**Therapeutic effects of *Cucurbita pepo* (Pumpkin) Fruit Pulp and Seeds Methanol extracts against CCl₄ induced Hepatotoxicity in Wistar Rats**

**ABSTRACT**

This study was aimed at assessing the therapeutic potential of *Cucurbita pepo* (Pumpkin) Pulp and Seeds Methanol extract against CCl4 induced hepatotoxicity in wistar rats. The phytochemical screening, analyses of liver function and haematological parameters were carried out following standard procedures. The study adopted experimental design. Forty two rats distributed into seven groups of six rats each. Group 1= normal control, group 2= positive control, group 7= standard control, groups 3-6= experimental groups administered different doses of the extracts. Results showed the presence of phenols, tannins, steroids and flavonoids in in both. Terpenoids in leaves, absent in pulp. Alkaloids in the leaves, absent in the pulp. phenols and saponins values of 17.99±1.47 and 13.07±0.30 (mg/100g). Similarly, the leaves showed the highest concentration in alkaloids, steroids, cardiac glycosides, and terpenoids (37.15±0.10, 28.42±1.17, 6.10±0.62, and 24.00±0.38 (mg/100g)) respectively. The fruit had the highest concentration of flavonoids and tannins (28.48±0.76 and 11.71±0.75 (mg/100g)). ALT, AST, increased significantly while protein and albumin decreased significantly in the untreated group but were significantly reversed in the treated groups. SOD and GPx also increased significantly in the treated groups with significant decrease in MDA and Platelets. The Red Blood Cell (RBC), PCV significantly (p<0.05) increased in the treatment groups. There was also an increased in levels of MCH in group 2. Significant (p<0.05) elevation of lymphocyte count (L) in group 2 when compared to the rest groups same observation with the monocyte differential count (M) and Eosinophils count. Group 2 shows an especially high level of lymphocytes. In conclusion, the administration of CCL4  expectedly lead to serious alterations of biochemical and haematological parameters in the rats which were however reversed and restored upon administration of the leaves and pulp extracts of *Cucurbita pepo.*

**Keywords:**Antioxidants,Hepatotoxicity, Haematology, Pumpkin, Therapeutic

**1.0 INTRODUCTION**

The plant*, Cucurbita pepo* (pumpkin) is a member of the *Cucurbitaceae* family. It is native to West Africa and predominantly grown in Sierra Leone, Ghana, and Nigeria (Ajuru&Nmom, 2017). *Cucurbita spp.* is one of the main vegetable crops cultivated in the Northern part of Nigeria, where it is known by the local name “kabewa” in Hausa. Pumpkin is a creeping or climbing plant, monoecious, annual although persistent for a certain period, giving the impression of being a short-lived perennial, without swollen reserve roots (Ajuru&Okoli, 2013). It is resistant to low temperatures but not to severe frosts. It has five vigorous, slightly angular stems and leaves with 5 to 25 cm petioles that are ovate–cordate to suborbicular–cordate, with or without white spots on the surface, and has three to five rounded or obtuse, apiculate lobules, the central one bigger than lateral ones (Ajuru & Okoli, 2013). Male flowers are long and pedicellate and have a campanulate calyx that is 5 to 10 mm long and almost as wide, 5–15 x 1–2 mm linear sepals and a tubular campanulate corolla that is rather broader towards the base, 6 to 12 cm long and yellow to pale orange. They have three stamens (Ajuru & Okoli, 2013). Female flowers have sturdy peduncles, 3 to 5 cm long, an ovoid to elliptical, multilocular ovary, sepals that are occasionally foliaceous, and a corolla that is somewhat larger than that of the male flowers. They have a thickened style and three lobate stigmas. The fruit is globose to ovoid–elliptical, with three colour patterns (Ajuru & Okoli, 2013).



Figure 1.Pumpkin plant(Sharma et al., 2013)

Hepatotoxicity refers to liver damage resulting from exposure to toxic substances, which can significantly impair the liver's essential functions, including metabolism and detoxification​.(Sayeed & Marwa, 2023). This damage sets off a series of pathological processes, potentially leading to the development of various liver diseases such as fibrosis, cirrhosis, fatty liver disease, and acute liver injury ​(Pooja *et al.,* 2021).

​Fibrosis is the initial stage of liver damage, characterized by the accumulation of excessive connective tissue in response to persistent injury and inflammation. Over time, this scarring can disrupt liver function, and if the underlying damage remains untreated, fibrosis may progress to cirrhosis—an advanced and irreversible condition marked by extensive scarring, loss of liver architecture, and impaired liver function(Yaseer *et al.,* 2022).​

Cirrhosis is a late-stage liver disease that occurs when scar tissue (fibrosis) replaces healthy tissue (Sojood *et al.,* 2022). It is a consequence of long-standing excessive fibrogenesis resulting in encapsulation and/or replacement of injured liver parenchyma by a collagenous scar.

The aetiology of cirrhosis varies geographically, with alcoholism, chronic hepatitis C virus infection, and non-alcoholic fatty liver disease (NAFLD) being the most common causes in Western countries whereas chronic hepatitis B is the primary cause of liver cirrhosis in the Asia-Pacific region. Numerous other conditions can induce liver cirrhosis, such as autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and hereditary disorders like hemochromatosis and Wilson's disease.

The liver is a vital organ responsible for many important functions, including detoxification, metabolism, and the production of proteins. When the liver is injured, inflammation is the body’s natural response to repair the damage and protect against infection(Gibert-Ramos *et al.,* 2021).Multiple cells play a role in liver cirrhosis, including hepatocytes and sinusoidal lining cells (Gibert-Ramos *et al.,* 2021). Damaged hepatocytes release reactive oxygen species these reactive intermediates can induce pro-fibrogenic cytokines and the release of several inflammatory markers and collagen synthesis during the progression of liver fibrosis (Ezhilarasan *et al.,* 2018). Under certain conditions, the oxidative to anti-oxidative balance shifts towards the oxidative status as a result of an increase in ROS production, or antioxidant depletion results in oxidative stress.

