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Hepatoprotective Effect of Combined Extracts of Turmeric and Curry Leaves on High Salt-Fed Wistar Rats

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Abstract

This experimental study aimed to explore the impact of a combined aqueous extract of turmeric and curry leaves (TCL) on biochemical markers and liver histology in high-salt diet-fed Wistar rats. Thirty-six adult male rats were divided into six groups and subjected to various treatments, including Vitamin C and different doses of TCL extracts. The animals were acclimatized for two weeks before the 28-day experimental period. Aqueous extracts were prepared following rigorous procedures, and a high-salt diet was administered based on systolic blood pressure measurements. Biochemical assays revealed elevated liver enzyme levels in the negative control group, aligning with previous research linking high salt intake to liver dysfunction. Treatment with Vitamin C and higher doses of TCL extracts demonstrated significant reductions in enzymes, indicating potential hepatoprotective effects. Notably, higher doses of TCL extracts positively influenced Total Protein and Albumin levels. Both Vitamin C and TCL extracts showed significant reductions in Total Bilirubin and Conjugated Bilirubin, suggesting protective effects against liver dysfunction. Histological examination on day 14 and day 28 elucidated nuanced structural changes. Group responses varied, with the high salt-fed group exhibiting severe damage and ongoing degeneration, emphasizing the detrimental effects of sustained high salt intake. Vitamin C and TCL extracts demonstrated varying protective effects, highlighting the importance of dosage consideration. The study contributes to understanding the complex interactions between interventions and liver health, urging further research for a comprehensive exploration of their hepatoprotective properties.

Keywords: Biochemical markers, Curry leaves, Liver histology, Turmeric, Vitamin C

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Introduction

Liver-related diseases have become a global health concern, affecting millions of individuals worldwide [1]. The liver plays a crucial role in various metabolic processes, including detoxification, protein synthesis, and regulation of cholesterol and blood sugar levels [2]. However, factors such as unhealthy lifestyle choices and dietary habits can lead to liver dysfunction.

Excessive intake of dietary salt, particularly in the form of sodium chloride, has been linked to the development of liver diseases, including fatty liver disease, liver fibrosis, and cirrhosis [3]. The prevalence of these conditions has increased due to the widespread consumption of processed foods and the excessive use of salt as a flavor enhancer. High-salt intake disrupts the balance between oxidative stress and the antioxidant systems, leading to a higher risk of liver damage, fibrosis, and apoptosis [4].

Making healthy lifestyle choices, such as reducing salt intake, can help prevent and manage liver diseases. Herbs and spices such as oregano, rosemary, fennel seeds, and cumin can help cut down on salt and are also a healthy source of antioxidants. Whole grains such as brown rice, oatmeal, barley, and millet contain high fiber content and prevent fatty liver conditions by reducing inflammation. On the other hand, fried and baked food items, such as fries, chips, pastries, and muffins, increase fat accumulation in the liver cells, which may cause fatty liver disease [5].

The treatment of liver diseases often involves the use of pharmaceutical products. However, the growing cost and potential side effects associated with these medications have driven researchers and healthcare professionals to explore alternative treatment options [6]. Herbal medicine, which utilizes plant-based remedies, has gained prominence due to its potential efficacy and lower occurrence of adverse effects. The World Health Organization (WHO) has been advocating for the integration of traditional and herbal medicine into national healthcare systems (World Health Organization [7]. This approach aims to provide affordable and accessible healthcare options, particularly in lower-income countries where the burden of liver diseases is significant. Consequently, there is an increasing interest in identifying medicinal plants with hepatoprotective properties to develop safer and cost-effective therapeutic interventions [6].

Considering the abundance of bioactive compounds in both turmeric and curry leaves, it is plausible to explore the combined extracts of these plants for their potential hepatoprotective effects. Scientific research has shown promising results regarding the individual hepatoprotective properties of turmeric and curry leaves. However, limited studies have investigated the synergistic effects of their combined extracts on liver diseases caused by high salt intake.

Curcuma longa Linn, popularly known as turmeric is the common tall herb that flourishes in tropical as well as in other Indian regions and is referred to as “Indian saffron or The Golden Spice of India” because of its use in a broad range of diseases in Indian homes as a spice, food preservative, and coloring source. *C. longa* belonging to the Zingiberaceae (ginger) family is a perennial plant commonly planted in Asian nations. It is among the oldest spices of India and have been used in Western and Southern parts for centuries and is a significant part of Ayurvedic

medicine. In Ayurveda, the therapeutic effects of *C. longa* have been well established and are discussed in Dashemani Lekhaniya (emaciating), Kusthagna (anti-dermatosis), and Visaghna (anti-poisonous) texts. It is known by many distinct names such as Haridra in Sanskrit, Haldi in Hindi, Jianghuang (yellow ginger in Chinese), manjal in South India, and Kyoo or Ukon in Japanese, which means an effective medication for jaundice [8].

In China, it is being used to relieve urticaria, dermatitis, hepatitis infection, inflammatory joints, sore throat, and wounds. It was mentioned in Hindu Mythology manuscripts as an aromatic stimulant and carminative. Turmeric powder combined with calcium hydroxide is indeed a popular home remedy for treating sprains and swelling induced by wounds or might be applied directly over the injury site. Traditional medicine has exploited dried curcumin powder to treat illnesses in history. *C. longa* is said to have antitoxic, anticancer, antibacterial, anti-inflammatory, and antioxidant effects [9]. The tuberous rhizome from which *C. longa* is formed has a coarse and segmented skin. In the ground soil, the rhizomes mature underneath the foliage. The matured rhizomes have a yellowish-brown color with a dull orange from inside. Small pointed or tapered tubers sprout off the main rhizome measuring 2.5–7.0 cm (1–3 inches) in length and 2.5 cm (1 inch) in diameter [10].

The dry rhizome is ground into a yellow powder form that has a bitter, yet sweet taste. A yellowcolored substance derived from the rhizome is curcumin (1,7-bis[4-hydroxy-3-methoxyphenyl]1,6-heptadiene-3,5-dione), a combined form of resin and oil. Rhizome powder is supposed to flavor various cuisines and treat numerous disorders, including inflammation, flatulence, jaundice, menstrual troubles, hematuria, and hemorrhage. It is also a useful ointment to treat several skin disorders. *C. longa* of India is particularly popular when compared with those from other countries due to its high curcumin concentration, which is the most essential and active biological ingredient responsible for its therapeutic potential [11]. Curcumin is a flavonoid having a lipophilic affinity that is practically water-insoluble [12] yet quite stable at the stomach's acidic pH. *C. longa* and curcumin show antioxidant features close to vitamins C and E in both aqueous and fatsoluble extracts.

The curry leaf is a chief seasoning ingredient in Indian dishes, it is often compared to a person who is used for a particular purpose / task and discarded immediately once it is done, in an old Indian saying [13-16]. The curry leaf / *Murraya Koeinigii* plant is cultivated throughout India, growing in moist well drained places of full sun as well as partly shade. It is widely used in Indian cookery for centuries and is considered valuable for its aromatic and medicinal value [17]. The parts used are leaves, flowers, bark, stems, roots and fruits. The phytochemical study of the plant shows presence of carbazole alkaloids [18], numerous chemical constituents, minerals, Iron, flavonoids, sterol [17], volatile oils and alkaloids like *Murraya canine*, Koenimbine, Koenine, Mahanimbine, Murrayazolidine, Murrayazoline, Murrayacine, Girinimbine, Mukoeic Acid, etc [18].These bioactive compounds possess antioxidant, antimicrobial, anthelmintic, analgesic, anti-inflammatory, antidiarrheal, hepatoprotective and antitumor properties.

