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# Antioxidant Properties and Dose-Dependent Effects of Monkey Fruits (*Artocarpus lakoocha*) against Paracetamol-Induced Hepato-Renal Toxicity in Rats

**[1]Md. Yousuf Ali, [1, 2]Md. Reaz Morshed, [1,3]Md. Sakib Hossen, [4]E.M. Tanvir, [1]Alamgir Kabir, [1]Md. Aminul Islam, [1]Nurul Karim, [1]Nadia Alam, [1]Md. Ibrahim Khalil and [5]Siew Hua Gan**

*[1]Laboratory of Preventive and Integrative Biomedicine, Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh*
*[2]Department of Biochemistry and Molecular Biology, Noakhali Science and Technology University, Noakhali-3814, Bangladesh*
*[3]Department of Biochemistry, Primeasia University, Banani, Bangladesh*
*[4]Veterinary Drug Residue Analysis Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka 1349, Bangladesh*
*[5]School of Pharmacy,Monash University Malaysia, Jalan Lagoon Selatan, 47500 BandarSunway, Selangor, Malaysia*

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Corresponding Author:
Md. Ibrahim Khalil
Laboratory of Preventive and Integrative Biomedicine, Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh
Tel: (+8802) 7708478-85/1379
Fax: (+8802) 7708069
Email: drmikhalil@gmail.com

Siew Hua Gan
*School of Pharmacy, Monash University Malaysia, Jalan Lagoon Selatan, 47500 Bandar Sunway, Selangor, Malaysia*
Tel: +60 3 55144918
Email: Gan.SiewHua@monash.edu

**Abstract:** In this study, the antioxidant potentials and protective effect of ethanolic extract of monkey fruits (*Artocarpus lakoocha*) (AL) was investigated against paracetamol-induced toxicity in rats. AL which contains high concentration of polyphenols, flavonoids, tannins and protein, exhibited high radical scavenging activity and ferric reducing antioxidant power. Administration of paracetamol (500 mg/kg) for seven consecutive days caused severe oxidative stress in liver and kidney, as observed by the significantly higher level of Lipid Peroxidation (LPO) and the associated biochemical markers compared to control rats. Pre-treatment with AL at 250, 500 and 1000 mg/kg prior to paracetamol administration for 30 days significantly improved hepatic and renal parameters in a dose-dependent manner. Silymarin (100 mg/kg) was administered as a standard drug for comparison over a similar treatment period. Moreover, AL exhibited the highest protective effect when administered at the highest dose, by lowering serum levels of alanine transaminase (28.25%), aspartate transaminase (29.0%), alkaline phosphatase (27.87%), lactate dehydrogenase (7.51%), γ-glutamyltransferase (31.0%), total bilirubin (69.38%), cholesterol (14.80%), triglycerides (27.52%), low-density lipoprotein cholesterol (76.12%), creatinine (36.84%), urea (41.08%) and uric acid (34.88%), In addition, significantly increased total protein (50.0%) and high-density lipoprotein cholesterol (55.79%) with administration of AL was seen when compared with paracetamol-controlled group. Decreased LPO levels in the liver (45.55%) and kidneys (32.0%) confirmed the hepatorenal protective effects of AL, as further confirmed by the histopathological findings. Overall, AL fruit is an excellent source of natural antioxidants and possess hepatorenal protective activity against paracetamol-induced liver and kidney injuries.

**Keywords:** *Artocarpus lakoocha*, Antioxidant, Hepatorenal Protective, Paracetamol, Dose Dependent

## Introduction

*Artocarpus lakoocha* (AL), also known as monkey fruit, Monkey Jack or barhar (Bengali: Dewa) is a tropical evergreen deciduas tree species of the family Moraceae. It is widely distributed in the regions of South East Asia including Nepal, Sri Lanka, Myanmar, India, Southern China, Vietnam, Thailand, Indonesia, Malaysia

and Bangladesh (Gardner *et al*., 2000; Hari *et al*., 2014). The AL fruits are generally eaten fresh (Fig. 1). The edible fruit pulp is believed to act as a liver tonic (Gautham and Patel, 2014). The spikes of the raw fruits and male flowers which are acidic and act as an astringent are often utilized in pickles and chutney. Traditionally, the aqueous extract prepared from the heartwood of this species known as 'Puag-haad' is used as an anthelmintic agent for the treatment of tapeworm infection in Thailand (Charoenlarp *et al*., 1981; Jacobsen and Salguero, 2014) which warrants its further investigation.

AL fruits and seeds are reported to be high in carbohydrates, proteins and minerals (Suwannalert *et al*., 2012). The fruits are also reported to contain alkaloids, flavanoids, phenols, tannins, steroids and saponins (Jahan *et al*., 2011; Suwannalert *et al*., 2012). Ethanolic extract of AL specifically contains bioactive rutin, pyrogallo, gallic acid, resorcinol, quercetin, catechin and caffeic acid (Singhatong *et al*., 2010). The heartwood of AL has been reported to contain artocarpin, norartocarpin, norcycloartocarpin, cycloartocarpin, resorcinol, oxyresveratrol and β-sitosterol (Sastry *et al*., 1972).

The largest gland and the main metabolic organ in an animal is the liver which may be damaged due to several reasons. To date, the pathophysiological mechanisms of chemical-induced liver toxicity are not well understood. It may be related with the metabolic conversion of xenobiotics into their Reactive Oxygen Species (ROS) which is considered as the main culprit for oxidative stress, resulting from an imbalance between ROS production and antioxidant protective mechanism (Tanikawa and Torimura, 2006; Zhu *et al*., 2012) finally leading to the damage of hepatocytes (Gu and Manautou, 2012). Furthermore, free radicals mainly act by attacking the unsaturated lipid molecule of plasma membrane that ultimately cause lipid peroxidation (a hallmark of liver damage) (Martin and Grotewiel, 2006).

Paracetamol (also known as acetaminophen)-induced toxicity in animals is one of the widely used experimental model to investigate the hepatoprotective activity of plant extract (Paul *et al*., 2016). It is an analgesic and antipyretic drug, but produces both acute liver and kidney toxicities in overdoses. Paracetamol-induced liver toxicity is attributed to the formation of highly reactive metabolite N-acetyl-p-benzo Quinine Imine (NAPQI), which acts via cytochrome p450 enzyme system and causes oxidative stress due to its ability to covalently react with protein, nucleic acids and to diminish Glutathione (GSH) (Jollow *et al*., 1973; Mitchell *et al*., 1973; Potter *et al*., 1974). Besides hepatotoxicity, paracetamol causes renal damage by simultaneously acting through necrosis of glomerulus (Mazer and Perrone, 2008; Orlić *et al*., 2014).

Due to the wide application of AL as a liver tonic by the locals, it is plausible that AL may confer hepato-and nephroprotective potentials against paracetamol-induced liver and kidney toxicities. For this reason, we designed a study to evaluate the antioxidant activity and hepato-and nephroprotective effects of ethanolic extract of AL at multiple doses against paracetamol-induced hepato-and nephrotoxicities in rat model.