Hepatotoxicity, is a significant concern in both clinical and environmental contexts, leading to liver damage and dysfunction. Nasarawa state is endowed with deposits of solid minerals which contain heavy metals.  Human activities such as mining, industrial processing, and various agricultural activities can lead to the release of these heavy metals into the environment, the continuous ingestion of these toxic heavy metals could cause liver injury and damage.

While various treatment strategies exist, the search for effective and accessible therapies with minimal side effects continues.

Given the rising interest in natural products, there is a pressing need to explore the therapeutic potentials of plant extracts. *Cucurbita pepo*, or pumpkin, is rich in bioactive compounds and has been suggested to possess hepatoprotective properties. This study aimed at assessing the potential therapeutic effects of pumpkin in mitigating hepatotoxicity induced by CCl₄ in Wistar rats (animal models). By doing so, we sought to address the gap in knowledge regarding the protective effects of pumpkin extracts on liver health, ultimately contributing to the development of safer, natural therapeutic options against hepatotoxicity.

# 2.0 MATERIALS AND METHODS

# 2.1 Materials

# 2.1.1 Plant Materials

The plant materials were the fruit and leaves of pumpkin*.* They were collected in a village called Dokari, which is situated in Keffi LGA, in Nasarawa state, North Central Zone of Nigeria. The plant was identified by a Botanist at the Department of Plant Science and Biotechnology, Nasarawa State University, Keffi.

# 2.1.2 Chemicals and Reagents

The chemical compound, carbon tetrachloride (CCl4) was obtained from the Chemistry Department, Nasarawa State University, Keffi, and was used to induce liver cirrhosis. All other chemicals and reagents were of analytical grade.

# 2.1.3 Equipment

The major equipment used for the study were:UV/Visible Spectrophotometer,CentrifugeMicroscope, Digital Camera, Digital Photo Colorimeter, Bench Centrifuge, Rotary Evaporator, Vortex Mixer, Electronic Balance,Homogenizer,pH Meter,Water Distiller,Electronic Incubator,Dry Oven

# 2.1.4 Experimental Animals

Forty two (42) male Wister albino rats weighing between 180- 200 were used for the study. The choice of this rat’s body weight was to ensure they could withstand the toxicity of CCl4. These rats werepurchased from the animal house of the National Veterinary Research Institute (NVRI), Vom, in Plateau State. They were housed in clean, well-ventilated metal cages in the animal house of the Department of Biochemistry, Nasarawa State University Keffi. The animals were kept under 24 hours of light/ dark cycling. They were allowed access to unlimited food and water supply and allowed to acclimatize for one (1) week before the commencement of the study.

# 2.2 Methods

# 2.2.1 Sample Collection and Preparation

# The pumpkin fruit was peeled and thinly sliced using a knife to get the pulp and also the leaves were handpicked.

The pumpkin parts (pulpand leaves) were properly rinsed in water to remove dust and sand particles and then dried at room temperature for 2 weeks and. The dried pumpkin parts were then ground into powder using an electric blender andused for the preparation of methanol extract.

# 2.2.2 Preparation of Extracts

Ground plant materials were extracted with methanol by soaking 100 g of the ground samples in 500 ml of 98% methanol (i.e., ratio 1:5; volume weight) for 48 hrs. The extracts were then filtered using filter paper and then concentrated by heating in a water bath and stored in airtight containers.

# 2.2.3 Qualitative Phytochemical Screening of the Pumpkin Extracts

Portions of the concentrated extracts were used for phytochemical screening using standard procedures of the Association of Analytical Chemists to identify the constituents as described by Fadeyi (1983), Odebiyi and Sofowora (1990), Sofowara (1993), and Harborne (1998).

# *Test for Alkaloids*: The plant extracts (0.5g) was dissolved in 2 ml of dilute Hydrochloric acid and filtered; the filtrate was treated with a few drop of Hager’s reagent (saturated picric acid solution). The presence of yellow colour precipitate indicated the presence of alkaloids.

# *Test for Sterols*: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drop of concentrated H2SO4, shaken, and allowed to stand. A reddish-brown colour at the interface indicated the presence of a steroid ring.

# *Test for Triterpenoids:*Crude extracts were dissolved in chloroform and filtered. The filtrates were treated with a few drop of concentrated H2SO4, shaken, and allowed to stand. The presence of yellow colour at the layer indicated the presence of triterpenoids

# *Test for Flavonoids*:A few drop of sodium hydroxide solution were added to (0.5 g) of the extract. The persistence of intense yellow colour with the addition of dilute acid indicated the presence of flavonoids.

# *Test for Saponins*: Half (0.5) grams of the plant extract were shaken vigorously with 2 ml of water in a test tube. The persistence of the foam formed indicated the presence of saponins.

# *Test of Tannins*: In a test tube, 1 ml of ethanol solution was added to 2 ml of water and 2-3 drops of diluted solution of FeCl3 and observed for a green colour. A blue-black or a blue-green colouration showed the presence of tannins.

# *Test for Terpenoids*: Acetic anhydride (0.5 ml) was added to the sample extract and a few drops of H2SO4 will be added. The presence of a bluish-green precipitate indicated the presence of terpenoids.

# *Test for Phenols*: To 0.5 g of extract, 1% FeCl3 in ethanol was added. The formation of a dirty green precipitate indicated the presence of phenol.

# *Test for Glycosides*: Two hundred milligrams (200 mg) of crude extract were mixed vigorously with 2 ml of diluted sulphuric acid and 5% aqueous ferric chloride solution boiled for 5 minutes was added. Oxidation to anthroquinones indicated the presence of glycosides.

# *Test for Anthraquinones*: The extract (0.2 g) was added to 10 ml Benzer, shaken, and filtered. A volume of 0.5 ml of 1% NH3 will be added. The persistence of pink, red, or violet colour at the lower phase indicated that anthraquinones are present.

# *Test for Cardiac Glycosides*: A quantity (0.5 g) of sample extract was dissolved in 2 ml of glacial acetic acid containing a drop of ferric chloride. 2 ml of concentrated Sulphuric acid was added. The formation of a brown ring at the interface indicated the presence of cardiac glycoside.

# 2.4 Experimental Design for in vivo studies

The animals were divided into seven(7) groups of 6 animals each.