Essential oils, fresh leaves and powder of dried leaves are widely used for curries, flavouring fish, soups and meat dishes, eggs dishes, ready to use and seasoning other food preparations. The essential oils are also used by cosmetic aromatherapy and soap industry [19]. *Murraya koenigii* leaves are used as a first-rate hair tonic for keeping natural hair tenor and increasing hair growth.

Leaves of this tree are cooked with coconut oil till a concentrated residue is formed. Parts or whole plant is traditionally used as a remedy for anti-emetic, blood purifier, depressant, anti-fungal, body aches, anti-inflammatory, anti-diarrhoea, in kidney pain and vomiting. Barks and roots have been used by the tribals to cure poisonous animals bite. Green leaves of *Murraya koenigii* when eaten raw helps in diarrhea and treating morning sickness along with lime juice. The paste of the leaves and juice of roots relives boils and renal pain respectively [20,21].

The prevalence of liver diseases has increased dramatically over recent years, posing a significant burden on global healthcare systems. Liver disorders, including fatty liver disease, hepatitis, cirrhosis, and liver cancer, have become major public health concerns worldwide. High salt intake, particularly in the form of processed and fast food, has been identified as a contributing factor to the rising cases of liver diseases. Unfortunately, the available treatment options for liver diseases are limited and often associated with various side effects. In light of these challenges, there is a pressing need to explore alternative treatment approaches that are effective, safe, and accessible. Herbal medicine has emerged as a promising field of research, offering potential solutions for liver diseases. The combination of Turmeric (*Curcuma longa*) and curry leaves (*Murraya koenigii*) has gained attention for its potential hepatoprotective properties. However, there is a paucity of scientific evidence on the efficacy of this herbal combination in mitigating liver damage caused by high salt intake.

This present study aimed to evaluate the hepatoprotective effect of combined extracts of turmeric and curry leaves on high salt-fed Wistar rats. By conducting comprehensive experiments and assessments, we seek to determine the efficacy of this herbal remedy in preventing or reversing liver damage induced by high salt intake. Such evidence could contribute to the development of alternative and affordable therapeutic interventions for liver diseases, particularly in populations with excessive salt consumption and limited access to costly pharmaceutical treatments.

Materials and Methods

Research design

The research design for this study was an experimental design involving male Wistar rats. The study aimed to investigate the effect of a combined aqueous extract of turmeric and curry on highsalt diet-fed rats. The rats were divided into different groups and subjected to various treatments to assess the biochemical and histological changes.

Procurement of Animals

Thirty-six (36) adult male Wistar rats weighing 180–200g were procured from the Animal House of the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt. The rats served as the animal model for the study. The rats were housed in clean, disinfected wooden cages with sawdust as bedding. The animal house provided a controlled environment with a 12-hour light/dark cycle, 50–60% humidity, and a temperature of roughly 30°C. These conditions were maintained throughout the acclimatization and experimental periods. The rats were allowed to acclimatize for two weeks in the animal house. During this period, they had free access to clean water and standard animal feed. This acclimatization period

ensured that the rats adapted to their new environment and stabilized before the commencement of the experiment.

Collection and Identification of Plant Materials

Fresh turmeric rhizomes and curry leaves were purchased from the local market within the University of Port Harcourt environment. Subsequently, these turmeric rhizomes and curry leaves were taken to the Department of Plant Science and Biotechnology for identification and authentication before their use in the study.

Preparation of Aqueous Extracts

The turmeric rhizomes were carefully cleaned to remove any dirt or debris using clean water and a soft brush, while the curry leaves were plucked from the stems and thoroughly washed to eliminate any impurities. Once cleaned, the turmeric rhizomes and curry leaves were spread out in a single layer on clean trays or mesh screens. They were placed in a well-ventilated area with controlled temperature and airflow to facilitate drying, and the natural drying process lasted for 14 days (2 weeks) until they became completely dry and brittle. After the drying process, the turmeric rhizomes and curry leaves were separately ground into fine powders using an electric grinder. The powders were then carefully sieved through a fine mesh to obtain a consistent and uniform texture, ensuring the removal of any coarse particles.

To prepare the aqueous extract, 60g of the powdered turmeric and curry leaves were accurately weighed using a digital scale. Distilled water (100ml) was heated in a clean container until it reached its boiling point. The measuredquantity of powdered turmeric and curry leaves was added to the boiling water in a ratio of 1:1. The mixture was continuously stirred using a glass rod or a magnetic stirrer to ensure efficient extraction of bioactive compounds. The heat was then reduced, and the mixture was allowed to simmer for 30-60 minutes, allowing the constituents to dissolve in the water effectively.

After simmering, the mixture was removed from heat and allowed to cool down to room temperature. The cooled mixture was filtered using a sterile muslin cloth or filter paper to separate the liquid extract from any solid residues or particles. The filtered aqueous extract was collected in sterilized amber glass bottles or airtight containers, ensuring proper labeling with relevant information such as the contents, date of preparation, and any other necessary details. The extract was stored in a cool, dry place, protected from light and moisture to maintain its stability and bioactive properties. The entire process was carried out following standard laboratory practices and adhering to relevant safety guidelines to ensure the production of a high-quality aqueous extract of turmeric and curry leaves for further research and experimentation.

Preparation of high salt diet

A high-salt solution containing 20% sodium chloride was prepared and 1ml of the solution was administered to the rats daily and were observed for signs of toxicity.

Experimental Design

Thirty-six (36) adult male Wistar rats were randomly divided into six (6) groups of six (n=6) animals each. The treatment protocol is presentedin Table 1 below;

Table 1: Experimental Protocol

Groups	Description	No. of Rats	Treatment Protocol
Group 1	Normal control	6	Administered 2ml of normal saline and feed for 28 days
Group 2	Negative control	6	Administered 1ml of 20% NaCl solution without treatment for 28 days
Group 3	Vitamin C	6	Administered 1ml of 20% NaCl solution and 30mg/kg of vitamin C for 28 days
Group 4	Low Dose	6	Administered 1ml of 20% NaCl solution and 250mg/kg of combined extract for 28 days
Group 5	Medium Dose	6	Administered 1ml of 20% NaCl solution and 500mg/kg of combined extract for 28 days
Group 6	High Dose	6	Administered 1ml of 20% NaCl solution and 1000mg/kg of combined extract for 28 days

The high-salt group was administered 1 ml of 20% NaCl solution by intragastric gavage [22]. The determined dose of the combined aqueous extract was orally administered to the rats in the experimental group.

Sample collection

Sample collection involved obtaining liver and blood samples from the rats on day 28 of the experiment. These samples were collected using standard techniques and handled with appropriate precautions to ensure accurate and reliable results.

Liver Function Marker Assay

Animals were sacrificed on the 28th day. The rats were anesthetized using diethyl ether. The thorax of the rats was opened, using the cardiac puncture procedure, blood samples were gotten from the heart using a needle. Also, rat blood (more than 6 ml) was drawn from the inferior vena cava under anesthesia. The biochemical parameters are; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), Gamma-Glutamyltransferase (GGT), Total
Protein (T.P), Albumin (ALB), Total Bilirubin (T.B) and Conjugated Bilirubin (C.B).

AST / ALT (Reitman and Frankel method)

Principle: (AST) x – Oxoglutarate + L – aspartate GOT L - glutamate oxalogcetate. AST is measures by monitoring the concentration of oxoloacete hydrazones formed with 2,4dinitrophenyl –hydrazine. **ALT:** x- oxogluterate + L- alanme GPT L- glutamate + ppyruvate. ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4 dinitrophynyl-hydrazine.