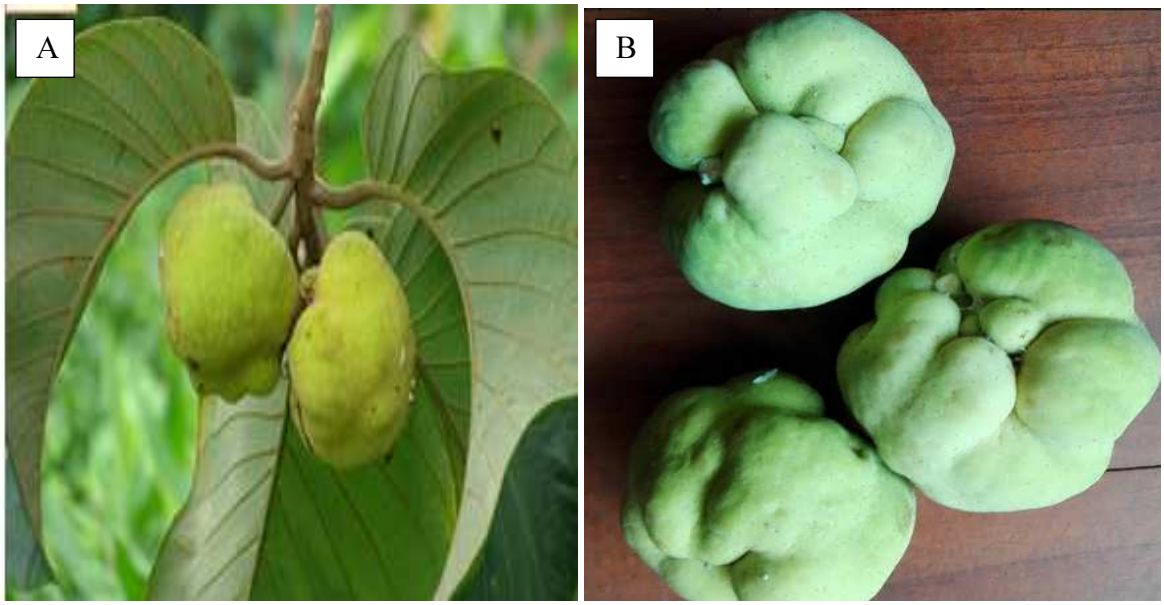


**Fig. 1:** (A) AL fruits with stem and (B) the plucked fruits

## Materials and Methods

### *Chemical and Reagents*

For phytoconstituents analysis, reference standards such as gallic acid; catechin; 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) and 2, 4, 6-tris (2-pyridyl)-1, 3, 5-triazine (TPTZ) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). L-ascorbic acid; tannic acid; Folin-Ciocalteu's phenol reagent and ferrous sulfateheptahydrate ($FeSO_4.7H_2O$) were purchased from Merck Co. (Darmstadt, Germany). Ketamine hydrochloride injection was purchased from Popular Pharmaceuticals Ltd., Dhaka, Bangladesh. Active paracetamol and silymarin were purchased from SQUARE pharmaceutical Ltd. Dhaka, Bangladesh. Silymarin was used as a herbal standard since it has a reputed hepatoprotective effect (Alam *et al*., 2017). All chemicals and reagents used in this study were of analytical grade.

### *Sample Collection*

Mature and fresh AL were collected from Perojpur district (22.5791°N, 89.9759°E), the Southern part of Bangladesh in June, 2016. The fruits were authenticated by Professor Nuhu Alam from Department of Botany, Jahangirnagar University, where a voucher specimen was deposited. After collection, the fruits were washed with clean sterile water in a laminar blower in order to remove all possible contamination and stored in the refrigerator (4°C) for one day before extract preparation.

### *Extract Preparation*

The ethanolic extract of AL was prepared according to the method described by Paul *et al*. (2016) with some slight modifications. Firstly, the fresh and cleaned mature fruits were thoroughly rinsed using cold sterile water. The edible parts were carefully isolated, cut into small pieces using a sharp stainless steel knife and were finally dried in the sunlight. When the samples were free from moisture and appeared crunchy, they were ground into a fine powder by using a blender (Jaipan Commando, Mumbai, India). Then, the mashed samples were dissolved with sufficient amount (20% w/v) of pure ethanol (100%) and were put into a shaker (IKA400i, Germany) at 150 rpm and 30°C for 72 h. The extract was filtered through a cotton plug followed by a Whattman No. 1. Subsequently, the crude extract was evaporated using a rotatory evaporator (R-215 BUCHI, Seitzerland) under a reduced pressure (100 psi) and controlled temperature at 40°C. It was finally concentrated and stored at -20°C until further use.

### *Phytochemical Analysis*

### *Estimation of Total Polyphenols Content*

The total polyphenol content (TPC) of AL ethanolic extract was estimated by a spectrometric method according to Folin-Ciocalteu method (Amin *et al*., 2006). TPC was determined as Gallic Acid Equivalent (GAE) and was expressed as mg of GAEs/100 g of AL.

### *Estimation of Total Flavonoid Content*

The Total Flavonoid Content (TFC) was estimated by using an aluminum chloride colorimetric assay (Chang *et al*., 2002). TFC was calculated as Catechin Equivalent (CE) and was expressed as mg of CEs/100 g of AL.

### *Estimation of Total Tannin Content*

The total tannin content of ALwas estimated using Folin-Ciocalteu's method (Folin and Ciocalteu, 1927). Tannic acid was used as a standard. The results were expressed as mg of Tannic acid Equivalents (TEs)/100 g of AL.

### *Estimation of Total Protein Content*

The total protein content of the ALextracts was estimated using Lowry's method of protein estimation (Lowry *et al*., 1951). Bovine Serum Albumin (BSA) was used as a standard. The results were expressed as g of BSA equivalents per 100 g of AL.

### *Antioxidant Activity*

The antioxidant potential of the AL was determined by conducting DPPH radical-scavenging activity and Ferric Reducing Antioxidant Power (FRAP) assays as described in the subsections below.

### *DPPH Radical-Scavenging Activity*

The antioxidant activities of AL extract was determined according to the DPPH radical-scavenging activity based method as established by Braca *et al*. (2002). Briefly, 1 mL of the extract was mixed with 1.2 mL of DPPH (0.003%) in methanol at various concentrations (2.5-80.0 µg/mL). Percentage of DPPH inhibition was calculated based on the following equation:

$$\% \text{ of DPPH inhibition} = [(A_{DPPH}-A_S/A_{DPPH})]\times 100$$

where, $A_{DPPH}$ is the absorbance of DPPH in the absence of a sample and $A_S$ is the absorbance of DPPH in the presence of either the sample or the standard. DPPH scavenging activity is expressed as the concentration of sample required to decrease DPPH absorbance by 50% ($IC_{50}$).

### *Ferric Reducing Antioxidant Power (FRAP) Assay*

FRAP assay was performed according to the method as established by Benzie and Strain (1999). An intense blue color is produced at low pH when ferric tripyridyltriazine complex was reduced into its ferrous form. The color was monitored by measuring the absorbance at 593 nm. FRAP

values were expressed as micromoles of ferrous equivalent (μM Fe [II] per 100 g of AL).

### *Experimental Animals*

Adult male Wistar rats weighing between 150 and 250 g at between 16-18 weeks old were used. The animals were bred and reared in the animal house facility of the Department of Biochemistry and Molecular Biology, Jahangirnagar University, at a constant temperature of 23±2°C and in an environment with humidity ranging from 44% to 56%. The rats were placed in sterile plastic cages with soft wood-chip bedding and received a natural 12h day-night cycle. The rats were provided with a standard laboratory pellet diet and water *ad libitum*. The study was conducted in accordance with the internationally established principles of the US guidelines (Council, 2010). The experimental protocol was approved by the Biosafety, Biosecurity and Ethical Committee of Jahangirnagar University, Savar, Dhaka.

### *Experimental Design*

Animal experiments were designed based on the findings of our previous studies (Paul *et al*., 2016) while the outcomes of the study were the effects of AL investigated at multiple doses with some modifications. A total of 42 rats were acclimatized one week prior to the experiment and were divided into seven groups of six animals in each group. During the experimental period the animals were also observed for behavioral changes in their feeding and drinking habits to detect abnormalities sign.