Groups 1, 2, 3, 4, 5, 6, and 7 with 6 rats. Each received intraperitoneally injection of CCl4 at a dose of 0.5 ml/ 100 g. body weight for the first time followed by a dose of 0.3 ml/ 100 g b.w. twice a week over eight weeks to induce liver cirrhosis (Zhou *et al.,* 2014). Four rats from the injected group of rats were chosen randomly and killed for pathological examination to determine liver cirrhosis. At the end of the experiment (8 weeks) the rats were fasted for 12 hours and then sacrificed under chloroform anaesthesia. The summary is given in Table 1.

Table 1: Experimental Design

|  |  |
| --- | --- |
| Groups | Substance Administered Orally |
| Group 1 (Normal control) | Water, feed, and 150 mg/ kg bw combined dose of the combined plant extract |
| Group 2 (Positive control) | Water, feed, and 0.5 ml/ 100 g of CCl4 |
| Group 3 (Pulp extract) low dose | Water, feed, 0.5 ml/ 100 g of CCl4, and treated 100 mg/ kg bw of pulp extract |
| Group 4 (Pulp extract) high dose | Water, feed, 0.5 ml/ 100 g of CCl4, and treated with 200 mg/ kg bw of pulp extract |
| Group 5 (leaf extract) low dose | Water, feed, and 0.5 ml/ 100 g of CCl4, and treated with 100mg/ kg bw of leaf extract |
| Group 6 (leaf extract) high dose | Water, feed, and 0.5 ml/ 100 g of CCl4, and treated with 200 mg/ kg bw of leaf extract |
| Group 7 (Standard)  | Water, feed, 0.5 ml/ 100 g of CCl4, and treated with 100mg/ kg Silybon |

# 2.5 Preparation of Biological Samples

# 2.5.1 Collection of Blood Samples

At the end of the experiment, the animals were made unconscious by exposure to chloroform in an enclosed c ontainer, according to the method described by Ekor, *et al.* (2006). Incisions were quickly made into the animals’ neck region with the aid of sterile blades. Blood samples were collected by decapitation into plain tubes. The serum was collected by centrifuging the clotted blood in an HSC (1000-4000 rpm) bench centrifuge at 3000 rpm for 10 minutes.

# 2.5.2 Preparation of Liver Homogenate

After bleeding, the livers were carefully removed, trimmed extraneous tissues, and rinsed in normal saline and 1.15% KCl. The livers were then blotted dry, and 2 grams (g) were weighed and homogenized in 8 millilitres (8 ml) of ice-cold phosphate buffer (100 mM, pH 7.4). The homogenate was then centrifuged first at 6,000 rpm for six minutes (6 mins) to remove nuclear debris after which the obtained supernatant was further centrifuged at 10,000 rpm for twenty minutes (20 mins) to obtain the post-mitochondrial supernatant (PMS), using a refrigerated centrifuge. This was used for the assay of the antioxidant enzymes (Superoxide dismutase, Catalase, and Glutathione Peroxidase).

# 2.5.3 Determination of the Liver Function indices

The prepared serum was analysed for various biochemical parameters - Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP), lipid profile (Triacyl glycerides, Total Cholesterol, High and low-density lipoproteins), total protein, albumin and bilirubin using spectrophotometric procedures.

# *Estimation of Alkaline Phosphatase (ALP):*The serum Alkaline Phosphatase activities of the experimental animals were estimated using the method of King (1965a).

Principle

The determination of alkaline phosphate (ALP) was based on the reactions below:

P-Nitrophenyl phosphate + H2O Alkaline phosphatase p-Nitrophenyl + inorganic phosphate

*Procedure:*One millilitre (1.0 ml) of serum (the ALP source) was pipetted into 1.5 ml of phosphate buffer (0.1M, pH 7.4) and made up to 3.0 ml with distilled water in the test tubes. The tubes were incubated at 37°C for 15 minutes. The reaction was arrested by the addition of 1.0 ml of Folin's phenol reagent. To the control tubes, the enzymes were added after arresting the reaction. The contents were then centrifuged and 1.0 ml of the supernatant, 1.0 ml of 15 % sodium carbonate, 1.0 ml of the substrate, and 0.1 ml of MgCl2 were added and incubated for 10 minutes at 37°C. The colour developed was read at 640 nm using a spectrophotometer against a blank. The standard solutions of phenol of varying concentrations were also treated similarly. The enzyme activity in serum and tissues was expressed as moles of phenol liberated/ minute/ mg of protein.

# *Estimation of Aminotransferase Activities*: The determination of Aspartate aminotransferase and Alanine aminotransferase were carried out using the King (1965b) method.

*Principle:*The transaminase enzymes, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) catalyse the transfer of the amino group of glutamic acid to oxaloacetic acid and pyruvate in reversible reactions. The transaminase activity is proportional to the amount of oxaloacetate and pyruvate formed over a definite period and is measured by a reaction with 2.4-dinitrophenylhydrazine (DNPH) in an alkaline solution.

*Estimation of Aspartate Aminotransferase (AST)*

*Principle:* L-Aspartate + α-ketoglutarate Aspartate amiontransferase Oxaloactate + L-Glutamate

Procedure: To 1.0 ml of the buffered substrate, 0.1 ml of enzyme source was added and incubated for one hour at 37°C. At the end of the incubation period, 0.07 ml of aniline-citrate reagent was added and incubated for another 20 minutes. Then, 1.0 ml of the dinitrophenyl hydrazine reagent was added and left for 20 minutes. At the end of 20 minutes, 10 ml of 0.4M NaOH were added and the colour developed was read at 640 nm in a spectrophotometer after 10 minutes. The standards were also treated similarly.

*Calculations:*The enzyme activity in serum was expressed as units per litre (moles of pyruvate/ hour/ mg of protein).

*Estimation of Alanine Amino Transferase (ALT)*

*Principle:*α -ketoglutarate +L-alanine Alanine amino transferase L-glutarate + pyruvate

Procedure: To 1.0 ml of the buffered substrate, 0.1 ml of enzyme source was added and incubated at 37°C for 30 minutes. The reaction was arrested by the addition of 1.0 ml of dinitrophenyl hydrazine and left aside for 20 minutes at room temperature. The colour developed by the addition of 10 ml of 0.4N sodium hydroxide was read at 540 nm in a spectrophotometer against the reagent blank.