Procedure: The procedure for ALT and AST is identical. The test tubes were labeled as sample blank and sample. Subsequently, 0.5ml of reagent 1 (R1) was added to all the test tubes, and 0.1ml of the sample was added to the tubes labeled sample. The mixture was then thoroughly mixed and incubated for precisely 30 minutes at 37°C. Following incubation, 0.5ml of reagent 2 (R2) was pipetted into all the test tubes, and 0.1ml of the sample was added to the tube labeled sample blank.
The mixture was again thoroughly mixed and allowed to stand for exactly 20 minutes at 2°C. Afterward, 5.0ml of NaOH was pipetted into all the test tubes, and the absorbance of the sample against the sample blank was read after 5 minutes at 540nm. The results are expressed in units per
liter (u/l).

ALP/GGT (Kochmar, J.F and moss, D.W method) Unit U/L
Principle: ALP/GGT acts upon buffered sodium thymolphthalein monophoshate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen which is measured photometrically.

Procedure: The tubes were labelled as STANDARD, blank and test**.** 0.5ml of Alp substrate was pipetted into all the test tubes and incubated at 37^{o}C for 3mins. 50ul of the Standard sample was added and d/w into appropriate tube**.** The spectrophotometer was zeroed at 590nm with the blank; the absorbance was read and recorded

Calculation = $\frac{\text{Absorbance (ABS) } of\ Test\ x\ Conc.\ of\ standard}{ABS\ of\ Standard}$

Bilirubin/Total Protein: (Jendrasik and Grof method) unit: Umoil/L

Principle: Total bilirubin is determined in the presence of caffeine, which releases albumin-bound bilirubin by the reaction with diazotized sulphanilic acid.

Procedure: The test tubes were labeled as samples and blank samples. Subsequently, 0.2ml of the reagent was pipetted into all the tubes. One drop of sodium nitrate was added to the tube labeled sample. Further, 1ml of caffeine was added to all the tubes. Then, 0.02ml of the sample was added to the tubes, and the mixture was thoroughly mixed and incubated for 10 minutes at 25°C. Following this, 1ml of tartrate was pipetted into all the tubes, mixed, and incubated for 5-30 minutes at 25°C. The absorbance was zeroed at 590nm, and the absorbance was read. The calculation involves multiplying the absorbance by 185 to determine the concentration of the test.

Histological Examination
The mice are dissected following diethyl-ether anaesthesia to access the kidneys and liver which were harvested into 10% chloroform with subsequent trimming of the later to a range of 2-4mm thick for fixation. These tissues were fixed, dehydrated, cleared, impregnated, embedded, sectioned and stained following circumspectly the method of [23].

Fixation

Fixation was performed with 10% phosphate buffered formaldehyde solution at a pH 7 for 6 to 12 hours to preserve the various cellular components in their normal micro-anatomical positions to prevent them from any degenerative or autolytic changes. After fixation, the samples were washed under running or stream of tap water.

Dehydration

Dehydration of the tissue was done to remove water from the tissue in an ascending order of 50% alcohol for 3 hours, 70% alcohol for 1-2 hours, 90-95% alcohol for 12 hours and 100% for 1-2 hours.

Clearing

After dehydration, the tissues were infiltrated with xylene for 2 hours for miscibility of solubility in paraffin and to remove the alcohol from the tissues.

Impregnation

This removes traces of the clearing agent from the tissues. The tissues were transferred from xylene to molten paraffin at a temperature of 60^{o}C for 2 hours each in two changes.

Embedding

During the embedding process, the tissue blocks were oriented so that the sections were cut in a desired plane of tissues. Tissues were then immersed in molten paraffin wax at 80^{o}C and then allowed to cool and solidify. Metallic embedding moulds were employed in this process. After embedding, the tissue blocks obtained were cast into wooden blocks. This supported the tissues, making it easier for sectioning. The sec blocks of paraffin with tissues were removed from moulds and trimmed to appropriate sizes and fixed on a metal object holder. The blocks were further trimmed and kept at a temperature of 0^{o}C.

Sectioning

The tissue blocks were taken to a microtome for sectioning. Sectioned ribbons ranging from 2-5µm were made using the microtome on albumenized slides.

Mounting

The sectioned ribbons were warmed at 37^{o}C to strengthen wrinkles while the best ribbons were removed with a forceps and dropped on slides. About 0.2ml of albumen was rubbed on the slide surface before placing the sectioned tissue serving as the adhering medium. The slides were labeled using diamond pencil and placed on a slide rack. Water was allowed to train away from the slides and was then placed in an incubator and incubated at 37-40^{o}C overnight, making the sections completely fixed on the slides and warm.

Staining

Hematoxylene and Eosine (H & E) were used to stain the slides and the slides were sent to a histopathology laboratory for examination, evaluation and histological changes.

Procedures for Staining: The slides were placed in xylene for 5-10 minutes, then transferred to absolute alcohol, followed by 95% alcohol, and finally to 70% alcohol in seconds. Afterward, they were rinsed in water. Subsequently, the slides underwent staining with hematoxylin stain for 1520 minutes, followed by rinsing in water to remove excess stain. Differentiation in 1% acid alcohol for 5 seconds was performed to eliminate excess stain and aid in nucleus stain

absorption. The slides were then subjected to two changes of water for 3 and 5 seconds, known as bluing, to provide the characteristic background for staining. Eosin staining was carried out on the slides for 5 to 10 minutes, followed by rinsing in water to remove excess stain. Dehydration in absolute alcohol ensued, and the slides were ultimately mounted with molten paraffin wax (DPX) and cover slip.

Photomicrography

Each of the slides was viewed under the light microscope and were photographed under magnification of x400 H & E and later printed into photomicrographs.

Ethical Considerations

The experimental protocol will adhere to the ethical guidelines and regulations set by the Animal Ethics Committee of the University of Port Harcourt. The welfare and well-being of the animals will be prioritized, and all procedures will be conducted in accordance with animal welfare

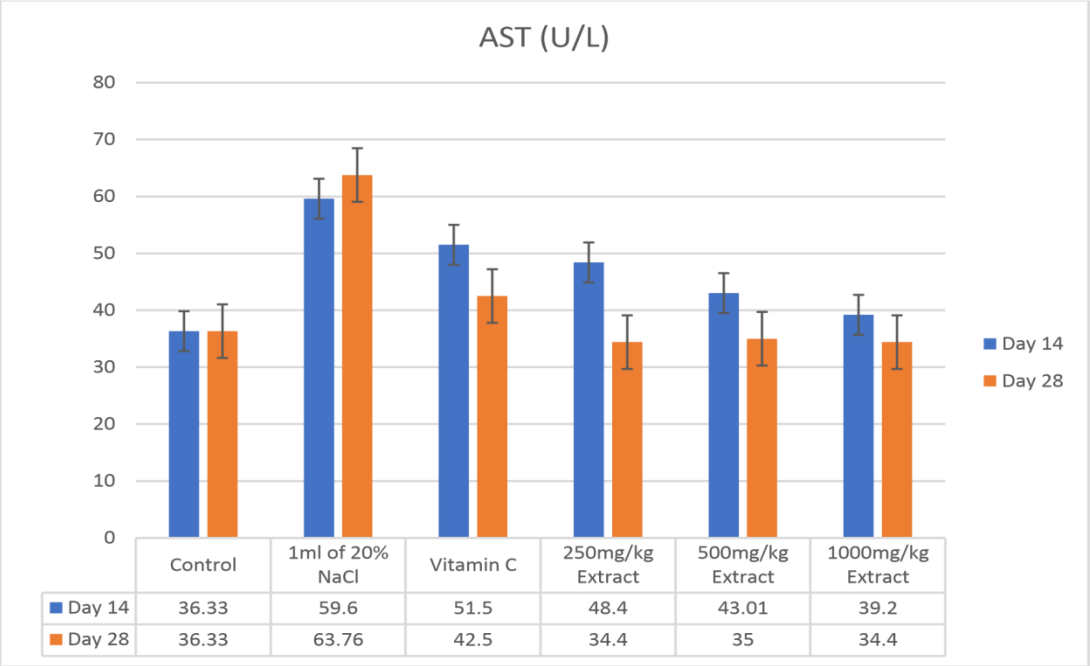
Data Analysis

The data obtained from the biochemical assays were subjected to appropriate statistical analyses using the Statistical Package for Social Sciences (SPSS) version 26.0. Statistical tests, such as descriptive statistics and analysis of variance (ANOVA) followed by a Tukey post hoc, were employed to determine significant differences between the experimental groups. The level of significance was set at $p < 0.05$.

