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Anti-nephrotoxic activity of aqueous extract of polyherbal mixture against renal toxicity induced by paracetamol in Wistar albino rats

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Abstract. *Abu MS, Mashi RL, Lawal JY, Samuel OS. 2023. Anti-nephrotoxic activity of aqueous extract of polyherbal mixture against renal toxicity induced by paracetamol in Wistar albino rats. Nusantara Bioscience 15: 112-117.* Medicinal plant materials as sources of therapeutic compounds continue to play an important role in maintaining human health for ages. This study investigated the effect of polyherbal extract (*Carica papaya* L., *Allium sativum* L., *Curcuma longa* L., and *Azadirachta indica* A.Juss.) on urea, creatinine, sodium ion, potassium ion, and chloride ion concentrations, and body weight of Wistar rats intoxicated with paracetamol. Thirty Wistar rats were randomly distributed into six groups, with five in each group. Group 1 is the normal control group; Group 2 is the negative control group (paracetamol-induced but untreated); Group 3 received 140 $mgkg^{-1}$ of silymarin; Groups 4, 5, and 6 received 100, 300, and 500 $mgkg^{-1}$ body of the aqueous polyherbal extract respectively for seven days. Therefore, the blood samples were collected and evaluated for creatinine, urea, sodium, potassium, and chloride ions concentrations to measure kidney function. The results revealed that the aqueous extract of the polyherbal mixture significantly ($p<0.05$) ameliorated the kidney function test parameters that were analyzed by lowering their concentrations previously elevated by the paracetamol intoxication comparable with the normal control rats. Therefore, it can be concluded that the polyherbal mixture extract expressed an anti-nephrotoxic effect against renal toxicity induced by paracetamol in Wistar albino rats.

Keywords: Acetaminophen, aqueous extract, nephrotoxicity, paracetamol, polyherbal, renal toxicity

INTRODUCTION

The liver, gastric tract, and kidneys metabolize drugs in the body. Drug and metabolite excretion can take one of two routes: renal or extra-renal (Perazella 2019). In terms of kidney elimination, medications can be removed via either glomerular filtering or tubular secretion. Each excretion pathway exposes the tubules and the interstitium to potentially harmful chemicals. The proximal tubules are exposed by apical contact with chemicals released into the tubular lumen, tubular epithelial cell absorption, or apical efflux from the peritubular circulation (the basolateral regions of tubular cells) into the tubular lumen (Perazella 2019). The chemicals are secreted from the proximal tubule into the loop of Henle and then into the distal tubule via glomerular filtration and tubular secretory traffic. In addition, drugs may precipitate, crystallize, or create casts in the distal tubules, resulting in tubular blockage (Luque et al. 2017). Another pathway involves interstitial tubule inflammation, which causes interstitial nephritis (Chamarthi et al. 2018). Therefore, drug-induced nephrotoxicity occurs through one of the three routes mentioned (Zuk and Bonventre 2016). Finally, a dose-dependent mechanism is caused by apical contact with drugs or their metabolites, transport of drugs and metabolites from the apical surface, and secretion of drugs from the basolateral surface into the tubular lumen (Chawla et al. 2017), which causes interstitial nephritis (Qu et al. 2017).

Allium sativum L., also known as garlic, is a member of the Amaryllidaceae family. Its biological qualities have been known to humans for many centuries. Garlic is native to Central Asia and has long been used as a crop in the Mediterranean region and as a flavoring in continents such as Africa and Europe. India is the world's second-largest producer of garlic after China (Raj et al. 2022). Garlic's anti-inflammatory, antioxidant, antibacterial, antiviral, antifungal, and antimutagenic effects are well known (Tsai et al. 2012).

Papaya (*Carica papaya* L.) is a popular and commercially significant species in tropical nations (Nugroho et al. 2017). Traditionally, it has been used to cure various conditions such as gastrointestinal problems, diarrhea, skin illnesses, male contraception, and cold household remedies. Numerous studies have found that papaya has anticancer properties for colon, prostate, cervical, and breast malignancies. Extracts of the chosen plant's fruit, seeds, and leaves have also been demonstrated to have strong cytotoxic activity against cancer cell lines such as breast, liver, and cancer of hematopoietic cell lines (Singh et al. 2020).