*Calculations:*The enzyme activity in serum was expressed as moles of pyruvate/ hour/ mg of protein

# *Determination of the Activities of the Antioxidant Enzymes in the Liver*

#  *Determination of Superoxide Dismutase (SOD) activity:* Superoxide Dismutase activity was determined by the method described by Sun and Zigma (1978).

*Principle:*Superoxide Dismutase can inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm.

*Procedure:*A reaction mixture (3 ml) containing 2.95 ml of 0.05 M sodium carbonate buffer pH 10.2; 0.02 ml of liver homogenate and 0.03 ml of 0.3 mM epinephrine in 0.005 N HCL was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water.

*Calculation:* Enzyme activity was calculated by measuring the change in absorbance at 480 nm after 5 minutes.

Σ= 4020M-1 cm-1

*Determination of Malondialdehyde Concentration:* MDA concentration was measured spectrophotometrically as described by Wallin et al, (1993).

Principle:The principle for the estimation is based on the fact that thiobarbituric acid (TBARS) reacts with malondialdehyde (MDA) to give a red or pink colour, which absorbs maximally at 532 nm.

*Procedure*: A known quantity of the serum sample, (0.1 ml) were mixed with 0.9 ml of distilled water in a test tube. Then, 0.5 ml of 25% trichloroacetic acid (TCA) and 0.5 ml of 1 ml thiobarbituric acid (TBARS) in 0.3% NaOH were also added to the mixture. The mixture was boiled for 40 minutes in a water bath and cooled in cold water. Then 0.1 ml of 20% sodium dodecyl sulphate (SDS) was added to the cooled solution and mixed properly. The absorbance was taken at wavelength 532 nm and 600 nm against a blank. MDA (mg/dL) = A532−A600 × 100 0.5271 x 1

*Determination of Catalase Activity*

The catalase activity was determined according to the method of Beers and Sizer as described by Usoh *et al.,* (2005).

*Principle:* Catalase is assayed by measuring the decrease in absorbance at 240 nm due to the decomposition of H2O2, using the UV spectrophotometer.

*Procedure:*The sample was prepared by homogenizing the liver in the phosphate buffer (1:4,), weight (in grams of liver) to volume (in millilitres of buffer). The reaction mixture (3 ml) contained 0.1 ml of homogenate in phosphate buffer (50 mM, pH 7.0) and 2.9 ml of 30 mM H2O2 in phosphate buffer pH 7.0.

*Calculations:*An extinction coefficient for H2O2 at 240 nm of 40.0 M-1cm-1 (Aebi, 1984) was used for the calculation. The specific activity of catalase was expressed as moles of H2O2 reduced per mg protein per minute

# *Determination of Glutathione Peroxidase (GSH-Px) Activity;* The method of Lawrence and Burk (1976) was employed to measure the activity of GSH-Px.

*Principle*: It is based on the oxidation of NADPH by the Glutathione peroxidise which is measured in terms of ‘n’ moles of NADPH oxidized/min/mg protein

*Procedure:*A hundred microliter (100 μl) of supernatant was mixed with 700 μl of reaction mixture containing, 1 mM EDTA, 1 mM NaN, 0.2 mM NADPH and 1 mM GSH in a phosphate buffer saline, pH 7.2, and 100 μl of glutathione peroxidase (containing 10 units of GPx). The tubes are vortexed and incubated for 5 min at room temperature. After incubation, 100 μl of 0.2 mM H2O2 was added to initiate the reaction and absorbance was recorded at 340 nm and after every 30 sec over 3 min, using a spectrophotometer.

*Calculations:* Changes in the rate of absorbance were converted into ‘n’ moles of NADPH oxidized/ min/ mg protein, using an extinction coefficient of 6.22 × 10 L/ mol/ cm.

# 3.7. Statistical Analysis

Statistical analysis was conducted, using the one-way ANOVA and data were expressed as mean ± standard deviation (SD). The differences between the mean values of the test groups were analysed for statistical significance using one-way ANOVA. The differences between groups were considered to be significant if p<0.05.

# 2.8. Ethical Considerations

The study complied with the Nasarawa State University guidelines for handling of experimental animals and conduct.

**3.0 RESULTS AND DISCUSSION**

**3.1 Comparative Qualitative Phytochemical Screening of the Leaves and Fruit of Pumpkin**

The result of the Qualitative Phytochemical Screening (Table 2.) showed that the pumpkin in its different parts is made of several phytochemical constituents. The comparative qualitative phytochemical screening between the leaves and fruit of pumpkin showed the presence of phenols, tannins, steroids and flavonoids in sufficient quantity in the leaves and fruits respectively. From the result of the screening, it was observed that there is presence of terpenoids in the leaves but not detected in the fruit. And also the alkaloids were observed to be sufficiently present in the leaves but deficit in the fruit.

Table 2 Comparative Qualitative Phytochemical Screening of the Leaves and Fruit of Pumpkin.

Phytochemicals Leaves fruit

Phenol + +

Tannins + +

Saponins + +

Alkaloid + ND

Steroids + +

Cardiacglyco. + +

Glycosides + +

Flavonoids + +

Terpenoids + ND

+= Detected, ND = Not Detected.

**3.1.2 Comparative Quantitative Phytochemical Screening of the Leaves and Fruit of Pumpkin**

The result of the Quantitative Phytochemical Screening (as shown in Table 1) showed different concentrations of phytochemicals in the parts of the pumpkin (leaves and fruits). The comparative quantitative phytochemical screening between the leaves and the fruits showed the presence of phenols, tannins, steroids and flavonoids in sufficient amounts in the leaves and fruits with the leaves having the highest concentration of phenols and saponins with values of 17.99±1.47 (mg/100g) and 13.07±0.30 (mg/100g). Similarly, the leaves showed the highest concentration in alkaloids, steroids, cardiac glycosides, and terpenoids with values of 37.15±0.10, 28.42±1.17, 6.10±0.62, and 24.00±0.38 (mg/100g) respectively. Furthermore, it was observed that the fruit had the highest concentration of flavonoids and tannins with values of 28.48±0.76 and 11.71±0.75 (mg/100g). Alkaloids and terpenoids were not detected in the fruit.