Results

Effect of Vitamin C and combined Extract of Turmeric and Curry leaves (TCL) on Liver function markers

The effect of Vitamin C and combined Extract of Turmeric and Curry leaves (TCL) on Aspartate Aminotransferase (AST) levels in High salt-fed Wistar rats is shown in Figure 1, demonstrating the impact on liver function. The normal control group showcases consistent AST levels on both Day 14 and Day 28. In contrast, the group treated with 1ml of 20% NaCl (negative control) exhibits notably elevated AST levels, indicating liver stress. The administration of Vitamin C and varying doses of TCL extracts demonstrates different effects, with higher doses (500mg/kg and 1000mg/kg) resulting in a significant decrease in AST levels compared to the negative control. These findings suggest a potential protective effect on the liver.



	Control	1ml of 20% NaCl	Vitamin C	250mg/kg Extract	500mg/kg Extract	1000mg/kg Extract
Day 14	36.33	59.6	51.5	48.4	43.01	39.2
Day 28	36.33	63.76	42.5	34.4	35	34.4

Figure 1. Effect of Vitamin C and combined extract of Turmeric and Curry leaves on Aspartate Aminotransferase (AST)levels in High salt-fed Wistar rats.

The effect of Vitamin C and combined Extract of Turmeric and Curry leaves (TCL) on Alanine Aminotransferase (ALT) levels in High salt-fed Wistar rats is shown in Figure 2. This table presents the levels of ALT, another liver enzyme, in Wistar rats. The negative control group (1ml of 20% NaCl) exhibits elevated ALT levels compared to the normal control, indicating liver damage, similar to AST. Treatment with Vitamin C shows a significant reduction in ALT levels, suggesting a protective effect on the liver. Similarly, the TCL extracts, particularly at higher doses (500mg/kg and 1000mg/kg), also demonstrate a significant decrease in ALT levels compared to the negative control, indicating a potential hepatoprotective effect.

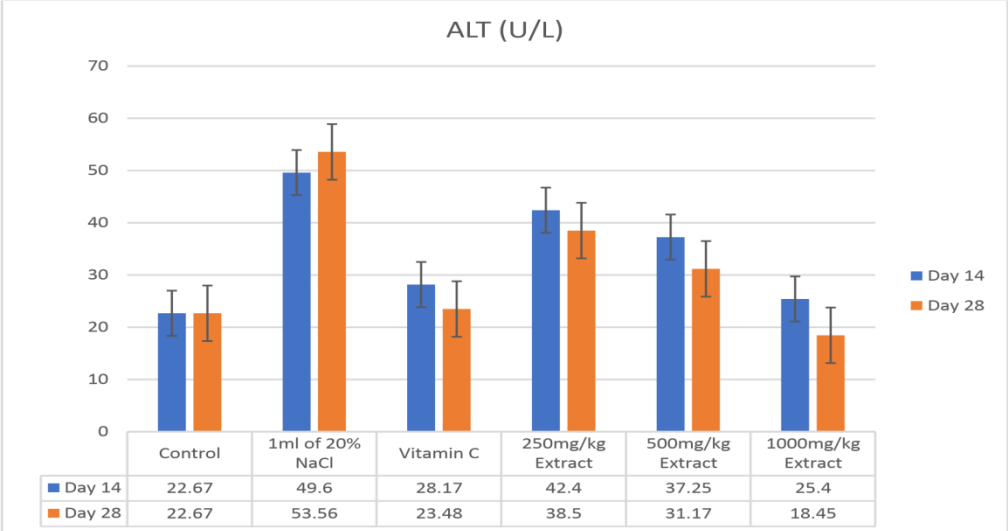


Figure 2. Effect of Vitamin C and combined extract of Turmeric and Curry leaves on Alanine Aminotransferase (ALT)levels in High salt-fed Wistar rats.

The effect of Vitamin C and combined Extract of Turmeric and Curry leaves (TCL) on Alkaline Phosphatase (ALP) levels in High salt-fed Wistar rats is presented in Figure 3. This table demonstrates the influence of these substances on ALP levels, an enzyme closely associated with liver and bone health. In the negative control group, ALP levels show an increase, indicating liver stress. On the other hand, Vitamin C displays a moderate reduction in ALP levels, suggesting a protective effect on the liver. Notably, TCL extracts, especially at higher doses of 500mg/kg and 1000mg/kg, significantly decrease ALP levels compared to the negative control, indicating a potential positive impact on liver health.

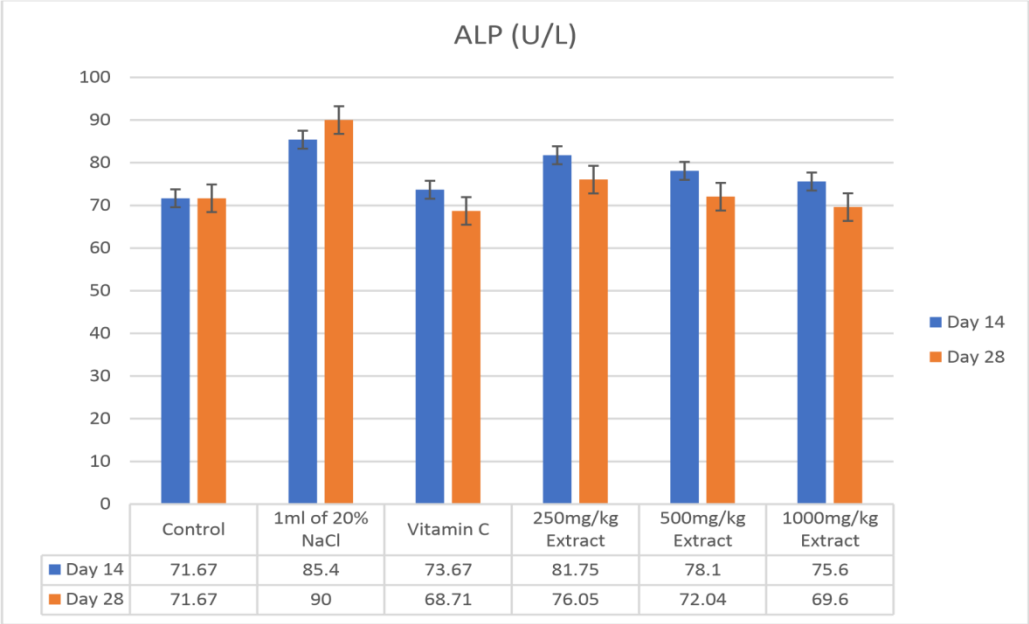
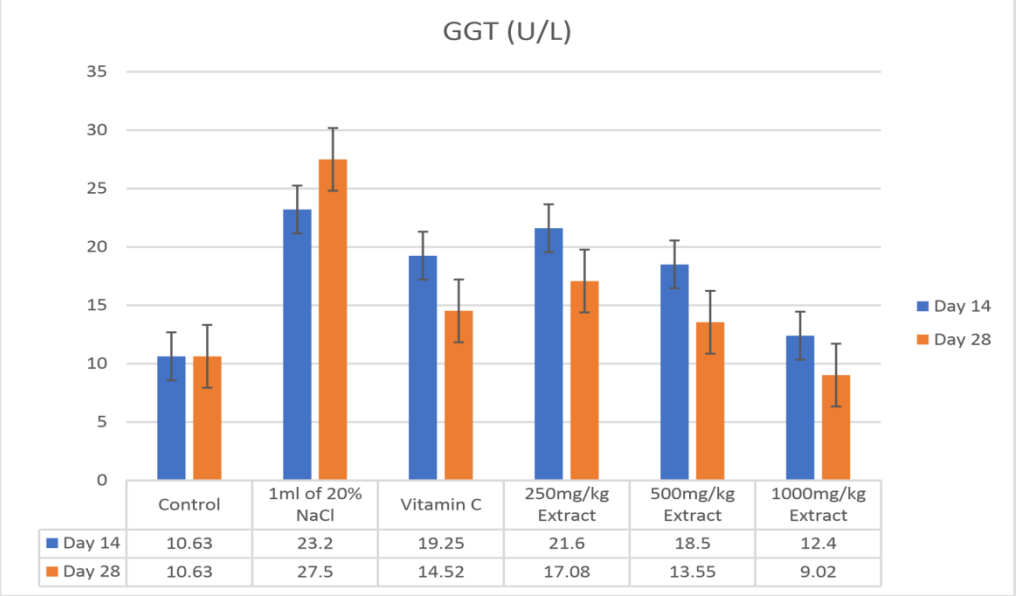