Although neem (*Azadirachta indica* A.Juss.) is native to India, the Meliaceae family is widely distributed globally, and it can thrive in most tropical and subtropical nations, including Indonesia. In Indonesia, Neem is known as Imba, Nimba, or Mimba (Sitasiwi et al. 2018). According to

previous studies, neem leaf extract has numerous biological and pharmacological activities such as antipyretic, analgesic, antihepatotoxic (Ogbuewu et al. 2011), spermicide, anti-implantation (Asif 2013; Sarkar et al. 2021), antihyperglycemic, antiulcer, antifungal, antibacterial, anti-inflammatory, immunomodulatory, antimutagenic, anticancer, antimalarial, antiviral, antioxidant (Alzohairy 2016; Gupta et al. 2017), antifertility (Gbotolorun et al. 2008), and contraception (Kumar et al. 2016).

Curcuma longa L. (turmeric or curcuma) is a rhizomatous monocotyledonous annual herbaceous plant in the ginger family (Zingiberaceae), native to tropical and temperate areas such as India, China, and Southeast Asia. India is the world's major producer, user, and exporter of turmeric (Trujillo et al. 2013). Curcuma is derived from the Arabic term Kourkoum, which was the original name for saffron (Benzie and Wachtel-Galor 2011). Curcuminoids have been consumed as therapeutic infusions over the years worldwide. In Ayurvedic medicine, curcumin is a well-documented treatment for various respiratory ailments such as asthma, bronchial hyperactivity, and allergy, as well as for liver disorders, rheumatism, anorexia, runny nose, cough, sinusitis, and diabetic wounds (Araújo and Leon 2001).

This study investigated the effect of the aqueous extract of a herbal mixture (*C. papaya, A. sativum, C. longa,* and *A. indica*) on urea, creatinine, sodium ion, potassium ion, chloride ion concentrations, and body weight of Wistar rats intoxicated with paracetamol.

MATERIALS AND METHODS

Study area

This study was conducted at the Biochemistry Department, Federal University Wukari Nigeria, Taraba State, Nigeria, from October 2022-2023.

Plant collection and identification

Matured and healthy-looking *C. papaya*, *A. indica, C. longa,* and *A. sativum* were collected from Wukari, Taraba State. The plants were washed with tap water thoroughly to remove the dust and soil particles. The leaves were air-dried under the shade to prevent ultraviolet rays from inactivating the chemical constituents (Ncube et al. 2008; Das et al. 2010). The dried planets were pulverized into a fine powder by a mortar and pestle, stored, and labeled in dry containers until needed.

Extract preparation

One hundred and twenty-five (125) grams of each pulverized plant powder was mixed and soaked for 48 hours in 250 mL of distilled water with periodic stirring and mixing (Abubakar and Haque 2020). The solution was subsequently sieved through Whatman filter paper. The extract, after filtration, was evaporated and concentrated using a water bath at 99°C. The percentage yield was calculated as 15.7%. The extract was stored at 4°C until further analysis.

Experimental animals

The rats weighing 150-230 g were purchased from Wukari, Taraba State, Nigeria, and were allowed to be acclimatized in the Animal House of the Department of Biochemistry, Federal University Wukari, for two weeks before the commencement of the experiment. All the rats were allowed free access to water ad libitum and feed throughout the experiment.

Animal grouping

Thirty (30) Wistar albino rats were used for the study. The rats were distributed into six groups of five rats in each group. The rats received the following treatment schedule:

Group 1: Non-paracetamol-induced rats (normal control).

Group 2: Paracetamol-induced rats (negative control), 500 $mgkg^{-1}$ body weight paracetamol.

Group 3: Paracetamol-induced (500 $mgKg^{-1}$) Nephrotoxic rats treated with 140 $mgkg^{-1}$ of silymarin (standard control) (Hamidian et al. 2020).

Group 4: Paracetamol-induced (500 $mgKg^{-1}$) Nephrotoxic rats treated with 100 $mgkg^{-1}$ body weight of extract.

Group 5: Paracetamol-induced (500 $mgKg^{-1}$) Nephrotoxic rats treated with 300 $mgkg^{-1}$ body weight of extract.

Group 6: paracetamol-induced (500 $mgKg^{-1}$) Nephrotoxic rats treated with 500 $mgkg^{-1}$ body weight of extract.