Table 3.Comparative quantitative phytochemical screening of the leaves and fruit of pumpkin.

Phytochemicals Leaves (mg/100g) fruit (mg/100g)

Phenol 17.99 ± 1.47b 15.14 ± 0.46a

Tannins 10.33 ± 1.5a 11.71 ± 0.75b

Saponins 13.07 ± 0.30b 8.41 ± 0.81a

Alkaloid 37.15 ± 0.10b ND

Steroids 28.42 ± 1.17b 19.03 ± 0.49a

Cardiacglyco. 6.10 ± 0.62b 3.95 ± 0.26a

Glycosides 4.76 ± 0.62a 3.54 ± 0.27a

Flavonoids 23.38 ± 0.65a 28.48 ±0.76b

Terpenoids 24.00 ± 0.38a ND

The results are expressed in Means ± SD (in triplicate).Mean values with different letters as superscripts across the column are considered significantat P< 0.05.ND = Not Detected.

**3.1.3. Effect of Pumpkin Leaves and Fruit Extract on Liver Function Parameters in Albino Rats**

In table 4. below it was observed that induction with CCl4 (group 2) increased significantly P< 0.05 the levels of ALT and AST, with concomitant decrease in protein and albumin levels. it was also noted that as we move down the groups for the extracts there was a significant decrease (p<0.05) in ALT and AST observed in groups 5 and 7 when compared with groups 1 and 2 respectively and an increased in protein and albumin.

**Table 4 Effect of pumpkin extract on liver function parameters in albino rats**

Groups ALT (µ/L) AST (µ/L) Protein (g/L) Albumin (g/L)

1 25.15 ± 1.06b 21.85 ± 2.90d 94.30 ± 8.06a 46.75 ± 4.17c

2 33.90 ± 0.14b 32.50 ± 3.54c 89.80 ± 1.19a 40.55 ± 0.49d

3 20.35 ± 0.78b 21.80 ± 5.66d 103.05 ± 0.64c 47.65 ± 10.53c

4 19.83 ± 2.72a 20.37 ± 3.25d 100.83 ± 3.17b 44.23 ± 3.17b

5 20.45 ± 2.05b 22.65 ± 2.62a 106.65 ± 3.32a 45.45 ± 1.77c

6 19.25 ± 1.63b 22.05 ± 1.48a 94.70 ± 9.62b 49.85 ± 2.19c

7 24.20 ± 6.88b 19.70 ± 3.87b 103.80 ± 1.20a 51.80 ± 2.65c

The results are expressed in Means ± SD (in triplicate).Mean values with different letters as superscripts down the groups are considered significantat P< 0.05.[1: (Positive control), 2: (Negative control), 3: (Low dose leaf extract 100mg), 4: (High dose leaf extract 200mg), 5: (Low dose fruit extract 100mg),6: (High dose fruit extract 200mg) 7: (Standard) Silybon 100 mg/kg.

**3.1.4. Effect of the pumpkin extracts on antioxidant enzymes in albino rats**

The antioxidant activity assessment revealed significant variations among the extracts. Notably the high dose fruit extract(200mg) group 6, demonstrated exceptional antioxidant potential, evident in its elevated SOD(309.42 ± 3.09) and its reduction in MDA (1.20 ± 0.06) levels. In contrast, the negative control(group 2)exhibited significantly lower SOD and GPX, alongside an elevated MDA. Both groups of leaf extract, 3 and 4,displayed moderate antioxidant activity.. The result showed almost no significant difference in all the groups for the MDA.

**Table 5 Effect of the pumpkin extracts on antioxidant enzymes in albino rats**

Groups SOD (µ/L) GPX (µ/L) MDA (mg/dl)

1 300.63 ± 5.74c 18.09 ± 1.08bc 1.36 ± 0.11a

2 266.59 ± 26.55a 14.61 ± 2.23a 1.89 ± 0.33c

3 271.54 ± 11.37b 16.24 ± 1.23b 1.46± 0.04b

4 286.11 ± 12.00bc 17.65 ± 0.70b 1.35 ± 0.18a

5 309.23 ± 4.77c 18.96 ± 0.8bc 1.23 ± 0.08a

6 309.42 ± 3.09c 18.74 ± 1.26bc 1.20 ± 0.06a

7 298.77 ± 10.14c 19.13 ± 1.47c 1.41 ± 0.13b

The results are expressed in Means ± SD (in triplicate).

Mean values with different letters as superscripts down the down are considered significant at P< 0.05. SOD= Superoxide dismutase, GPX, Glutathione peroxidase and MDA= Malonadehyde.

[1: (Positive control), 2: (Negative control), 3: (Low dose leaf extract 100mg), 4: (High dose leaf extract 200mg), 5: (Low dose fruit extract 100mg),6: (High dose fruit extract 200mg) 7: (Standard) Silybon 100 mg/kg.

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**3.1.5 Effect of the Leaves and Fruit Extract on Haematological parameters in Albino Rats**

The result in table 6, showed a varying significant difference in the studied parameters in the entire group. For the White Blood Cell (WBC) there were observed significant differences among the groups, Group 2 had the highest concentrations.

For the (Platelets) PLT there was an observed significant decrease at (p< 0.05) in groups 4 and 7 while group 2 had the highest concentration (increase). The Red Blood Cell (RBC) had a significant decrease in group 2 with concentrations of 3.70 ± 0.57a while groups 4 and 7 have the highest respective values. For the Packed Cell Volume (PCV) it was observed from the result that a significant decrease in group 2 when compared to the rest of the groups. The haemoglobin (HB) had no obvious significant decrease among the groups at (p<0.05) except for groups 2 and 6.