### *Group A (Normal Control)*

Animals administered with saline water, a solution of 0.90% sodium chloride (5 mL/kg) by oral gavage needle for once daily 30 days.

### *Group B (AL Control)*

Animals administered with AL (1000 mg/kg) doses by oral gavage needle for 30 days once daily.

### *Group C (Paracetamol-Controlled)*

Animals administered with saline water (5 ml/kg) with normal diet for 30 days once daily followed by oral administration of single dose of paracetamol (500 mg/kg) in the final 7 days of the experimental period.

### *Group D (Treatment 1)*

Animals administered with AL (250 mg/kg) for 30 days once daily followed by oral administration of single dose of paracetamol (500 mg/kg) for last 7 days.

### *Group E (Treatment 2)*

Animals administered with AL (500 mg/kg) for 30 days once daily followed by oral administration of single dose of paracetamol (500 mg/kg) for last 7 days.

### *Group F (Treatment 3)*

Animals administered with AL (1000 mg/kg) for 30 days once daily followed by oral administration of single dose of paracetamol (500 mg/kg) for last 7 days.

### *Group G (Standard)*

Animals administered with a standard drug silymarin (100 mg/kg) by oral gavage needle for 30 days once daily followed by oral administration of single dose of paracetamol (500 mg/kg) in the last 7 days.

### *Sacrifice of Animals*

At the end of the experimental period, all rats were fasted for 24 h. The animals in each group were deeply anaesthetized by ketamine hydrochloride injection (500 mg/kg) (Ringer, 1979) via the intraperitoneal veins and were sacrificed prior to dissection. Blood samples (5 mL) were collected from the inferior vena cava and were placed into plain tubes for serum separation by allowing it to coagulate at ambient temperature for 30 min. Serum was separated by centrifugation at 2000 rpm for 10 min and were stored at -20°C for subsequent biochemical analyses.

Changes in the Body Weights (BW) of rats were recorded on a weekly basis. The liver and kidney tissues were excised and weighed immediately after sacrifice. The relative organ weight was calculated by dividing the individual weight of each organ with the final body weight of each rat according to the following formula (Liu *et al*., 2004): Relative organ weight (%) = wet organ weight/body weight ×100.

### *Tissue Homogenate Preparation*

Immediately following collection, the tissue samples (liver and kidney) were washed with ice cold Phosphate-Buffered Saline (PBS). The samples were homogenized in phosphate buffer (25 mM, pH 7.4) to make approximately 10% w/v homogenates. The homogenates were centrifuged at 1700 rpm for 10 min and the supernatant was collected and stored at −20°C LPO analysis. A portion of each tissue sample was preserved in formalin solution (10%) for histopathological examination.

### *Biochemical Analysis*

Serum biochemical parameters for liver function tests including Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), γ-glutamyltransferase (GGT), Total Bilirubin (TB), Total Protein (TP), Albumin (ALB) and Albumin/Globulin (A/G) ratio, the parameters for kidney function including creatinine, urea and uric acid, parameters for lipid profile, Total Cholesterol (TC), Triglycerides (TG) and High-Density Lipoprotein Cholesterol (HDL-C) were estimated following standard procedures using an automated chemistry analyzer

(Dimension EXL with LM Integrated Chemistry System, Siemens Medical Solutions Inc., USA). Another lipid profile parameter, serum Low-Density Lipoprotein Cholesterol (LDL-C) level was estimated based on the following Friedewald formula (Friedewald *et al*., 1972):

$$LDL\text{-}C = TC\text{-}HDL\text{-}C\text{-}TG/5$$

### *Oxidative Stress Parameters LPO in Liver and Kidney Tissues*

Malondialdehyde (MDA) levels were investigated for LPO products in the liver and kidney tissues. MDA, which is also referred to as Thiobarbituric Acid Reactive Substance (TBARS), was measured at 532 nm according to a method described by Ohkawa *et al*. (1979). The levels of TBARS were expressed as mmol of MDA per mg of protein.

### *Histopathological Analysis*

For histopathological analysis, the tissue samples (liver and kidney) were fixed in 10% neutral formalin and were processed by a paraffin embedding technique. The histopathological specimens were cut into 5-µm-thick sections using a rotary microtome and subsequently stained with hematoxylin and eosin (H and E) dye (Carleton *et al*., 1980). Photomicrographs at 6100 X magnification were captured by a normal-spectrum fluorescence microscope (Olympus DP 72) with an attached digital camera (Olympus, Tokyo, Japan). The pathologist performing the histopathological evaluation was blinded to the treatment assignments of the different study groups in order to minimize bias.

### *Statistical Analysis*

All results were presented as mean ± Standard Deviation (SD). Data were analysed by using SPSS (Statistical Packages for Social Science, version 16.0, IBM Corporation, New York, USA) and Microsoft Excel 2007 (Redmond, Washington, USA). All the data of treatment groups were compared with the control group by using a one-way ANOVA followed by Dunnett's multiple comparison tests. A p value of $< 0.05$ was considered as statistically significant.

## Results

### *Phytochemical Analysis*

Phytochemical analysis indicated that AL contains substantial amount of bioactive polyphenols, flavonoids, tannins and protein (Table 1).

### *Antioxidant Activity*

The antioxidant potential of AL extracts was investigated by estimating the DPPH free radical-scavenging activities as well as FRAP values. The percentage inhibition values of DPPH were plotted against concentrations of sample extract. The value of the sample extract producing 50% inhibition ($IC_{50}$ value) was calculated (Fig. 2). The calculated $IC_{50}$ value for AL was 1.06 µg/mL while standard ascorbic acid was 0.82 µg/mL. The calculated FRAP values of AL fruit was 4.1±0.2 [µM Fe (II)] per g.