Treatment and induction

Nephrotoxicity was induced by oral administration of paracetamol 500 $mgkg^{-1}$ body weight after dissolution of paracetamol in distilled water. The paracetamol administration was continued for ten days. After three days of paracetamol induction, treatments with the extract of the mixture of *C. papaya, A. indica, C. longa, A. sativum*, and standard drug (silymarin) was carried out concomitantly with the induction for seven days. At the end of the experimental period, the rats were fasted for twelve (12) hours, anesthetized using chloroform, and sacrificed. Consequently, blood was collected from the heart via cardiac puncture using sterile syringes and needles to analyze kidney function tests.

Estimation of serum sodium, potassium, and chloride ions

A flame photometer Model 143, equipped with an automatic diluter Model 144 (ratio of the dilution of 200:1) (Instrumentation Laboratory, Inc., Lexington, Mass., USA), was used. The flame photometer was calibrated with twice distilled water and a standard with a Na^+ concentration of 140 mequiv/L and a K^+ concentration of 5 mequiv/L (Instrumentation Laboratory, Inc., Lexington, Mass., USA). After each sample measurement, the instrument's stability was checked with the standard solution.

Determination of serum urea concentration

The serum urea concentration was assessed using the method described by Fawcett and Scout (1960).

Principle: Urease breaks down urea into ammonia and carbon dioxide. In an alkaline medium, ammonia reacts with hypochlorite and salicylate to form dicarboxy indophenol, a colored compound. The reaction is catalyzed

by sodium nitroprusside. The intensity of color produced is measured spectrophotometrically at 578 nm.

$$\text{Urea} + H_2O \xrightarrow[\text{Urease}]{} 2NH_3 + CO_3$$

$$NH_3 + \text{Hypochlorite} + \text{salicylate} \rightarrow \text{dicarboxyindophenols (blue compound)} + CO_3$$

Procedure: Reagent (1 mL) containing sodium nitroprusside and urease was added into three clean test tubes labeled as a test sample, standard, and reagent blank containing 0.01 mL sample, 0.01 mL standard reagent, and 0.01 mL distilled water, respectively. The content in each test tube was mixed and incubated at room temperature (25-30°C) for 10 minutes. The absorbance of the test sample and standard were read against the reagent blank at 578 nm.

Calculation: The serum urea concentration was calculated using the formula below:

$$\text{Urea Conc. (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \; x \; \text{Concentrationof Standard}$$

BUN Concentration (mg/dL) = 0.467 x Urea Concentration (mg/dL)

Determination of serum creatinine concentration

The colorimetric method was used to determine serum creatinine concentration, according to Bertels et al. (1973).

Principle: Creatinine in the serum reacts with alkaline picrate to form a colored complex. The rate of formation of the colored complex is directly proportional to creatinine concentration. This reaction rate (intensity of orange color produced) is measured colorimetrically at 510 nm and compared with the standards.

$$\underset{\text{(Yellow)}}{\text{Creatinine} + \text{Picric acid}} \xrightarrow[\text{(Alkaline medium)}]{\text{NaOH}} \underset{\text{(Orange)}}{\text{Creatinine picrate (510 nm)}}$$

Procedure: Working reagent (1 mL) containing picric acid and sodium hydroxide was added into two clean test tubes labeled sample test and standard, containing 0.1 mL of the test sample and 0.1 mL of standard solution. The content in each test tube was mixed, and after 20 seconds, the absorbance of the standard (ST1) and test sample (TS1) was read at 510 nm. Exactly 80 seconds later, absorbance for (ST2) and (TS2) of the standard and sample were read at 510 nm against distilled water (blank).

Calculation: The Concentration of creatinine in serum (mg/dL) was calculated using the formula below:

$$\text{Creatinine Conc. (mg/dl)} = \frac{TS2 - TS1}{ST2 - ST1} \; x \; \text{Concentration of Standard}$$

Where:
ST : Standard
TS : Test sample

Statistical analysis

The biochemical results were subjected to statistical analysis using One-Way Analysis of Variance (ANOVA) and further duncan multiple comparisons using Statistical Package for Social Science (SPSS) version 21. The means were compared for significance at $p<0.05$, and the group results were presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Effect of polyherbal (*Carica papaya, Allium sativum, Curcuma longa,* and *Azadirachta indica*) on electrolytes levels of experimental rats

The study revealed a significant ($p<0.05$) increase in the concentration of sodium ions in Group 2 compared to Group 1. In contrast, Groups 3, 4, and 5 showed a significant ($p>0.05$) decrease in sodium ion levels compared to Groups 1 and 2. However, Group 6 did not display significant differences compared with Group 1 but decreased significantly ($p<0.05$) compared to Group 2. This implies that the paracetamol's adverse effects on the kidney were experimentally reversed, as shown in Table 1.