Table 6 Effect of the leaves and fruit extract on Haematological Parameters

Groups WBC (cells/L) PLT (plt/L) RBC (RBC/L) PCV (%) HB (g/L)

1 4.95 ± 0.92a 337.00 ± 93.33c 5.30 ± 0.38a 45.50 ± 0.71c  14.85 ± 0.35b

2 6.70 ± 0.57c 474.50 ± 44.55d 3.70 ± 0.57a 41.50 ± 2.12b 13.60 ± 0.85a

3 4.35 ± 0.49a 269.00 ± 181.02a 4.25 ± 0.35b 44.00 ± 1.41a 14.05 ± 0.35b

4 5.00 ± 0.75b 310.67 ± 109.50b 5.63 ± 0.35c 45.33 ± 5.13c 14.10 ±0.44b

5 5.70 ± 4.81b 333.00 ± 48.08b 5.40 ± 0.99c 43.00 ± 4.24b 14.20±1.27b

6 5.20 ± 0.57b 323.00 ± 55.15b 5.10 ± 0.99c 44.00 ± 1.44b 13.90 ± 0.42a

7 6.37 ± 2.87c 271.33 ± 69.01a 5.60 ± 1.23c 48.00 ± 6.25c 15.60 ± 1.20c

The results are expressed in Means ± SD (in triplicate, N= 7).Mean values with different letters as superscripts down the groups are considered significantat P< 0.05.WBC= White-Blood Cell, PLT= Platelets, RBC= Red Blood Cell, PCV= Packed Cell Volume and BH= Haemoglobin([1: (Positive control), 2: (Negative control), 3: (Low dose leaf extract 100mg), 4: (High dose leaf extract 200mg), 5: (Low dose fruit extract 100mg),6: (High dose fruit extract 200mg) 7: (Standard) Silybon 100 mg/kg.)

**3.1.6 Effect of the Leaves and Fruit Extract on Red Blood Cell Differential count in Albino Rats**

For the MCV, MCH, and MCHC respectively in Table 7, it revealed a significant difference across the groups. Group 2 having the most elevated value of MCV which notably showed a sign of macrocytosis. There was also an increased in levels of MCH in group 2..

Table 7 Effect of the Leaves and Fruit Extract on Red Blood Cell indices in Albino Rats

Groups MCV(fL) MCH (pg) MCHC (g/dL)

1 85.50 ± 3.54a 28.05 ± 0.78c 32.25 ± 0.21a

2 97.50 ± 3.54b 31.95 ± 0.78d 32.75 ± 0.35b

3 96.00 ± 1.44a 28.75 ± 4.60a 33.10 ± 0.57a

4 93.67 ± 7.51b 28.50 ± 2.19b 32.77 ± 0.57c

5 88.00 ± 2.83c 26.95 ± 2.47b 32.85 ± 0.21c

6 88.0 ± 0.57b 27.15 ± 1.06b 33.20 ± 0.14c

7 87.00 ± 8.66c 28.40 ± 2.78a 32.93 ± 0.61c

The results are expressed in Means ± SD (in triplicate, N= 7).

Mean values with different letters as superscripts down the groups are considered significant at P< 0.05.

([1: (Positive control), 2: (Negative control), 3: (Low dose leaf extract 100mg), 4: (High dose leaf extract 200mg), 5: (Low dose fruit extract 100mg),6: (High dose fruit extract 200mg) 7: (Standard) Silybon 100 mg/kg.)

**3.1.7 Effect of the Leaves and Fruit Extract on white Blood Cell Differential count in Albino Rats**

There was an observed significant elevation of lymphocyte count (L) in group 2 when compared to the rest groups same observation with the monocyte differential count (M) and Eosinophils count. Group 2 shows an especially high level of lymphocytes, suggesting an inflammatory response. It also has the highest mean of lymphocytes (82.00), which could suggest an immune response or inflammation. However, Group 3 and Group 4 (low and high dose leaf extracts) show similar lymphocyte levels, which are closer to the baseline of the positive control.

Group 2 has the highest monocyte count, suggesting significant inflammation. Groups 5 and 6 (low and high dose fruit extract) show lower levels of monocytes, again suggesting anti-inflammatory effects, as seen in eosinophil and lymphocyte levels.

Table 8 Effect of the Leaves and Fruit Extract on white Blood Cell Differential count in Albino Rats

|  |  |  |  |
| --- | --- | --- | --- |
| Groups | Eosinophils (cells/ µL) | Lymphocytes (cells/ µL) | Monocytes (cells/ µL) |
| 1 | 2.00 ± 1.14c  | 67.00 ± 11.31b | 4.50 ± 0.71 |
| 2 | 2.00 ± 0.00b | 82.00 ± 1.41  | 8.50 ± 2.12 |
| 3 | 1.50 ± 0.71a | 68.00 ± 1.41b | 3.50 ± 2.12 |
| 4 | 2.00 ± 1.00c | 66.67 ±3.51b | 4.00 ± 1.00 |
| 5 | 1.00 ± 0.00b | 65.50±10.61b | 4.00 ± 2.83 |
| 6 | 1.50 ± 0.71b | 67.50 ± 4.95a | 3.50 ± 0.71  |
| 7 | 2.67 ± 1.53c | 70.67 ± 10.69c | 5.67 ± 0.589 |

The results are expressed in Means ± SD (in triplicate, N= 7).

Mean values with different letters as superscripts down the groups are considered significant at P< 0.05.

([1: (Positive control), 2: (Negative control), 3: (Low dose leaf extract 100mg), 4: (High dose leaf extract 200mg), 5: (Low dose fruit extract 100mg),6: (High dose fruit extract 200mg) 7: (Standard) Silybon 100 mg/kg.)

E(Eosinophils)L(lymphocyte)M(monocyte)

**3.2 DISCUSSION**

Pumpkin, belonging to the genus Cucurbita, is a well-known plant utilized for both culinary and medicinal purposes. Its leaves and fruit are rich in various bioactive compounds, contributing to their health benefits. This study aims to investigate the therapeutic effect of pumpkin leaves and fruits in ameliorating liver cirrhosis. In this report the qualitative phytochemical screening of pumpkin leaves and fruit extracts revealed the presence of key phytochemicals such as phenol, tannins, saponins, alkaloids, steroids, cardiac glycosides, glycosides, flavonoids, and terpenoids .