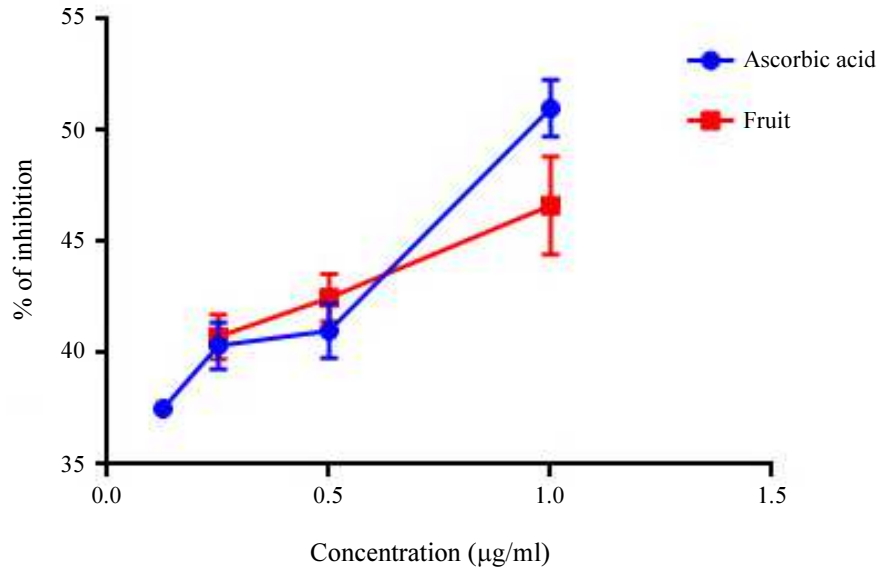


**Fig. 2:** $IC_{50}$ values of AL fruit against standard ascorbic acid indicating a dose-dependent inhibition of DPPH

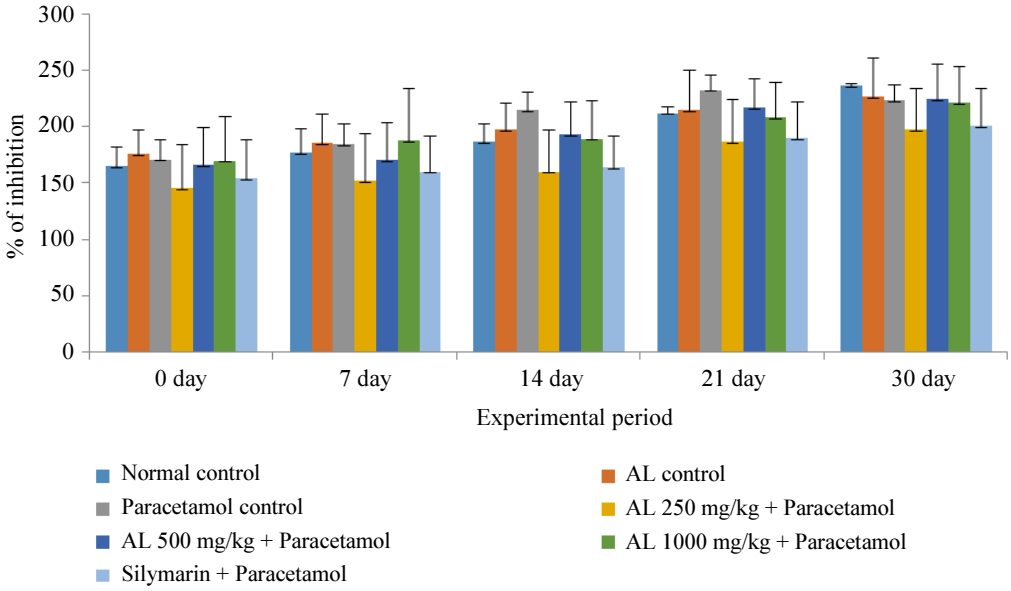


**Fig. 3:** The effects of AL fruit extract, paracetamol and silymarin on body weight gain; Results were expressed as mean ± SD. (n = 6). Paracetamol was administered at (500 mg/kg) while silymarin was at 100 mg/kg. Non-significant changes were found when all groups were compared against normal control and paracetamol-controlled group ($p<0.05$)

**Table 1:** The recorded total polyphenol, flavonoid, tannins and protein contents in AL fruit

| | |
|---|---|
| Polyphenols (mg/100 g GAEs) | 325.63±2.99 |
| Flavonoids (mg/100 g CEs) | 521.98±0.01 |
| Total tannins (mg/100 g TEs) | 124.03±0.46 |
| Total protein (g/100 g BSA) | 4.00±0.14 |

Data are presented as the mean ± SD, n = 3, GAE: Gallic Acid Equivalents; CE: Catechin Equivalents; TE: Tannic acid Equivalent, BSA: Bovine Serum Albumin

**Table 2:** The effects of AL fruit extract, paracetamol and silymarin on relative organ weight profile

| | Relative organs weight (%) | |
|---|---|---|
| | Liver | Kidney |
| Normal control | 2.66±0.12 | 0.29±0.05 |
| AL control | 2.42±0.54 | 0.31±0.05 |
| Paracetamol-controlled | 2.79±0.67 | 0.26±0.02 |
| AL 250 mg/kg + Paracetamol | 2.68±0.43 | 0.36±0.07 |
| AL 500 mg/kg + Paracetamol | 2.94±0.32 | 0.23±0.01 |
| AL 1000 mg/kg + Paracetamol | 2.93±0.21 | 0.30±0.02 |
| Silymarin + Paracetamol | 2.86±0.76 | 0.32±0.05 |

Results were expressed as mean ± SD. (n = 6). Non-significant changes were found when all groups were compared against normal control and paracetamol-controlled group ($p<0.05$)

### *Effects of AL Extract, Paracetamol and Silymarin on Body Weight and Relative Organ Weight Parameters*

There were no significant changes in both body weight gain (Fig. 3) and relative organ weight (Table 2) observed between the different experimental groups for the liver and the kidneys. In addition, during the experimental period, the body weight increased gradually although the changes were not significant as observed from the first to the fourth week.

### *Effects of AL Extract, Paracetamol and Silymarin on Serum Hepatic Markers*

The effects of oral administration of AL fruit extract, paracetamol and silymarin on liver function in experimental rats were investigated by measuring serum activities of ALT, AST, ALP and LDH (Fig. 4) as well as GGT, TB, TP, ALB levels and A/G ratio (Table 3). Serum ALT, AST, ALP, GGT and LDH activities and TB levels were significantly higher while serum TP and ALB level were significantly lower in paracetamol-controlled group as compared to normal control and other experimental groups. Rats pre-treated with AL extract at 250, 500 and 1000 mg/kg or silymarin had significantly reduced AST, ALP, GGT, LDH and TB levels and increased TP level as compared with paracetamol-controlled rats. Significant reductions in serum ALT was observed only in the *A. lakoocha* (at 1000 mg/kg) and silymarin administered groups when compared with the paracetamol-controlled group.

### *Effects of AL Fruit Extract, Paracetamol and Silymarin on Serum Lipid Profiles*

A marked increase in circulating levels of TG, TC and LDL-C and a decrease in HDL-C level were observed in paracetamol-controlled rats as compared with normal control rats (Fig. 5). Treatment with AL extract and silymarin were observed to significantly

reduce the levels of TC, LDL-C (for silymarin and all the three doses of AL) and TG (only 500 mg/kg of AL dose) while increasing the HDL-C levels compared with the parameter measured in the paracetamol groups.

### *Effects of AL Fruit Extract, Paracetamol and Silymarin on Serum Renal Biomarkers*

The effects of AL extract, paracetamol and silymarin on renal function were investigated by measuring serum creatinine, urea and uric acid levels (Table 4). There were significant increase in serum creatinine and urea and a non-significant increase in uric acid levels in paracetamol-controlled rats when all groups were compared with normal controls. However, serum biomarkers of kidney function were improved in animals which received AL. Moreover, animals treated with AL and silymarin had significantly reduced serum creatinine and urea levels but non-significantly reduced serum uric acid compared with paracetamol-controlled rats.

### *Effects of AL Fruit Extract, Paracetamol and Silymarin on Lipid Peroxidation (LPO) in Liver and Kidney*

Based on the investigation of oxidative stress biomarkers, there was a significant increase in LPO levels in paracetamol-controlled rats as evidenced by increases in liver MDA levels compared with those of the normal control group. However, AL tended to confer a protective effect where the animals treated with fruit extracts had a dose-dependent reduction in MDA levels as compared with the paracetamol-controlled group. Significantly reduced LPO levels were observed in the liver tissue of rats treated with AL and with silymarin compared with those of paracetamol-controlled rats and the rats treated with AL had significantly reduced LPO levels in kidney tissue compared with those of normal control rats (Fig. 6).

### *Histopathological Examination*

Normal control and AL control rats [Fig. 7A and 7B] had normal liver findings with a uniform pattern of polyhedral hepatocytes radiating from the Central Vein (CV) toward the periphery in the hepatic lobules. Paracetamol-controlled rats showed disrupted arrangement of the hepatocytes around the CV, congestion in the CV, degeneration of hepatocytes at the peripheral area of the central vein and massive vacuolization in the lobules [Fig. 7C and 7D] indicative of liver damage. AL treatment at all three doses (250, 500 and 1000 mg/kg) and silymarin-treated rats showed remarkable degree of preservation of the cellular arrangement with only mild inflammation [Fig. 7E-7H].