Figure 3. Effect of Vitamin C and combined extract of Turmeric and Curry leaves on Alkaline Phosphatase (ALP) levels in High salt-fed Wistar rats.

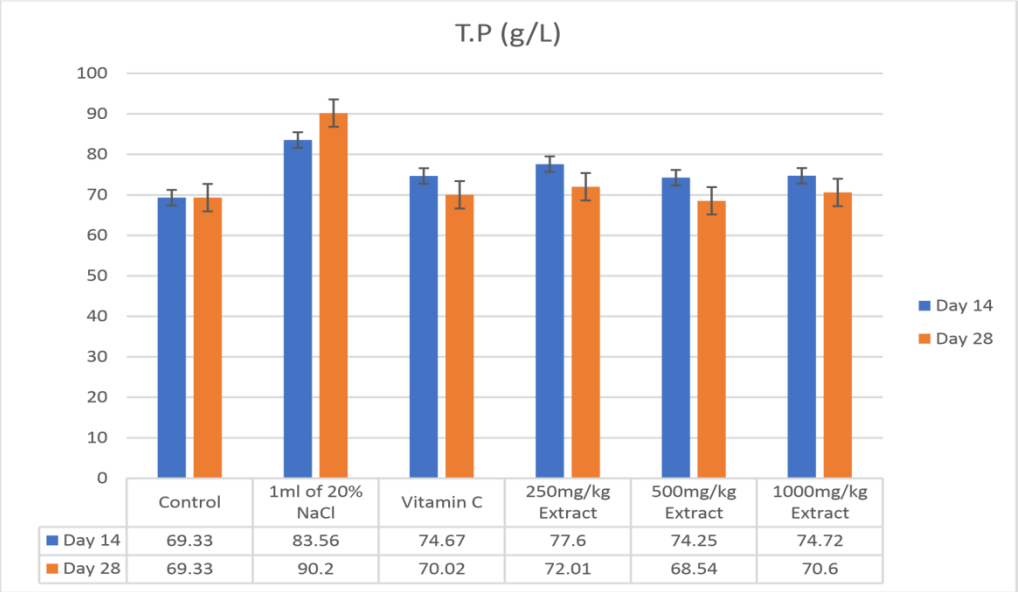
The effect of Vitamin C and combined Extract of Turmeric and Curry leaves (TCL) on Gamma-Glutamyl transferase (GGT) levels in High salt-fed Wistar rats is shown in Figure 4, which serves as a marker for liver and bile duct function. The negative control group exhibits elevated GGT levels, indicative of liver and bile duct stress. In contrast, the administration of Vitamin C and TCL extracts, particularly at higher doses, exhibits a significant reduction in GGT levels compared to the negative control group, suggesting a potential protective effect on the liver and bile ducts.



	Control	1ml of 20% NaCl	Vitamin C	250mg/kg Extract	500mg/kg Extract	1000mg/kg Extract
Day 14	10.63	23.2	19.25	21.6	18.5	12.4
Day 28	10.63	27.5	14.52	17.08	13.55	9.02

Figure 4. Effect of Vitamin C and combined extract of Turmeric and Curry leaves on Gamma-Glutamyl transferase (GGT) levels in High salt-fed Wistar rats.

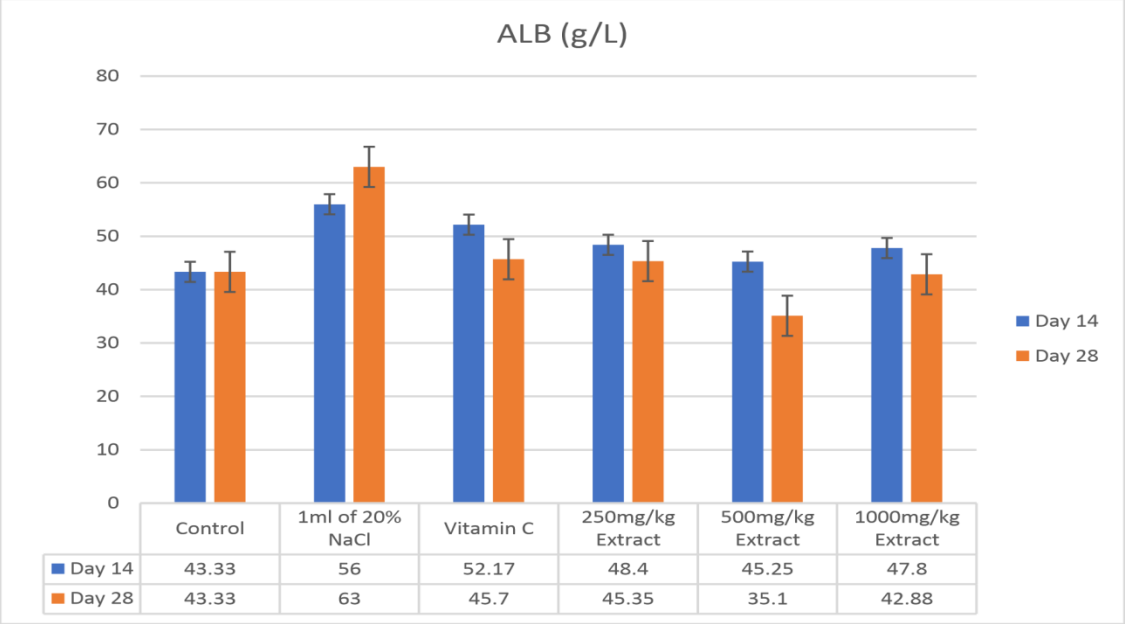
The impact of Vitamin C and combined Extract of Turmeric and Curry leaves (TCL) on Total Protein (T.P) levels in High salt-fed Wistar rats is illustrated in Figure 5. This table provides insights into the overall protein content in the blood of Wistar rats. The negative control group demonstrates an elevation in T.P levels, potentially indicative of inflammation or dehydration. Notably, Vitamin C and TCL extracts, especially at higher doses, show a substantial decrease in T.P levels compared to the negative control, implying a potential normalization of protein levels.