The presence of a wide range of phytochemicals in both pumpkin leaves and fruit extracts suggests their potential health benefits. Alkaloids are known for their analgesic and antibacterial properties (Roy, 2017), while flavonoids and phenols possess strong antioxidant activities (Ferreira *et al.,* 2015; Husain *et al.,* 2021). Saponins are beneficial for their cholesterol-lowering properties, and tannins have anti-inflammatory effects (Singh *et al.,* 2017). Glycosides, terpenoids, and steroids also contribute to various therapeutic effects, making pumpkin a valuable plant in both nutritional and pharmaceutical contexts (Mastropasqua*et al.,* 2020). These findings highlight the potential health benefits of pumpkin and underscore the need for further research to isolate and characterize these bioactive compounds for potential therapeutic applications.

The quantitative screening of the leaves and fruits showed a significantly high concentration of phenols, saponins, alkaloids, steroids, cardiac glycosides, and terpenoids in the leaves compared to the fruit. However, the concentration of the flavonoid was significantly higher in the fruits from previous pages.Pumpkin leaves and fruit are rich in phenolic compounds and carotenoids, which exhibit strong antioxidant properties (Perez-Alvarez *et al.,* 2020). These antioxidants neutralize free radicals, reducing oxidative stress and preventing cellular damage. The high β-carotene content in both leaves and fruit contributes to this antioxidant effect, which is crucial in reducing the risk of chronic diseases such as cancer and cardiovascular diseases (Perez-Alvarez *et al.,* 2020).

The flavonoids and phenolic acids present in pumpkin extracts have significant anti-inflammatory properties. These compounds inhibit the production of pro-inflammatory cytokines and enzymes, such as COX-2 and TNF-α, thus reducing inflammation (Perez-Alvarez *et al.,* 2020). Phenolic compounds in pumpkin extracts have been shown to induce apoptosis (programmed cell death) in cancer cells and inhibit tumour growth (Ferreira *et al.,* 2015; Husain *et al.,* 2021).

Liver enzymes such as ALT and AST are crucial indicators of liver function. Elevated levels of these enzymes typically signify liver damage or inflammation. Protein and albumin levels are also significant markers of liver health, reflecting the liver's synthetic capacity. Pumpkin leaves and fruit extracts have shown promising hepatoprotective effects, potentially normalizing these biochemical markers. Studies have shown that pumpkin extract can significantly reduce elevated levels of SGPT, suggesting a hepatoprotective effect (Ghahremanloo *et al.,* 2018; Salehi *et al.,* 2021). Similar to ALT, AST levels also decrease with pumpkin extract treatment.

Total protein levels in the serum often decline in liver dysfunction due to impaired protein synthesis. Pumpkin extract has been observed to normalize these levels, indicating improved liver function. As a major protein synthesized by the liver, albumin levels directly reflect liver synthetic activity (Sun *et al.,* 2019). Pumpkin extracts were found to maintain and increase serum albumin levels, signifying better liver health and function. The reduction in liver function parameters by the pumpkin extract was equivalent to the mechanism of Silybon at 100 mg/kg administered daily. The extracts exhibit significant anti-inflammatory properties. Inhibiting inflammatory mediators and cytokines reduce liver inflammation and consequent enzyme leakage into the bloodstream (Kulczynski *et al.,* 2020).

Both the fruit and leaves of the pumpkin plant have been studied for their potential health benefits, particularly their antioxidant properties. Antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidases (GPx), and markers of oxidative stress like malondialdehyde (MDA) are critical in understanding the biochemical effects of pumpkin extracts (Salehi *et al.,* 2021).

Superoxide dismutase is a critical enzyme in the defence against oxidative stress. It catalyses the dismutation of the superoxide radical (O2•−) into oxygen and hydrogen peroxide. In this current study, the SOD levels were significantly elevated in groups 5 and 6 supplemented with low and high doses of pumpkin fruit extract when compared with the normal control fed with normal feed and water and the standard group that received 100 mg/ kg Silybon (Table 9). This is in line with other reported studies that revealed extracts of pumpkin fruits and leaves to upregulate the activity of SOD (Salehi *et al.,* 2021; Balgoon *et al.,* 2021; Almohaimeed *et al.,* 2022). This is primarily due to the high content of phenolic compounds and flavonoids in pumpkin, which enhance the expression of SOD genes. These compounds can scavenge free radicals directly and also induce the expression of endogenous antioxidant enzymes. Increasing the activity of SOD, which helps in the dismutation of superoxide radicals, thereby reducing oxidative stress.

Glutathione peroxidases are a family of enzymes that reduce lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water (Pei *et al.,* 2023). Pumpkin extracts have been shown to increase GPx activity (Chen *et al.,* 2020a). In this study, the levels of GPx levels slightly increased in the group supplemented with low and high doses of pumpkin fruit extracts and had similar effect with the standard treated with Silybon 100 mg/kg. This effect is attributed to the presence of selenium and other trace elements in pumpkin, which are essential cofactors for the activity of GPx. Moreover, the antioxidant compounds in pumpkin can maintain the levels of reduced glutathione (GSH), the substrate for GPx, thereby supporting the enzyme's activity. Boosting GPx activity, lowers hydrogen peroxide and lipid hydroperoxide levels, protecting cells from oxidative damage.

Malondialdehyde is a biomarker for oxidative stress and lipid peroxidation (Maurya *et al.,* 2021). High levels of MDA indicate significant oxidative damage to cell membranes. Studies have demonstrated that pumpkin extracts can significantly reduce MDA levels (Ghahremanloo *et al.,* 2018; Balgoon *et al.,* 2021). The pumpkin leaves and fruit extracts of varying doses significantly reduced the levels of MDA. The action of the pumpkin extract was comparable to the standard (Silybon) at 100 mg/kg. The mechanism behind this reduction is the enhancement of antioxidant defences by pumpkin compounds, which reduces the initiation and propagation of lipid peroxidation.

Recent studies have focused on the health benefits of pumpkin leaves and fruit, particularly their effects on various blood parameters such as White Blood Cells (WBC), Chartlets (PLT), Red Blood Cells (RBC), Packed Cell Volume (PCV), and Haemoglobin (HB). The understanding of these effects will help provide insights into the potential role of pumpkins in promoting haematological health and preventing disorders (Achilonu *et al.,* 2018; Shaikh *et al.,* 2022).