**Table 3:** The effects of AL fruit extract, paracetamol and silymarin on serum GGT, TB, TP, ALB levels and A/G ratio

| | Parameters | | | | |
|---|---|---|---|---|---|
| Groups | GGT (U/L) | TB (mg/dL) | TP (g/L) | ALB (g/L) | A/G |
| Normal control | 4.93±0.32[b] | 0.16±0.01[b] | 6.67±0.39[b] | 2.53±0.20[b] | 0.62±0.10 |
| AL control | 5.69±0.21[b] | 0.14±0.01[b] | 5.70±0.08[b] | 1.93±0.02[a,b] | 0.51±0.02 |
| Paracetamol-controlled | 9.31±0.41[a] | 0.49±0.04[a] | 4.02±0.71[a] | 1.95±0.11[a] | 0.51±0.09 |
| AL 250mg/kg + Paracetamol | 6.97±0.22 | 0.17±0.00[b] | 5.92±0.37[b] | 2.10±0.17[b] | 0.56±0.11 |
| AL 500 mg/kg + Paracetamol | 6.37±0.24[b] | 0.16±0.00[b,c] | 5.58±0.32[b] | 1.98±0.15[a] | 0.53±0.20 |
| AL 1000 mg/kg + Paracetamol | 5.98±0.11[b] | 0.15±0.00[b,d,e] | 6.00±0.27[b] | 1.86±0.28[a] | 0.44±0.07 |
| Silymarin + Paracetamol | 4.88±0.50[b] | 0.15±0.00[b] | 5.34±0.67[b] | 1.96±0.33[a] | 0.64±0.26 |

Data are presented as mean ± SD (n = 6). [a]denotes a significant difference ($p<0.05$) when all groups were compared against the normal control group. [b]denotes significant difference ($p<0.05$) when all group were compared against paracetamol-controlled group. [c]denotes significant difference ($p<0.05$) between AL 250 + paracetamol and AL 500 + paracetamol groups. [d]denotes significant difference ($p<0.05$) between AL 500 + paracetamol and AL 1000 + paracetamol groups. [e]denotes significant difference ($p<0.05$) between AL 250 + paracetamol and AL 1000 + paracetamol groups

**Table 4:** The effects of AL fruit extract, paracetamol and silymarin on serum renal function markers

| | Parameters (mg/dL) | | |
|---|---|---|---|
| Groups | Creatinine | Urea | Uric acid |
| Normal control | 0.44±0.02[b] | 25.5±1.11[b] | 0.75±0.04 |
| AL control | 0.40±0.01[b] | 28.33±2.62[b] | 0.63±0.03 |
| Paracetamol-controlled | 0.76±0.08[a] | 46.25±2.38[a] | 0.86±0.08 |
| AL 250 mg/kg + Paracetamol | 0.46±0.03[b] | 28.75±2.38[b] | 0.63±0.12 |
| AL 500 mg/kg + Paracetamol | 0.40±0.04[b,c] | 26.25±1.30[b] | 0.63±0.23 |
| AL 1000 mg/kg + Paracetamol | 0.48±0.01[b,d] | 27.25±2.27[b] | 0.58±0.19 |
| Silymarin + Paracetamol | 0.33±0.01[b] | 23.25±1.47[b] | 0.56±0.18 |

Data are presented as the mean ± SD (n = 6). [a]denotes a significant difference ($p<0.05$) when all groups were compared against the normal control group. [b]denotes significant difference ($p<0.05$) when all group were compared against paracetamol-controlled group. [c]denotes significant difference ($p<0.05$) between AL 250 + paracetamol and AL 500 + paracetamol groups. [d]denotes significant difference ($p<0.05$) between AL 500 + paracetamol and AL 1000 + paracetamol groups

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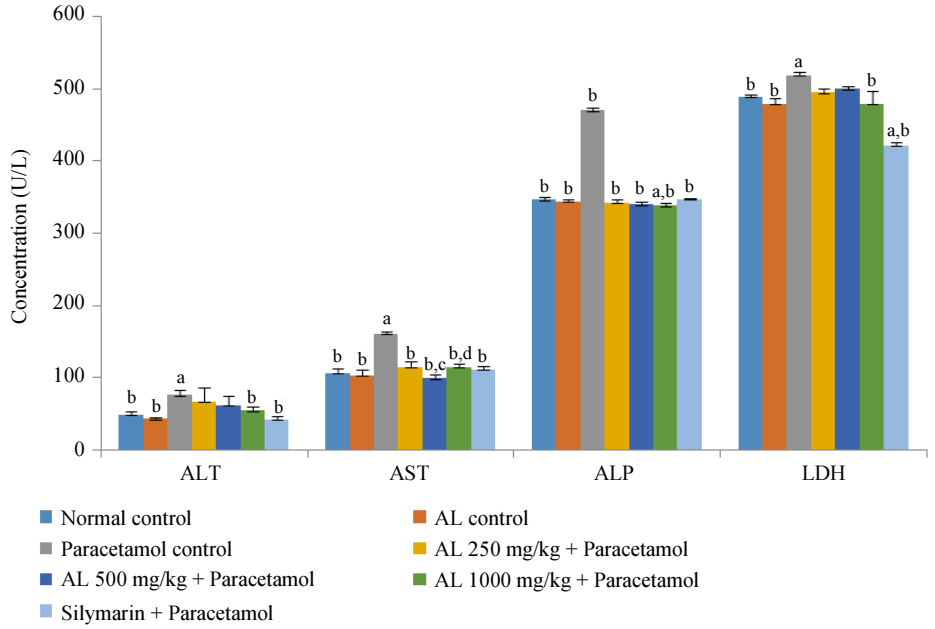



**Fig. 4:** The effects of AL fruit extract, paracetamol and silymarin on serum ALT, AST, ALP and LDH levels. Data were represented as the mean ± SD. (n = 6). [a]denotes a significant difference ($p<0.05$) when all groups were compared against the normal control group. [b]denotes significant difference ($p<0.05$) when all group were compared against paracetamol control group. [c]denotes significant difference ($p<0.05$) between AL 250 + paracetamol and AL 500 + paracetamol groups. [d]denotes significant difference ($p<0.05$) between AL 500 + paracetamol and AL 1000 + paracetamol groups.

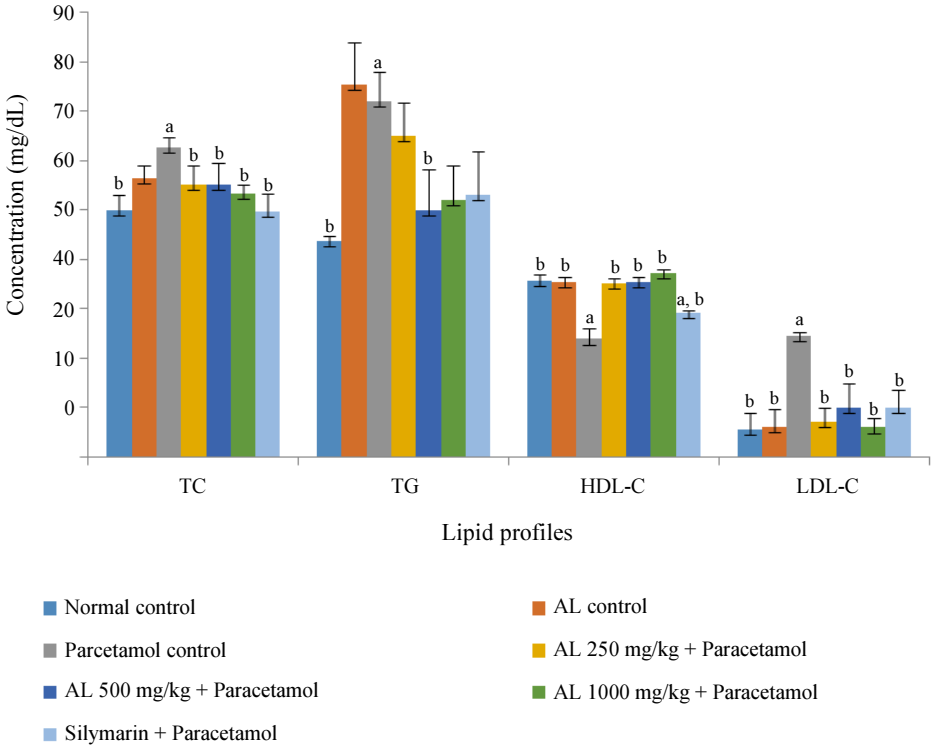



**Fig. 5:** The effects of AL fruit extract, paracetamol and silymarin on serum lipid profiles; Data are presented as the mean ± SD (n = 6). [a]denotes a significant difference ($p<0.05$) when all groups were compared against the normal control group. [b]denotes significant difference ($p<0.05$) when all group were compared against paracetamol-controlled group