Also, Groups 2 and 3 showed a significant ($p<0.05$) rise in potassium ion concentration compared to Groups 1, 4, 5, and 6. Although, numerically, Group 3 has a lower potassium ion value than Group 2. Meanwhile, Groups 1, 4, 5, and 6 were all observed to be significantly ($p<0.05$) lower in potassium concentration when compared to Group 2.

Moreover, a significant ($p<0.05$) increase in the chlorine level in Group 2 compared to Groups 1, 3, 4, 5, and 6 was observed. The experiment demonstrated a significant ($p< 0.05$) reduction in chlorine concentration in Groups 3, 4, 5, and 6, comparable with Group 1 due to the extracts' administration.

Effect of polyherbal (*Carica papaya, Allium sativum, Curcuma longa,* and *Azadirachta indica*) extract on urea level of experimental rats

There was a significant ($p<0.05$) rise in the concentration of urea in Group 2 as compared with the other groups. The urea level in Groups 3, 4, 5, and 6 was ($p<0.05$) significantly ($p<0.05$) lowered after the treatments with both the silymarin and the polyherbal extract when compared with Group 2. However, the values in these groups were significantly higher compared to Group 1, which served as the normal control in the experiment. Repairing the paracetamol damage increases the urea's excretion rate and reduces its accumulation in the system, as evident in Figure 1.

Effect of polyherbal (*Carica papaya, Allium sativum, Curcuma longa,* and *Azadirachta indica*) extract on creatinine level of experimental rats

The creatinine concentration demonstrated a significant ($p<0.05$) increase in Groups 2, 3, and 4 compared to Groups 1, 5, and 6. However, upon treatments, Groups 5 and 6 significantly ($p<0.05$) reduced compared to Groups 1 and 2. The increased creatinine concentration indicated renal obstruction in Groups 2, 3, and 4, while the evidential reduction in Groups 1, 5, and 6 was attributed to kidney clearance, as shown in Figure 2.

Table 1. Effect of polyherbal (*Carica papaya, Allium sativum, Curcuma longa,* and *Azadirachta indica*) extract on serum electrolytes of albino rats

Groups	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)
Group 1	175.77± 13.11^b	8.70 ± 2.83^c	118.37 ± 3.90^b
Group 2	250.06 ± 1.43^a	17.89 ± 1.15^a	139.82 ± 27.61^a
Group 3	145.03 ± 63.62^c	15.90 ± 6.51^a	121.16 ± 2.05^b
Group 4	102.65 ± 7.67^d	6.55 ± 0.92^c	113.35 ± 030^c
Group 5	140.58 ± 91.19^c	11.95 ± 5.16^b	118.69 ± 0.33^b
Group 6	188.52 ± 13.44^b	7.65 ± 2.05^c	109.93 ± 12.05^c

No = 5; the result is represented as mean ± standard deviation. Results within a column with the same superscript indicate no level of significance (p>0.05), while result within a column with different superscripts indicates a level of significance (p<0.05). Group 1: Non-paracetamol-induced rats (Normal control), Group 2: Paracetamol-induced rats (negative control), 500 mgkg^{-1}body weight paracetamol; Group 3, Paracetamol-induced (500 mgKg^{-1}) Nephrotoxic rats treated with 140 mgkg^{-1} of silymarin (standard control) (Hamidian et al. 2020), Group 4: Paracetamol-induced (500 mgKg^{-1}) Nephrotoxic rats treated with 100 mgkg^{-1} body weight of extract, Group 5: Paracetamol-induced (500 mgKg^{-1}) Nephrotoxic rats treated with 300 mgkg^{-1} body weight of extract, Group 6: paracetamol-induced (500 mgKg^{-1}) Nephrotoxic rats treated with 500 mgkg^{-1} body weight of extract.