	Control	1ml of 20% NaCl	Vitamin C	250mg/kg Extract	500mg/kg Extract	1000mg/kg Extract
Day 14	69.33	83.56	74.67	77.6	74.25	74.72
Day 28	69.33	90.2	70.02	72.01	68.54	70.6

Figure 5. Effect of Vitamin C and combined extract of Turmeric and Curry leaves on Total Protein (T.P) levels in High salt-fed Wistar rats.

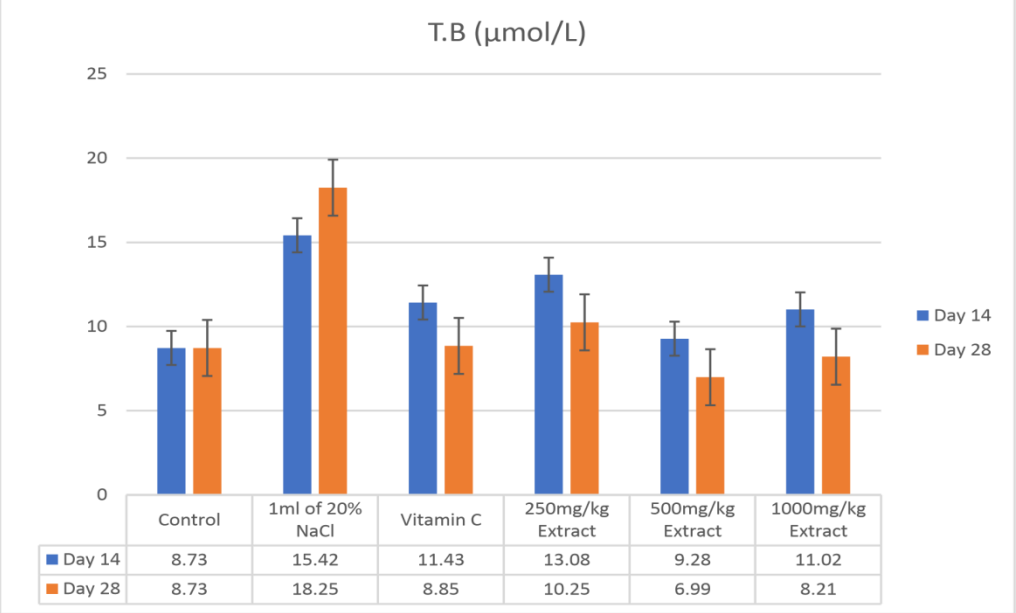
The impact of Vitamin C and combined Extract of Turmeric and Curry leaves (TCL) on Albumin (ALB) levels in High salt-fed Wistar rats is illustrated in Figure 6. This table provides insights into the levels of Albumin, a liver-produced protein. The negative control group exhibits elevated ALB levels, likely indicative of a response to liver stress. Notably, Vitamin C and TCL extracts, particularly at higher doses, show a significant decrease in ALB levels compared to the negative control, implying a potential positive influence on liver function.



	Control	1ml of 20% NaCl	Vitamin C	250mg/kg Extract	500mg/kg Extract	1000mg/kg Extract
Day 14	43.33	56	52.17	48.4	45.25	47.8
Day 28	43.33	63	45.7	45.35	35.1	42.88

Figure 6. Effect of Vitamin C and combined extract of Turmeric and Curry leaves on Albumin (ALB) levels in High salt-fed Wistar rats.

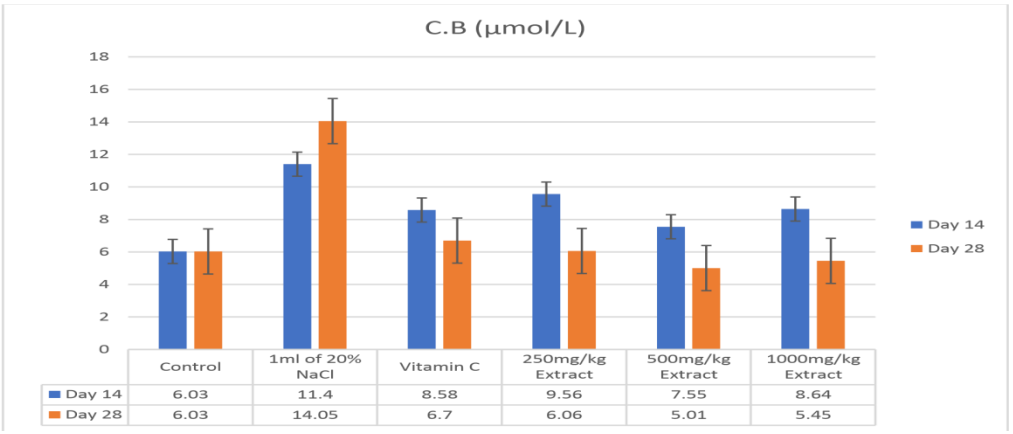
The impact of Vitamin C and combined Extract of Turmeric and Curry leaves (TCL) on Total Bilirubin (T.B) levels in High salt-fed Wistar rats is demonstrated in Figure 7. The table presents the levels of Total Bilirubin (T.B), a byproduct of red blood cell breakdown, in Wistar rats. The negative control group exhibits elevated T.B levels, indicating potential liver dysfunction. Notably, Vitamin C and TCL extracts, particularly at higher doses, show a substantial reduction in T.B levels compared to the negative control, suggesting a potential protective effect on liver function.



	Control	1ml of 20% NaCl	Vitamin C	250mg/kg Extract	500mg/kg Extract	1000mg/kg Extract
Day 14	8.73	15.42	11.43	13.08	9.28	11.02
Day 28	8.73	18.25	8.85	10.25	6.99	8.21

Figure 7. Effect of Vitamin C and combined extract of Turmeric and Curry leaves on Total Bilirubin (T.B) levels in High salt-fed Wistar rats.

The impact of Vitamin C and combined Extract of Turmeric and Curry leaves (TCL) on Conjugated Bilirubin (C.B) levels in High salt-fed Wistar rats is shown in Figure 8. The table presents the concentrations of C.B, a liver-processed form of bilirubin, in Wistar rats. In the negative control group, elevated C.B levels are observed, indicative of potential liver dysfunction. Notably, the administration of Vitamin C and TCL extracts, particularly at higher doses, demonstrates a significant decrease in C.B levels compared to the negative control. These findings suggest a potential protective effect on liver function associated with the supplementation of Vitamin C and TCL extracts in the experimental rats.



	Control	1ml of 20% NaCl	Vitamin C	250mg/kg Extract	500mg/kg Extract	1000mg/kg Extract
Day 14	6.03	11.4	8.58	9.56	7.55	8.64
Day 28	6.03	14.05	6.7	6.06	5.01	5.45

Figure 8. Effect of Vitamin C and combined extract of Turmeric and Curry leaves on Conjugated Bilirubin (C.B) levels in High salt-fed Wistar rats.