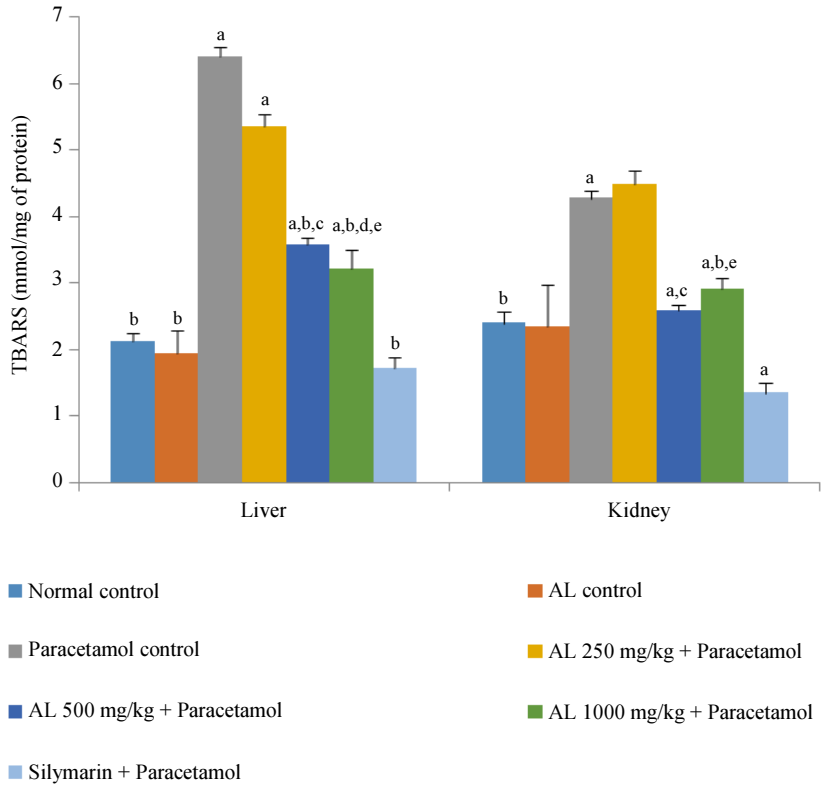
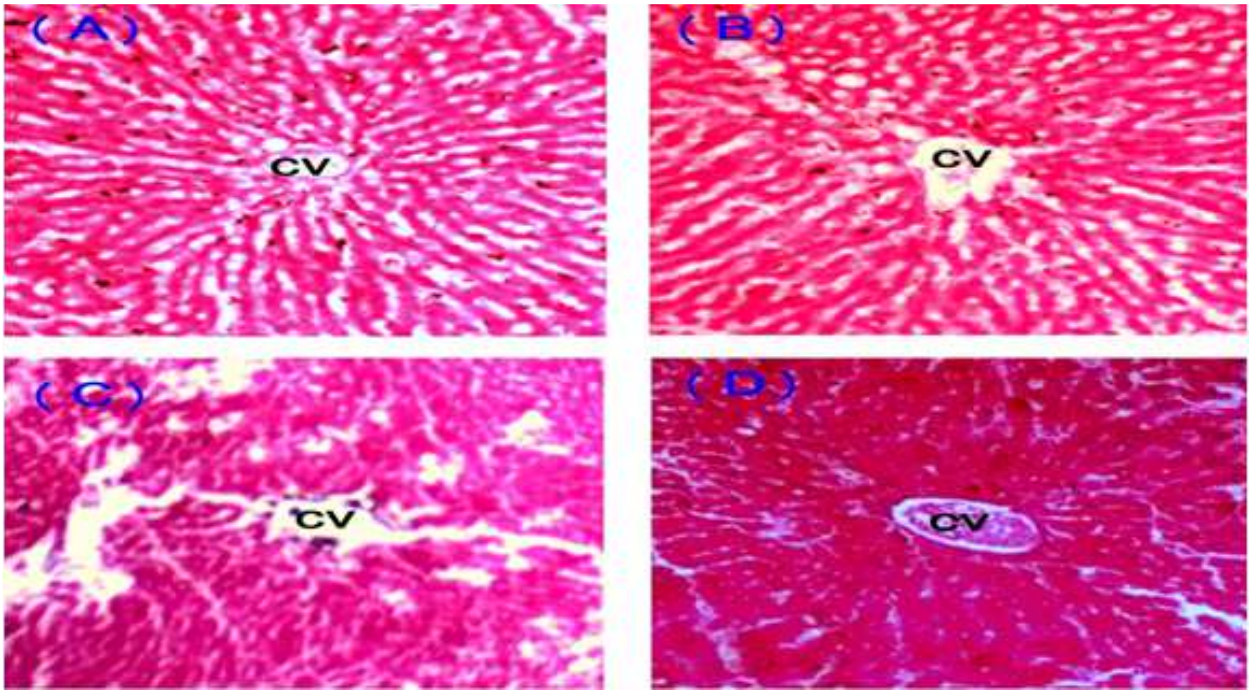



**Fig. 6:** The effects of AL extract, paracetamol and silymarin on lipid peroxidation in the liver and kidneys; Data are presented as the mean ± SD (n = 6). [a]denotes a significant difference ($p<0.05$) when all groups were compared against the normal control group. [b]denotes significant difference ($p<0.05$) when all group were compared against paracetamol-controlled group. [c]denotes significant difference ($p<0.05$) between AL 250 + paracetamol and AL 500 + paracetamol groups. [d]denotes significant difference ($p<0.05$) between AL 500 + paracetamol and AL 1000 + paracetamolgroups.[e]denotes significant difference ($p<0.05$) between AL 250 + paracetamol and AL 1000 + paracetamol groups.