White Blood Cells are essential for the body's immune response, defending against infections and foreign invaders (Glenn & Armstrong, 2019). In this study, the pumpkin extract especially the fruit extract (groups 5 and 6) significantly maintained the WBC levels when compared to the standard Silybon (100 mg/kg) this is indicative of the phytochemical constituents in the pumpkin. Phytochemicals' antioxidant and anti-inflammatory properties in pumpkin, particularly flavonoids and vitamin C, have been shown to enhance immune function. Studies suggest that regular consumption of pumpkin leaves and fruit may increase WBC count, improving the body's ability to fight infections (Salehi *et al.,* 2021; Hussain *et al.,* 2022). This immunomodulatory effect is particularly beneficial in conditions where WBC count is compromised, such as in immune deficiencies or chronic illnesses.

Platelets are critical for blood clotting and wound healing. The phytochemicals in pumpkin, especially saponins and alkaloids, have been reported to have a regulatory effect on platelet aggregation (Haruna *et al.,* 2021). This can help prevent conditions such as thrombocytopenia (low platelet count) or thrombosis (excessive clotting). Red Blood Cells are responsible for oxygen transport in the body, and Haemoglobin is the protein in RBCs that binds to oxygen (Haruna *et al.,* 2021). Iron, an essential component of Haemoglobin, is abundantly found in pumpkin leaves and fruit this was evidenced in the increase in RBC levels of rats treated with high and low doses of the pumpkin leaves and fruit extract. Regular consumption of pumpkin has been linked to increased RBC count and Haemoglobin levels, which are crucial in preventing anaemia and ensuring efficient oxygen transport to tissues (Haruna *et al.,* 2021).

Packed Cell Volume, also known as haematocrit, measures the proportion of blood that is made up of RBCs. A higher PCV indicates better oxygen-carrying capacity of the blood. In this study, the pumpkin extract slightly increased the PCV levels when compared to the standard treated with Silybon at 100 mg/kg. This is indicative of the iron and folate content in pumpkins contributes to an increase in PCV by promoting RBC production. Additionally, the antioxidant properties of pumpkin's phytochemicals protect RBCs from oxidative damage, ensuring their longevity and functionality, which in turn helps maintain a healthy PCV (Salehi *et al.,* 2021).

The phytochemical content in pumpkin leaves and fruit exerts a positive influence on various blood parameters, including WBC, PLT, RBC, PCV, and HB. By enhancing immune function, supporting platelet regulation, and boosting RBC production and haemoglobin levels, pumpkins can play a significant role in maintaining and improving haematological health (Nwidi & Oboma, 2019).

The Mean Corpuscular Volume (MCV) is a measure of the average volume of a red blood cell. Phytochemicals in pumpkin, particularly flavonoids and polyphenols, are known to enhance red blood cell production and integrity (Fathima *et al.,* 2024). Consumption of pumpkin leaves and fruit may lead to an increase in MCV by supporting the synthesis and maintenance of healthy red blood cells (Ikwebe & Onuche, 2023). This effect can be attributed to the antioxidant properties of these phytochemicals, which protect erythrocytes from oxidative stress and damage (Ikwebe & Onuche, 2023). Phytochemicals like carotenoids, present in high concentrations in pumpkin fruit, contribute to improved haemoglobin synthesis (Table 10). group 2 which had the highest noted value exhibited macrocytosis, which is indicated by elevated levels of MCV, suggesting potential effects on red blood cell size. Additionally increased levels of MCH in group 2 implied an enhanced haemoglobin content per red blood cells. This is due to their role in iron metabolism and their antioxidant properties, which ensure the stability and functionality of haemoglobin (Ikwebe & Onuche, 2023). Regular consumption of pumpkin products could potentially increase MCH levels, improving oxygen transport efficiency in the body.

Mean Corpuscular Haemoglobin Concentration (MCHC) measures the concentration of haemoglobin in a given volume of packed red blood cells. The rich phytochemical content in pumpkin leaves and fruit, especially carotenoids and flavonoids, supports the integrity and function of haemoglobin (Table 11). By reducing oxidative stress and supporting erythropoiesis, these phytochemicals can help maintain or even increase MCHC levels, contributing to better overall blood health (Ikwebe & Onuche, 2023). Erythrocytes, or red blood cells, are critical for oxygen transport throughout the body. The presence of essential vitamins and minerals, such as iron and folate in pumpkin leaves and fruit, further enhances this effect (Ikwebe & Onuche, 2023).

Lymphocytes are a type of white blood cell crucial for the immune response. Pumpkin leaves and fruit contain phytochemicals that exhibit immunomodulatory effects, which can enhance the function and proliferation of lymphocytes. Regular consumption of pumpkin products may lead to an increase in monocyte count, improving the body’s inflammatory response and its ability to clear infections.

The negative control exhibits the highest levels of immune markers (lymphocytes and monocytes), likely indicating inflammation or immune activation.

The low and high doses of fruit extracts seem to modulate the immune response by reducing levels of eosinophils, lymphocytes, and monocytes compared to the negative control, suggesting potential anti-inflammatory or immunomodulatory effects.

The data suggest that pumpkin fruit extracts (both low and high doses) may offer some protection or anti-inflammatory effects compared to the negative control.

**4.0 Conclusion**

Liver cirrhosis, a late stage of liver fibrosis characterized by the replacement of healthy liver tissue with scar tissue, poses significant health challenges globally. While traditional medical treatments focus on managing symptoms and slowing the progression of the disease, recent research highlights the potential therapeutic benefits of natural foods, such as pumpkin, in ameliorating liver cirrhosis.

While pumpkin alone cannot cure liver cirrhosis, its incorporation into a balanced diet can provide significant supportive benefits. The antioxidant, anti-inflammatory, hepatoprotective, and anti-fibrotic properties of pumpkin make it a valuable addition to dietary strategies aimed at ameliorating liver cirrhosis. As research continues to unfold, pumpkin's role in liver health could become even more prominent, offering a natural and accessible means to support liver function and overall health.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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