ASIAN J TROP BIOTECHNOL
Volume 20, Number 1, June 2023
Pages: 37-41

ISSN: 0216-6887
E-ISSN: 2301-8658
DOI: 10.13057/biotek/c200106

Effects of aqueous extract of polyherbal formulation on acetaminophen-induced hepatotoxicity in albino rats

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Manuscript received: 11 April 2023. Revision accepted: 3 June 2023.

Abstract. *Abu MS, Mashi RL, Lawal JY, Nathan F. 2023. Effects of aqueous extract of polyherbal formulation on acetaminophen-induced hepatotoxicity in albino rats. Asian J Trop Biotechnol 20: 37-41.* The present study investigated the hepatoprotective activity of aqueous extract of the polyherbal mixture (*Carica papaya* L., *Allium sativum* L., *Curcuma longa* L., and *Azadirachta indica* A.Juss.) against acetaminophen-induced liver damage in rats. The Wistar albino rats of either sex were divided into every five animals into six groups. They were given the following seven-day treatment, i.e., paracetamol 500 g/kg BW p.o. to induce hepatotoxicity; Silymarin (140 mg/kg BW p.o.) as a reference standard; and three doses of polyherbal extract (100 mg/kg BW p.o., 300 mg/kg BW p.o., and 500 mg/kg BW p.o.). Blood collection by cardiac puncture was carried out after 24 h of the last administration and analyzed for various serum parameters (Serum Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Total Bilirubin (TB), Albumin (ALB), Total Protein (TP), Triglycerides (TGL) and Total Cholesterol (TCHOL). Treatment of the aqueous extract of the polyherbal mixture reduced the elevated levels of AST, ALT, ALP, TB, TGL, and TCHOL. It increased ALB and TP levels to indicate the repair of hepatic damage and demonstrated the aqueous extract hepatoprotective activity of the polyherbal mixture. The aqueous extract of the polyherbal mixture at doses of 100 mg/kg BW, 300 mg/kg BW and 500 mg/kg BW have significant ($p<.005$) effects on the liver of the paracetamol-induced hepatotoxicity rat model.

Keywords: *Allium sativum*, aqueous extract, *Azadirachta indica*, *Carica papaya*, *Curcuma longa*, hepatoprotective, polyherbal

INTRODUCTION

Hepatotoxicity often arises from cellular necrosis associated with oxidative stress generated by elevated free radicals, which can directly cause cell membrane breakdown with consequent changes in metabolic pathways (Sahreen et al. 2011). ROS causes degenerative cellular alterations that impact various main organs, including the heart, liver, lung, and kidney (Pizzino et al. 2017). Furthermore, the injured liver may decrease the antioxidant defense system.

Radiation (e.g., UV radiation, X-rays, etc.), contaminants, and endogenous metabolites produce free radicals in the cells. Large dosages of paracetamol, a frequently used analgesic and antipyretic medication, have been shown in humans and experimental animals to induce hepatotoxicity. Increased lipid peroxidation in the liver is a common characteristic following a hepatotoxic overdose drug (Begum et al. 2022).

According to FAO 2004, more than 6.8 million tons of papaya (*Carica papaya* L.) fruits were produced worldwide on 389,990 hectares of papaya cultivation (Sagadevan et al. 2019). Green papaya fruits, leaves, young shoots, and papaya flowers are consumed as vegetables in Asian nations, while ground dry papaya seeds are used as pepper (Papaya Australia 2017). Papaya also has various therapeutic benefits. Papain, a proteolytic enzyme obtained in milky papaya latex, has several pharmacological and commercial uses (Sharma et al. 2022). For example, Papain is used as an enzymatic debridement for necrotic tissue in burns, ulcers, and other wounds in US FDA-approved topical therapies, as well as the manufacture of vaccines and medications for different digestive illnesses (Sagadevan et al. 2019).

Garlic (*Allium sativum* L.) belongs to the Alliaceae and is the second most extensively used *Allium* after onions. It is cultivated worldwide and utilized as a spice, additive, and medicinal herb (Mazengia et al. 2019; Tesfaye 2021). Garlic contains several sulfur compounds (allicin, diallyl disulfide, S-allylcysteine, and diallyl trisulfide are responsible for their therapeutic benefits. It is eaten raw (fresh leaves or dried cloves) or processed (garlic oil, garlic extracts, and garlic powder), with various chemical compositions and quantities of bioactive components. It has long been recognized as a popular remedy for various ailments and physiological disorders and a useful spice (Shang et al. 2019).