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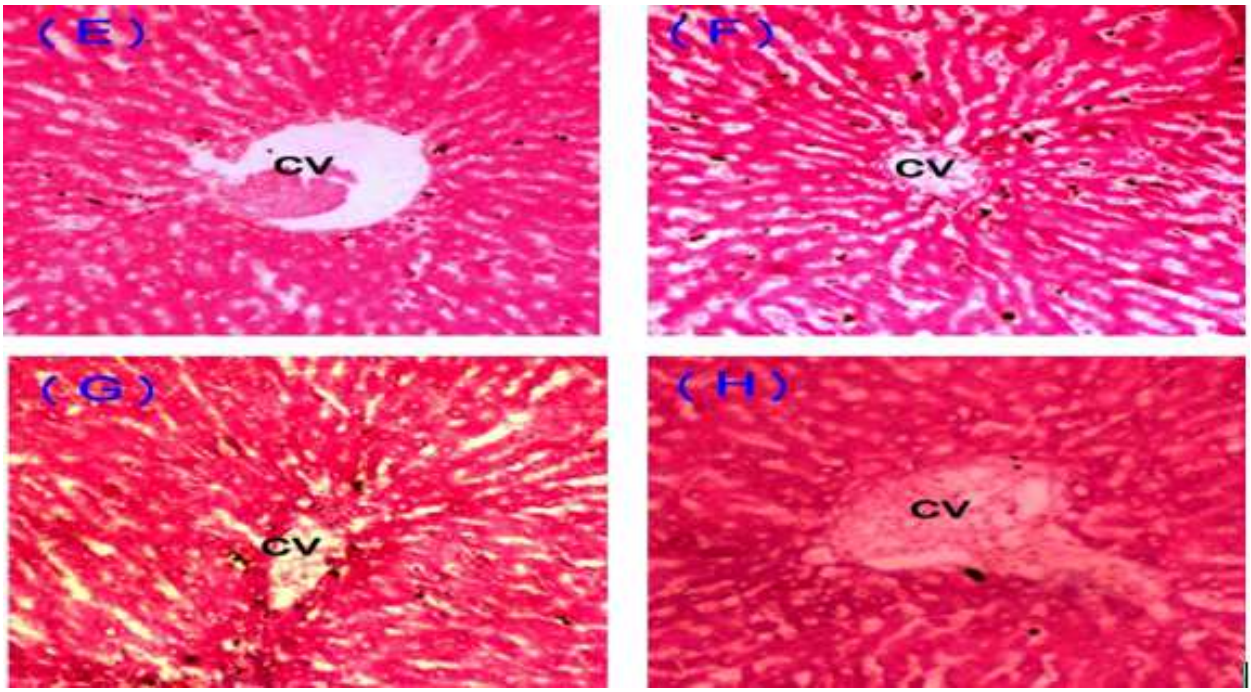
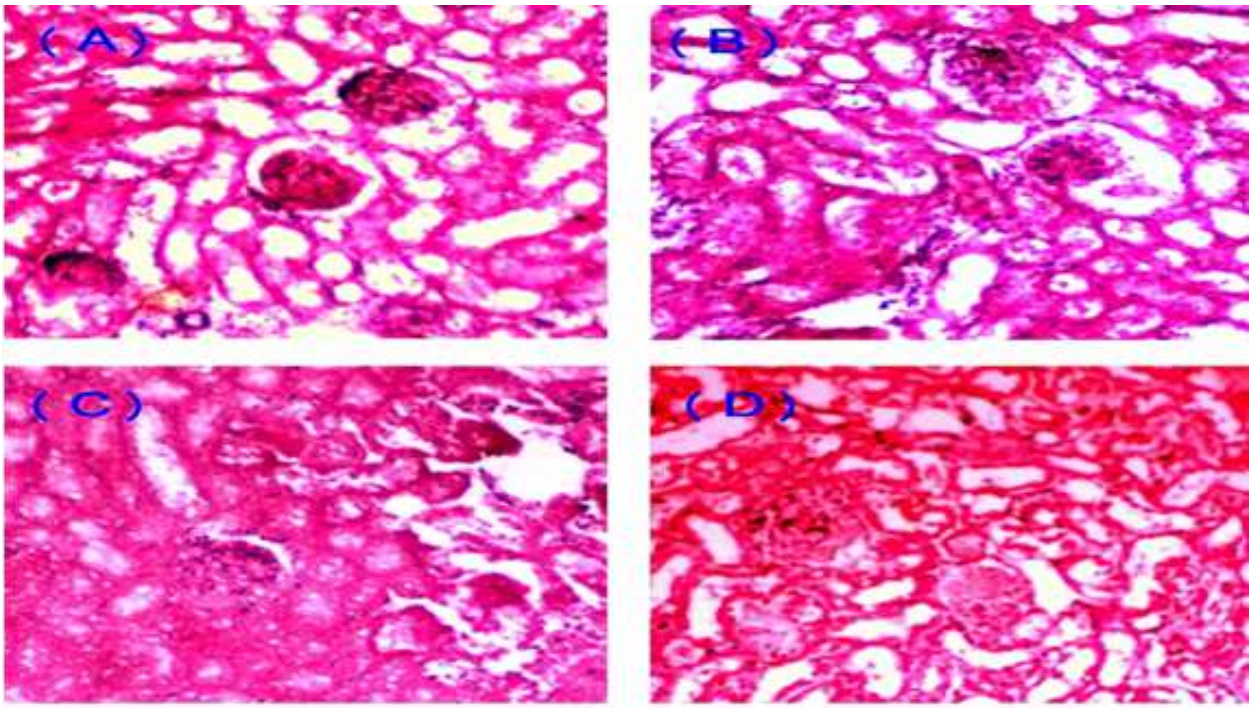



**Fig. 7:** Histopathological photomicrographs (40X magnification) of liver sections. (A-B) Normal control and AL control rats showing normal structure of hepatic lobules with a uniform pattern of polyhedral hepatocytes radiating from the Central Vein (CV) toward the periphery. (C and D) paracetamol-controlled rats showing disrupted arrangement of the hepatocytes around the CV, congestion in the CV, degeneration of hepatocytes at the peripheral area of the central vein and massive vacuolization in the lobules. (E-H) AL treatment at three dose levels (250, 500 and 1000 mg/kg) and silymarin treatment of rats showing remarkable degree of preservation of the cellular arrangement with only mild inflammation.



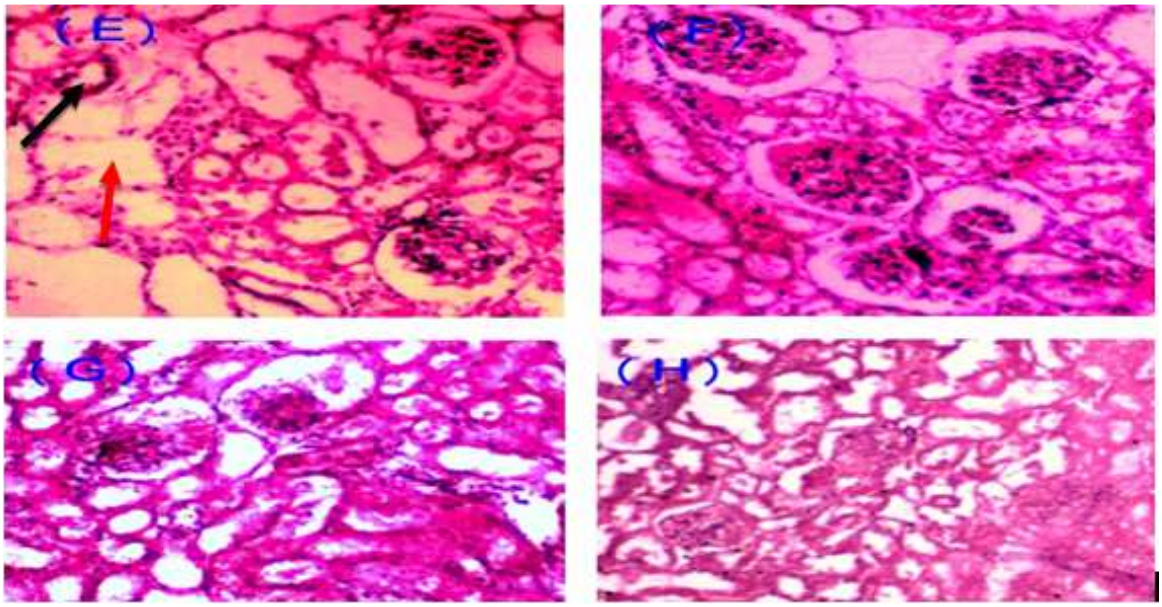



**Fig. 8:** Histopathological photomicrographs (40X magnification) of kidney sections. (A-B) Normal control and AL control rats showing a normal structure of renal parenchyma with good appearance of glomeruli and renal tubules; (C-D) paracetamol-controlled showing marked abnormalities of the tubular epithelium, vacuolization and severe glomerular necrosis. (E-H) AL (250, 500 and 1000 mg/kg) and silymarin-treated rats showing a remarkable degree of morphological preservation with only moderate to mild degenerative changes.