Effect on the Liver Histology (day 14)

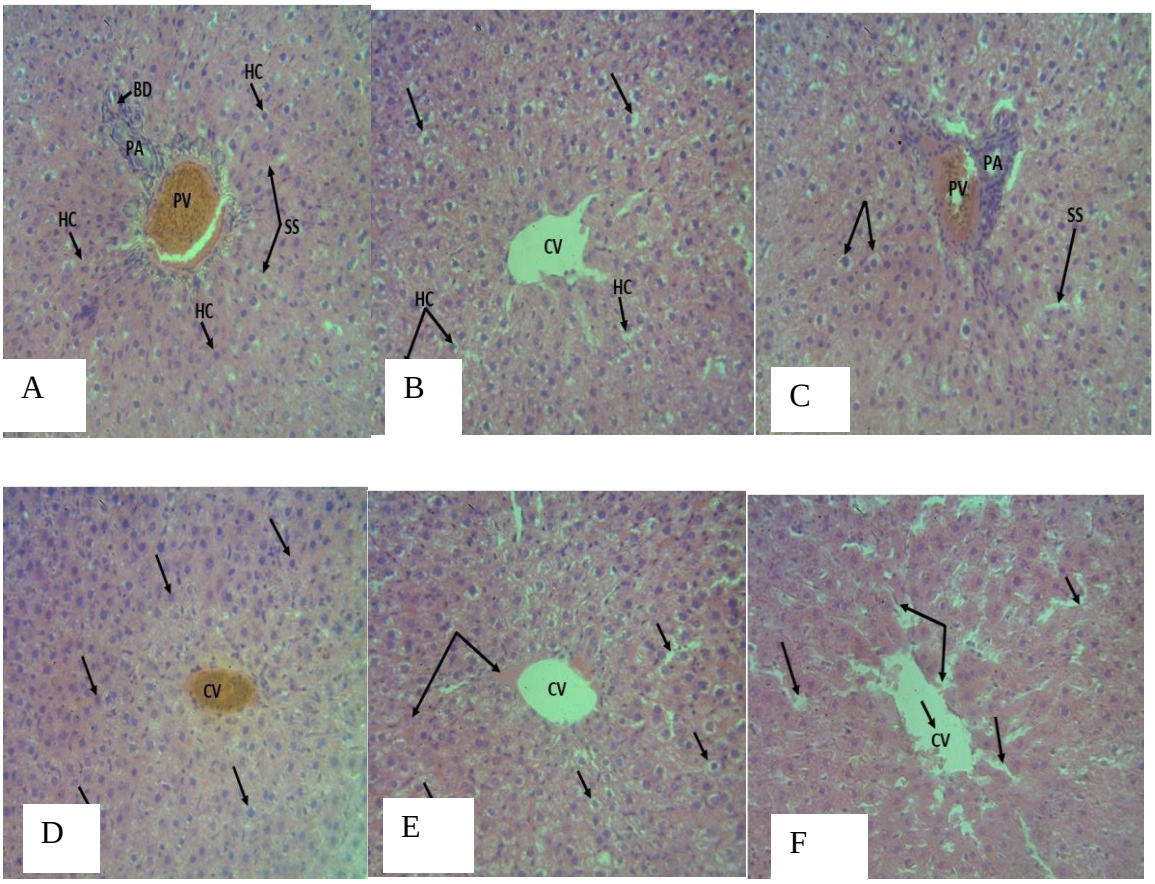


Figure 9. Photomicrograph (H&E X400) of the liver tissue: A - group 1 (control group) on day 14 showing the structure of the portal triad with visible periportal area of the portal vein (PV), portal artery (PA), bile duct (BD): visible hepatocytes (HC), and sinusoids (SS). **Diagnosis:** Normal appearance of the liver; B - group 2 (high salt-fed only) showing severely distributed hepatic lobules vacoulation of the parenchyma with distortion of the central vein (arrows) **Diagnosis:** Severe vacoulation of the liver tissue; C - group 3 (30mg/kg vitamin C treated) showing

hepatic lobules vacoulation of the liver parenchyma with constriction of the portal triad (arrows) **Diagnosis:** Portal vessel Congestion and vacoulation of the liver tissue; D - group 4 (250mg/kg of combined extract of Turmeric and Curry leaves) showing engulfed central vein with hepatocyte hypercellularity of the liver parenchymal (arrows) **Diagnosis:** moderate toxicity of the liver parenchymal; E - group 5 (500mg/kg of combined extract of Turmeric and Curry leaves) showing dilation and hemosiderin deposit in the sinusoids with mild hepatic lobules vacoulation of the liver parenchyma (arrows) **Diagnosis:** mild vacoulation and hemosiderin deposit in the liver tissue; F - group 6 (1000mg/kg of combined extract of Turmeric and Curry leaves) showing moderate sinusoidal and central vein distortion with lobular vacoulation of the of the liver (arrows) **Diagnosis:** Moderate distortion of the liver tissue.

Effect on the Liver Histology (Day 28)