The neem tree (*Azadirachta indica* A.Juss.) has been thought to have excellent health-promoting characteristics for generations (Rudra et al. 2019). Evidence shows that neem has been used to aid healing since 4,500 years ago; Their use originated in ancient India and neighboring countries, where it has long been revered as the most adaptable plant. Even today, the neem tree is recognized as the "Village Pharmacy," with all components having exceptional therapeutic potential.

Curcuma longa L., commonly known as turmeric (Zingiberaceae), is widely recognized in herbal medicine as

a panacea with a broad range of pharmacological properties. Turmeric plant is growing in tropical and subtropical regions worldwide and is widely grown in Asian countries, mainly in China and India. Turmeric may grow up to 1 m tall and has a short stem. Turmeric is an important spice worldwide, with a long history of human use, especially in the East (Nasir et al. 2014). Traditional medicine has used this powder to treat gastrointestinal problems, particularly biliary and hepatic disorders, diabetic wounds, rheumatism, inflammation, sinusitis, anorexia, colitis, and coughs (Ammon et al. 2012).

The study investigated the hepatoprotective activity of aqueous extract of a polyherbal mixture (*C. papaya, A. sativum, C. longa,* and *A. indica*) against acetaminophen-induced liver damage in rats.

MATERIALS AND METHODS

Study area

The study was conducted at the Department of Biochemistry, Federal University Wukari Nigeria, Taraba State, Nigeria, from October 2022 to January 2023.

Sample collection and preparation

Fresh papaya and neem leaves were collected within the Wukari Local Government of Taraba State, then cleaned and dried under shade. Garlic bulbs and turmeric rhizomes were bought in the Wukari market. They were brought to the Federal University Wukari, Biochemistry Laboratory, Nigeria, chopped into smaller pieces, and shade dried under standard laboratory conditions to prevent nutrient loss.

Preparation of crude extracts and polyherbal formulation

Exactly 125 g of each pulverized plant powder was mixed and soaked for 48 hours in 250 mL of distilled water with periodic stirring and mixing. The solution was subsequently filtered through the Whatman filter paper. After filtration, the extract was evaporated and concentrated using a water bath at 99°C. The yield percentage was 15.7%, and the extract was stored at 4°C until further analysis.

Experimental animal

Healthy male Wistar rats of about 120-150 g in weight were used in this study. They were purchased from the animal house of the College of Health Science, Benue State University, Nigeria, and transported to the Animal House of the Department of Biochemistry, Federal University Wukari, Nigeria. They were acclimatized for two weeks and weighed again before starting the experiment (Yakubu et al. 2014).

Animal grouping

Thirty (30) Wistar albino rats were distributed into six groups of five rats in each group. The rats received the following treatment: (i) Group I: Non-paracetamol-induced rats (normal control). (ii) Group II: Paracetamol-induced rats (negative control) 500 mg kg^{-1} BW. (iii) Group III: Paracetamol-induced Nephrotoxic rats treated with 140 mg kg^{-1}BW of silymarin (standard control). (iv) Group IV: Paracetamol-induced Nephrotoxic rats treated with 100 mg extract kg^{-1} BW. (v) Group V: Paracetamol-induced Nephrotoxic rats treated with 300 mg extract kg^{-1} BW. (vi) Group VI: paracetamol-induced Nephrotoxic rats treated with 500 mg extract kg^{-1} BW.

Treatment and induction

Nephrotoxicity was induced by oral administration of paracetamol (500 mg kg^{-1} BW). Paracetamol was dissolved in distilled water, and the paracetamol administration was continued for ten days. After three days of paracetamol induction, each group was given the appropriate treatments, namely administration of the extract of the mixture of *C. papaya, A. indica, C. longa,* and *A. sativum,* and standard drug (silymarin) carried out concomitantly with paracetamol induction for seven days. The rats were fasted for twelve (12) hours, anesthetized using chloroform, and sacrificed at the end of the experimental period. In addition, blood was collected from the heart via cardiac puncture using sterile syringes and needles for further analysis of kidney function.