Normal control AL control [Fig. 8A and 8B] rats showed a normal structure of renal parenchyma with good appearance of glomeruli and renal tubules. Paracetamol-controlled rats showed marked abnormalities of the tubular epithelium with vacuolization and severe glomerular necrosis [Fig. 8C and 8D]. AL treatment at three dose levels (250, 500 and 1000 mg/kg) and silymarin treatment showed a remarkable degree of morphological preservation with only moderate to mild degenerative changes [Fig. 8E-H].

## Discussion

Medicinal plants contain a wide variety of natural antioxidants including phenolic acid, flavonoids and tannins (Cai *et al*., 2004). Therefore, scientists have a great interest in their bioactive compounds due to their potential antioxidant, antimicrobial, antifungal, anti-inflammatory and anti-cancer activities (Djeridane *et al*., 2006; Sofowora, 1982). Our findings indicated that AL fruit is an abundant source of polyphenols and flavonoids, tannins and proteins. FRAP and DPPH free radical scavenging tests of AL ethanolic extract further confirmed its significant role as singlet oxygen quencher as well as free radical scavenger which may be highly effective against molecular damage of the cells.

Paracetamol is a widely used analgesic and antipyretic drug undergoes oxidation to produce N-acetyl-P-benzoquinone-imine at overdoses and covalently binds to sulfhydryl groups causing cell necrosis and lipid peroxidation by depleting reduced glutathione in the liver and kidney, subsequently leading to hepato-nephrotoxicity (Goldin *et al*., 1996; Jones and Vale, 1993).

Liver damage is associated with elevation of serum ALT, ALP, AST, GGT, LDH and TB levels which are among the most sensitive biomarkers for liver function (El-Demerdash, 2004). In our present experimental study, administration of paracetamol causes significantly higher serum levels of ALT, ALP, AST, GGT, LDH and TB and lower levels of TP and ALB confirming liver toxicity. However, the rats pre-treated with AL extract at three different doses as well as those which received silymarin have significantly lowered AST, ALP, GGT, LDH, TB and increased TP levels when compared with the paracetamol-controlled rats. Significant reductions in serum ALT was also observed in animals which received the highest dose of AL indicating its hepatoprotective effect. It is plausible that the hepatoprotective effect occur due to the high levels of its antioxidant compound such as oxyresveratrol, polyphenols (rutin, pyrogallo, gallic acid, resorcinol and quercetin) alkaloids, flavanoids (catechin) tannins, steroids and saponins (Singhatong *et al*., 2010; Suwannalert *et al*., 2012) which may confer the protective effect by preserving liver membrane integrity.

Serum levels of TC, TG and LDL-C were significantly increased while HDL-C level was decreased in animals which received paracetamol, suggesting that paracetamol affects liver cell membrane permeability. Moreover, blockage of liver bile ducts and

subsequent decreased secretion of cholesterol into the intestine may result in increased serum cholesterol, another important sign of liver injury (Kalender *et al*., 2010; Samir *et al*., 2000). Pre-treatment with ethanolic extract of AL significantly reduced serum levels of TC, TG and LDL-C and increased HDL-C level indicating its hepatoprotective effects.

Another important highlight of our research was the possible protective effect of AL on oxidative damage as generated by paracetamol-induced nephrotoxicity which was further confirmed by the kidney histopathology. Creatinine, urea and uric acid are waste products of protein metabolism requiring excretion via the kidney. The excessive increase in the kidney marker levels in the serum of paracetamol-controlled group rats confirmed the functional damage of the kidneys (Al-Attar and Al-Taisan, 2010; Yazar *et al*., 2003). It is plausible that considerable production of reactive NAPQI occur due to paracetamol overdose which covalently binds to the macromolecules on cellular proteins, leading to the disruption of homeostasis, tissue necrosis and eventually kidney dysfunction (Bessems and Vermeulen, 2001; Cekmen *et al*., 2009). The results of this study indicated that AL can confer protective effect against kidney damage induced by paracetamol. Again, it is plausible that the nephroprotective effect of AL is conferred by its high antioxidant content.

Oxidative stress is considered an important contributing factor to the development of liver and kidney diseases. Oxidative stress mainly develops due to increased generation of Reactive Oxygen Species (ROS) and a decrease in ROS scavenging capacity (Yousef *et al*., 2010; Zhu *et al*., 2012). The excessive levels of MDA are attributed to increased production of ROS ($O_2^-$, $H_2O_2$, $^-OH$). Excessive lipid peroxidation causes lipid degradation that impairs cell membrane function, resulting in tissue damage and failure of antioxidant defence mechanisms to prevent the formation of excessive free radicals in paracetamol-intoxicated rats (Halliwell and Gutteridge, 2015; Souza *et al*., 1997). Pre-treatment with AL successfully quenched the free radicals thereby inhibiting lipid peroxidation and protecting the membrane lipids from oxidative damage in rats' liver and kidney. Animals treated with fruit extracts however, showed a dose-dependent reduction in MDA levels as compared with the paracetamol-controlled group. AL is a good source of natural antioxidant since it is rich in oxyresveratrol, polyphenols alkaloids, flavanoids, tannins, steroids and saponins which may act synergistically through free radical scavenging, metal ion chelating, hydrogen donation, singlet oxygen quenching and neutralization of free radical reactions (Robards *et al*., 1999; Tanvir *et al*., 2015). Silymarin is widely used as a standard drug for hepatoprotective study. It is well established that silymarin protects the liver via multiple mechanism of actions against different hepatotoxic agents (Afroz *et al*., 2014). There was no mortality or toxicity sign in the AL control rats suggesting that the extract has no toxicity and is well tolerated by the animals at the doses tested.

Although the various phytoconstituents of AL may be responsible for its hepato-nephro protective activities, more detailed biochemical and molecular studies should be conducted to confirm the mechanistic roles of the phytoconstituents.

## Conclusion

The findings of this study indicated that the ethanolic extract of AL possess excellent antioxidant activity and protective effect against paracetamol-induced liver and kidney damaged in male Wister rats due to its ability to act as free radical scavenger as evident by the *in vitro* and *in vivo* antioxidant potentials. The findings on serum biomarkers were further confirmed by the histopathological examination of liver and kidney sections which reveals the normal architecture of liver and kidney were damaged by liver and kidney toxin intoxication. The hepato-nephro protective activities of AL would be due to the presence of bioactive compound bioactive rutin, pyrogallo, gallic acid, resorcinol, quercetin, catechin and caffeic acid all of which have hepatoprotective activity. Therefore, this study shows experimental evidence and justifies the traditional claims and use in the treatment of liver and kidney diseases.

## Conflicts of Interest

We declare that there are no conflicts of interest.

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## Author's Contributions

Md. Yousuf Ali, Md. Reaz Morshed, Md. Sakib Hossen, E. M. Tanvir, Alamgir Kabir, Md. Aminul Islam, Nurul Karim and Nadia Alam done the experimental work. Md. Yousuf Ali and E. M. Tanvir analysed and presented data. Md. Yousuf Ali wrote the manuscript. Md. Ibrahim Khalil and Siew Hua Gan revised and approved the final version of the manuscript.

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