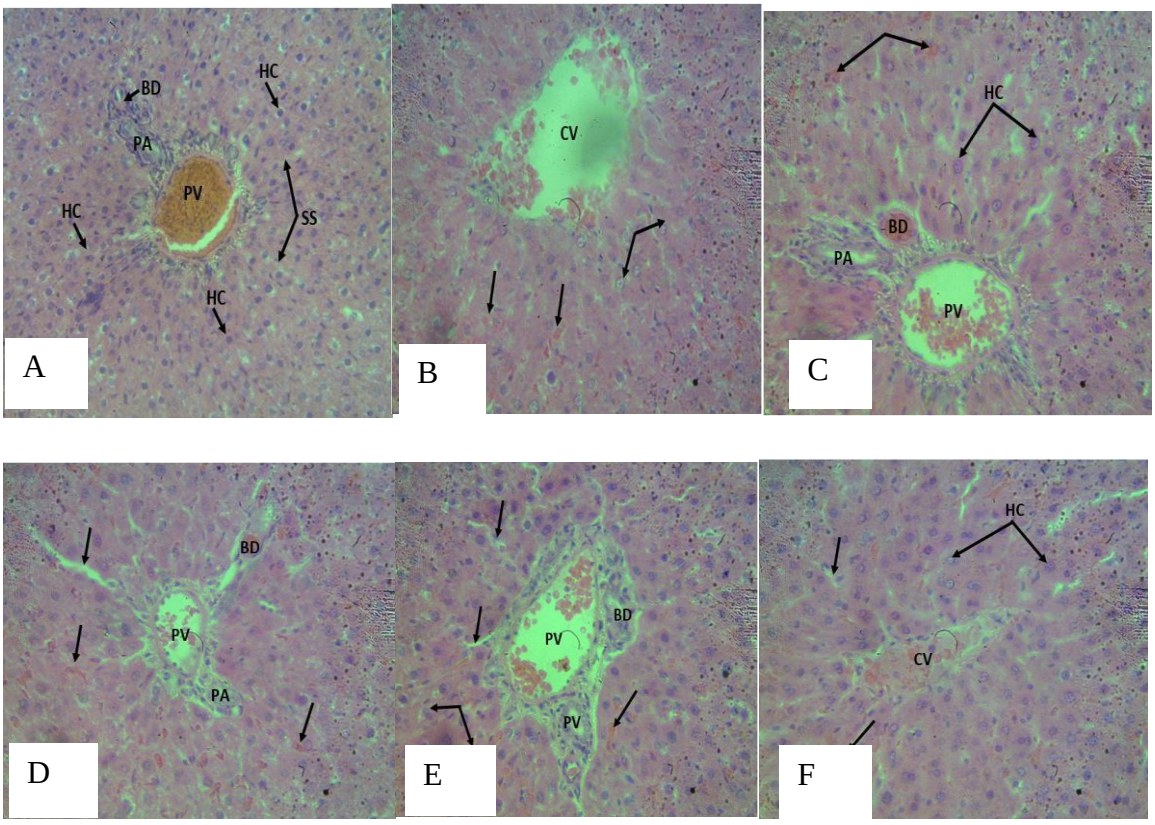


Figure 10. Photomicrograph (H&E X400) of the liver tissue: A - group 1 (control group) showing the structure of the portal triad with visible periportal area of the portal vein (PV), portal artery (PA), bile duct (BD): visible hepatocytes (HC), and sinusoids (SS); B - group 2 (high salt-fed) showing hemosiderin deposit within the liver parenchymal and hepatocyte hypocellularity (arrows) **Diagnosis:** Degeneration of the liver tissue; C - group 3 (30mg/kg vitamin C) showing mild hepatocyte enlargement with sinusoidal enlargement and hemosiderin deposit within the liver parenchymal (arrows). **Diagnosis:** Mild degeneration of the liver tissue; D - group 4 (250mg/kg of combined extract of Turmeric and Curry leaves) with moderate portal vessel congestion; sinusoidal dilatation and hemosiderin deposit within the liver parenchymal associated with hepatocyte ballooning (arrows) **Diagnosis:** moderate degeneration of the liver tissue; E - group 5 (500mg/kg of combined extract of Turmeric and Curry leaves) showing sinusoidal constriction; reduced hemosiderin deposit within the liver parenchymal and hepatocyte degeneration (arrows) **Diagnosis:** mild degeneration and sinusoidal congestion of the liver tissue; F - group 6 (1000mg/kg of combined extract of Turmeric and Curry leaves) on day 128 showing mild sinusoidal dilatation; congested and engulfed central vein with hepatocyte hypercellularity of the liver parenchymal (arrows) **Diagnosis:** moderate hepatocyte degeneration.

Discussion

This study rigorously explored the repercussions of Vitamin C and Combined Extract of Turmeric and Curry leaves (TCL) on biochemical markers indicative of liver function in Wistar rats subjected to a high-salt regimen. The investigation divulged heightened levels of liver enzymes (AST, ALT, ALP, and GGT) in the negative control group, indicative of considerable liver stress. This observation aligns with previous research findings of [24,25], associating high salt intake with liver dysfunction and increased liver enzyme levels.

In contrast, treatment with Vitamin C and higher doses of TCL extracts (500mg/kg and 1000mg/kg) manifested substantial reductions in these enzymes, strongly suggesting potential hepatoprotective effects. Prior studies have demonstrated the hepatoprotective attributes of Vitamin C in animal models [16,26]. Similarly, the combined extract of turmeric and curry leaves has exhibited hepatoprotective effects in various studies [27,28]. These findings substantiate our current observation that Vitamin C and TCL extracts could mitigate liver stress by diminishing liver enzyme levels.

Furthermore, the application of higher doses of TCL extracts exhibited positive effects on Total Protein (T.P) and Albumin (ALB) levels. This suggests a potential normalization of protein levels and a positive influence on liver function. Studies have reported the hepatoprotective properties of turmeric and curry leaves, including the normalization of protein levels [29,30]. This underscores the possibility that the TCL extracts employed in our study contribute to the enhancement of liver function by restoring protein levels.

Moreover, both Vitamin C and TCL extracts demonstrated significant reductions in Total Bilirubin (T.B) and Conjugated Bilirubin (C.B), indicating potential protective effects against liver dysfunction. Earlier research has indicated that Vitamin C supplementation can reduce bilirubin levels and improve liver function in animal models [31,32]. Turmeric and curry leaves have also exhibited hepatoprotective properties, including the reduction of bilirubin levels [33,34]. These cumulative findings suggest that both Vitamin C and TCL extracts may positively impact liver function by mitigating bilirubin levels.

Moving on to the histological assessment of the liver on day 14, the images present a nuanced understanding of structural changes in response to various interventions. The control group (Group 1) maintains a normal liver structure, serving as a baseline for comparison. In stark contrast, Group 2 (High salt-fed) exhibits severe vacuolation and distortion, indicative of substantial damage associated with elevated salt intake. This aligns with existing literature linking high salt intake to non-alcoholic fatty liver disease (NAFLD) and consequential liver damage [4,35].

Group 3, treated with 30mg/kg of Vitamin C, shows milder alterations, including vacuolation and portal vessel congestion. This aligns with the suggested protective effects of Vitamin C on the liver, as demonstrated in previous studies [18,36]. However, it's crucial to acknowledge the potential dose-dependent variations in Vitamin C's impact on liver histology.

On the other hand, Groups 4, 5, and 6, subjected to varying concentrations of TCL extracts, exhibit diverse toxic effects, with Group 6 showing moderate sinusoidal distortion. This aligns

with existing literature cautioning about potential histological impairments and biochemical alterations in the liver due to exposure to certain substances [37]. The varying responses emphasize the importance of carefully considering dosage and concentration when exploring the effects of these extracts.

As we progress to day 28, Group 2 (High salt-fed) continues to experience liver degeneration, marked by hemosiderin deposits and hepatocyte hypocellularity. This ongoing degeneration reinforces the detrimental impact of sustained high salt intake on liver health. Group 3 (30mg/kg Vitamin C) demonstrates milder degeneration, suggesting a persistent but diminished protective effect of Vitamin C. Group 4 (250mg/kg TCL extract) exhibits moderate portal congestion and hepatocyte ballooning, indicating a complex response to TCL extracts. Group 5 (500mg/kg TCL extract) reflects mild degeneration and sinusoidal congestion, possibly indicating a more balanced response. Group 6 (1000mg/kg TCL extract) displays moderate hepatocyte degeneration, suggesting potential toxicity at higher concentrations. These histological findings substantiate the impact of high salt intake and toxic substances on liver histology, as well as the potential protective role of Vitamin C. The nuances observed in the responses to TCL extracts underscore the importance of careful consideration of dosage and concentration in understanding their effects on liver health.

The findings of our study on liver histology find resonance in existing literature. The severe vacuolation and distortion in the high salt-fed group (Group 2) are consistent with research linking high salt intake to non-alcoholic fatty liver disease (NAFLD) and liver damage [4,35]. The milder alterations observed in the Vitamin C-treated group (Group 3) align with the suggested protective effects of Vitamin C on the liver [18,36].

Furthermore, the toxic effects observed in groups subjected to varying concentrations of TCL extracts are in line with existing literature cautioning about potential histological impairments and biochemical alterations due to exposure to certain substances [37].

These comprehensive findings emphasize the intricate interplay between high salt intake, interventions, and liver histology. The observed effects of Vitamin C and TCL extracts on biochemical markers and liver structure underscore their potential roles in mitigating liver stress and dysfunction induced by a high-salt diet. However, the varying responses to TCL extracts highlight the need for cautious consideration of dosage and concentration in exploring their effects on liver health. Further research is warranted to delve deeper into the mechanisms underlying these observations and to refine the understanding of the potential hepatoprotective properties of Vitamin C and TCL extracts.

Conclusion

In conclusion, Vitamin C and TCL extracts hold promise in mitigating liver stress induced by a high-salt diet. The observed reductions in liver enzyme levels, improvements in protein markers, and mitigated bilirubin levels suggest potential hepatoprotective effects. However, the varying histological responses, especially at higher doses of TCL extracts, indicate the importance of careful consideration of dosage and concentration. The ongoing liver degeneration in the high salt-fed group underscores the detrimental effects of sustained high salt intake. The findings

contribute to the understanding of the complex interplay between interventions and liver health. Further Research should be conducted to elucidate the underlying mechanisms of the observed effects, especially the varying histological responses to different doses of TCL extracts, investigate optimal dosage and concentration levels for Vitamin C and TCL extracts to maximize their hepatoprotective effects while minimizing potential toxicities and explore the long-term effects of Vitamin C and TCL extracts on liver health to assess sustained benefits and potential risks.

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