Table 2. Effect of polyherbal (*Carica papaya, Allium sativum, Curcuma longa,* and *Azadirachta indica*) extract on body weight of albino rats

Groups	Body weight		
	Week 0 (g)	Week 1 (g)	Week 2 (g)
Group 1	142.68±15.72^b	170.00±11.78^a	178.00±14.79^a
Group 2	162.34±17.51^a	158.60±13.46^a	163.20±12.58^a
Group 3	136.26±5.93^b	144.00±3.63^a	150.00±15.14^a
Group 4	158.80±14.72^b	174.60±14.92^a	172.60±14.32^a
Group 5	140.66±5.45^b	148.40±4.8^{ab}	156.60±12.66^a
Group 6	137.40±13.19^b	152.00±15.4^a	158.00±16.88^a

No = 5; the result is represented as mean ± standard deviation. Results within a row with the same superscript indicate no level of significance (p>0.05) difference, while different superscripts indicate a level of significance (p< 0.05)

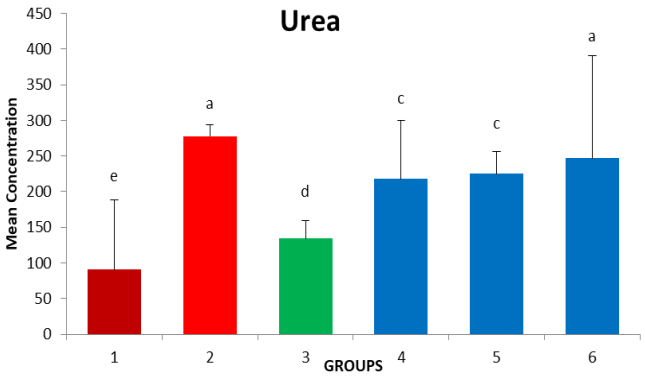

Figure 1. Effect of polyherbal (*Carica papaya, Allium sativum, Curcuma longa,* and *Azadirachta indica*) extract on urea of albino rats. No = 5; the result is represented as mean ± standard deviation. Results with the same superscript indicate no level of significance (p>0.05) difference, while different superscripts indicate a level of significance (p< 0.05)

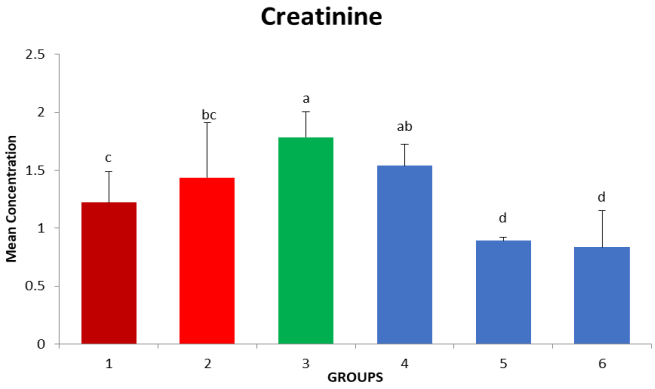

Figure 2. Effect of polyherbal (*Carica papaya, Allium sativum, Curcuma longa,* and *Azadirachta indica*) extract on creatinine of albino rats. No = 5; the result is represented as mean ± standard deviation. Results with the same superscript indicate no level of significance (p>0.05) difference, while different superscripts indicate a level of significance (p< 0.05)

Effect of polyherbal (*Carica papaya, Allium sativum, Curcuma longa,* and *Azadirachta indica*) extract on body weight of experimental rats

The average body weight of Group 2 rats in week 0, showed no significant (p<0.05) difference compared to the week 2 body weight. However, Groups 1, 3, 4, 5, and 6 in week 0 displayed a significant (p<0.05) lower body weight compared to week 2. The weight gain experienced in the extract-treated groups was attributed to kidney damage restoration, as shown in Table 2.

Discussion

In this research, upon induction of the rats with 500 mgkg^{-1} of paracetamol, the levels of the various kidney function parameters determined were significantly (p<0.05) increased. Meanwhile, administration of the different concentrations of the polyherbal extract (*C. papaya, A. sativum, C. longa,* and *A. indica*) and silymarin considerably reduced the levels of these parameters in the intoxicated rats, which were comparable with the normal control rats.

One of the most essential electrolytes in extracellular fluid is sodium, an osmotically active cation. It is in charge of controlling extracellular fluid volume as well as regulating cell membrane potential. The bulk of sodium reabsorption occurs in the kidney's proximal tubule (Shrimanker et al. 2022). Sodium transport occurs via sodium-chloride symporters activated by the hormone aldosterone; potassium functions primarily as an intracellular ion. The sodium-potassium adenosine triphosphatase pump is the major regulator of sodium and potassium homeostasis, pumping sodium out in exchange for potassium, which goes into the cells (Udensi and Tchounwou 2017). The glomerulus in the kidneys is where potassium is filtered. Chloride is an anion that is mostly present in extracellular fluid. The kidneys are the primary regulators of serum chloride levels. Most chloride filtered by the glomerulus is reabsorbed by both the proximal and

distal tubules (mostly the proximal tubule) via active and passive transport (Gattineni and Baum 2015).

