alabi, Q. K., Akomolafe, R. O., Olukiran, O. S., Adeyemi, W. J., Nafiua, A. O., Adefisayo, M. A., Omole, J. G., Kajewole, D. I., & Odujoko, O. O. (2017). The Garcinia kola bi-flavonoid kolaviron attenuates experimental hepatotoxicity induced by diclofenac. *Pathophysiology*, *07*, 003
10. Catala, A. (2011). Lipid peroxidation of membrane phospholipids in the vertebrate retina. *Front. Biosci.*, *3*, 52–60.
11. Jakovljevic, B., Novakov-Mikic, A., Brkic, S., Bogavac, M. A., Tomic, S., & Miler, V. (2012). Lipid peroxidation in the first trimester of pregnancy. *Journal of Maternal, Fetal and Neonatal Medicine*, *25*, 1316–1318.
12. Uraku, N., Ezeani, C., Ezeali, F. U., Ali, C. A., Afiukwa & Ebenyi, L. N. (2016). Effects of Extract of Spilanthesuliginosa (SW) on Total Protein, Albumin, Total and Conjugated Bilirubin in Ethylene Glycol Exposed Albino Rats. *Asian Journal of Biochemistry*, *11*(1), 53-58.

13. Pohl, K., Moodley, P., & Dhanda, A. D. (2021). Alcohol's Impact on the Gut and Liver. *Nutrients*, *13*(9), 3170. https://doi.org/10.3390/nu13093170
14. Geokas, M. C., Lieber, C. S., French, S., & Halsted, C. H. (1981). Ethanol, the liver, and the gastrointestinal tract. *Ann Intern Med*, *95*(2), 198-211. doi: 10.7326/0003-4819-95-2-198. PMID: 7196192.
15. Keary, C., Terence, R., & Anna, M. D. (2000). Increased gastrointestinal ethanol production in obese mice: Implications for fatty liver disease pathogenesis. *Gastroenterology*, *119*(5), 1340-1347,
16. Rahman, Z., Dwivedi, D. K., & Jena, G. B. (2020). Ethanol-induced gastric ulcer in rats and intervention of tert-butylhydroquinone: Involvement of Nrf2/HO-1 signalling pathway. *Hum Exp Toxicol*, *39*(4), 547-562. doi: 10.1177/0960327119895559. Epub 2019 Dec 26. PMID: 31876185.
17. Chou, S. P. (1994). An examination of the alcohol consumption and peptic ulcer association--results of a national survey. *Alcohol Clin Exp Res*, *18*(1), 149-53. doi: 10.1111/j.1530-0277.1994.tb00895.x. PMID: 8198212.