Usually, nephrology defects caused by xenobiotics, such as CCl_4 and DEN toxicity, may truncate renal functions and result in irregular distribution of these ions in the ECF (Ganie et al. 2011), as it was the case of this research where paracetamol was able to cause similar alteration. However, the treatments with 100, 300, and 500 $mgkg^1$ of the polyherbal extract significantly ($p<0.05$) lowered the levels of sodium, potassium, and chloride ions of the paracetamol-intoxicated rats compared with the normal control and the standard drug groups after seven days of daily oral administration. This result can be deduced that administering the evaluated polyherbal extract can ameliorate the elevated level of serum electrolytes orchestrated by paracetamol toxicity, probably due to some bioactive components such as polyphenol, glycoside, and flavonoid in the different plants. The normalization of electrolytes in this study could liken to the findings of Abu et al. (2022), that used an n-butanol fraction of *F. glumosa* to reverse the distorted levels of sodium, chloride, and potassium ions in albino rats.

Amino acid deamination occurs in the liver, which is also the site of the urea cycle, where ammonia is converted into urea and excreted through urine (David et al. 2014). The urea excretory function of the kidney is often obstructed by injuries arising from toxic substances, as experienced with paracetamol in the present report. Whereas creatinine is a breakdown waste product formed in the muscle by creatinine phosphate metabolism, its retention in the blood is evidence of kidney impairment. However, it was revealed that the administration of 100, 300, and 500 $mgkg^{-1}$ of the polyherbal extract reduced urea and creatinine levels significantly ($p<0.05$) compared to the untreated Group 2. However, the reductions were not sufficiently comparable with the Group 1 and Group 3 rats. A similar finding was observed by the administration of *A. marmelos* (Kore et al. 2011), *T. terrestris* (Abdel-Kader et al. 2016), and *B. diffusa* (Olaleye et al. 2010) in rats have also been reported. This report has established that the aqueous extract of the polyherbal mixture examined can ameliorate the high urea and creatinine levels in the paracetamol-intoxicated rats by exploring the wound healing and antioxidant capacities of the varying phytoconstituents of the plants. On the other hand, the increased serum creatinine and urea level in the paracetamol-induced rats decreased the glomerular filtration rate in rats (Jesurun and Lavakumar 2016). The mechanism behind elevated serum urea and creatinine might be that the paracetamol increases calcium ions' entry into the mesangial cells, leading to a reduced glomerular filtration rate (Stojiljković et al. 2008). However, urea can also increase other ailments such as upper gastrointestinal bleeding, dehydration, catabolic process, and a high-protein diet (Gounden et al. 2023). Consequently, the near-normal reversal of the elevated creatinine and urea in this report might result from the mechanical reversal or obstruction of the paracetamol nephrotoxicity pathway in the experimental rats by the polyherbal extract.

Furthermore, Kpela et al. (2013) also found that the rat fed with the neem leaf extract at 500 $mgkg^{-1}$ body weight dose for 14 days caused a decrease in urea and creatinine concentrations in the rat induced by cisplatin for kidney damage. Based on the result obtained, induction of paracetamol without treatment showed no significant ($p<0.05$) increase in the body weight in Group 2 in week 0 compared to week 2, indicating a weight gain restriction. However, the administration of the polyherbal extract in the other groups showed a significant ($p>0.05$) increase in body weight between weeks 0 and 2, which may be an indication of restoration of normalcy from the nephrotoxic effects of the paracetamol. These findings also support the previous studies (Ali et al. 2005). The insignificant impart in the body weight in Group 2 could be attributed to the decrease in the animal's oral food intake due to acidosis and anorexia caused by acute kidney injury (Basile et al. 2012). Reduced body weight may have also been due to impaired water reabsorption by damaged renal tubules and consequent dehydration (Ali et al. 2005).

In conclusion, based on the result obtained, aqueous extract of polyherbal extract (*C. papaya, A. sativum, C. longa*, and *A. indica*) ameliorated the lost body weight. In addition, it reversed kidney functions of nephrotoxic albino rats, as evidenced by urea, creatinine, and electrolyte results.

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