Parameters for assessing liver function

Remi centrifuge separated serum from the clotted blood at 2,500 rpm for 15 min at 30°C. Serum samples were immediately subjected to biochemical analysis of Serum Aspartate Transaminase (ASP), Serum Alanine Transaminase (ALT), Alkaline Phosphatase (ALP) Total Bilirubin (TB), Total Protein (TP), Albumin (ALB), Total Glycerides (TGL) and Total Cholesterol (TCHOL) by a microplate reader (Power wave XS), using auto-analyzer (DT 60 Ã Chemistry Analyzer).

Statistical analysis

Data from the biochemical analysis were reported as mean ± standard error. One-way ANOVA and Dunnet's test performed the statistical analysis. A 95% confidence interval, $P<0.05$, and $P<0.01$ were considered significant.

RESULTS AND DISCUSSION

Effect of the extract of the polyherbal mixture (*Curcuma longa, Carica papaya, Allium sativum,* and *Azadirachta indica*) on liver function

In the acetaminophen-treated rats, the levels of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Alkaline Phosphatase (ALP) increased significantly ($p<0.05$) compared to the normal control Group 1 rats in Table 1. Similarly, the levels of Total Bilirubin (TB) increased significantly ($p<0.05$) while that of the Total Protein (TP) and Albumin (ALB) decreased significantly ($p<0.05$) in the acetaminophen-induced but not treated (Group 2) compared to the normal control Group 1 and the standard drug Group 3 as presented in Table 1. Furthermore, the Total Glycerides (TGL) and Total Cholesterol (TCHOL) were significantly ($p<0.05$) increased in the acetaminophen-induced but not

treated Group 2 compared to the normal control Group 1 and the standard drug Group 3 (Figures 1 and 2). However, treatments with the aqueous extract of the polyherbal mixture lowered the elevated levels of AST, ALT, ALP, TB, TGL, and TCHOL and increased the levels of ALB and TP in Groups 4, 5 and 6 when compared with Group 2.

Discussion

This study investigated the hepatoprotective effect of mixed polyherbal aqueous extract on rats induced with acetaminophen. Acetaminophen-induced hepatotoxicity is the most commonly used screening method for testing the hepatoprotective properties of plant extracts. The hepatic damage causes increased serum levels of enzymes, such as Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), and other serum parameters such as Total Bilirubin (TB), Total Glycerides (TGL) and Total Cholesterol (TCHOL). In addition, it indicates cellular damage and loss of functional integrity of the cell membrane of the hepatocytes (Ilukho et al. 2022). The hepatocytes' acetaminophen-induced damage was also observed in impaired protein metabolism, which was indicated by decreased Total Protein (TP) and Albumin (ALB) beyond the normal control group.

The serum ALT, AST, and ALP levels could be used to determine liver function alteration by increasing levels in the cytoplasm of hepatocytes (Giannini et al. 2005). However, they are usually present in low concentrations in the serum under normal physiological conditions; Their functions are unknown and hence are called non-functional enzymes in the serum (Vasudevan et al. 2011). These enzymes leak into the bloodstream while hepatopathy and rupture of cell or organelle membranes occur; therefore, their concentrations are used to determine the damage to liver tissues (Ze et al. 2020).

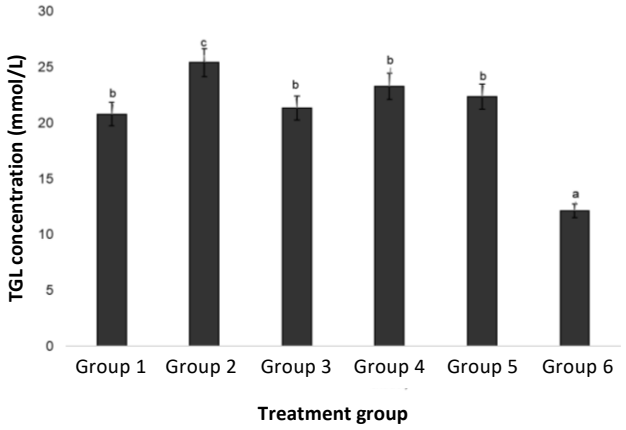

Figure 1. Effect of the administration of the polyherbal extract (*Curcuma longa*, *Carica papaya*, *Allium sativum*, and *Azadirachta indica*) on triacylglycerides levels in Wistar albino rats. N = 5; Result presented as mean ± standard deviation. The same superscripts indicate no significance level, while different alphabets indicate significant differences

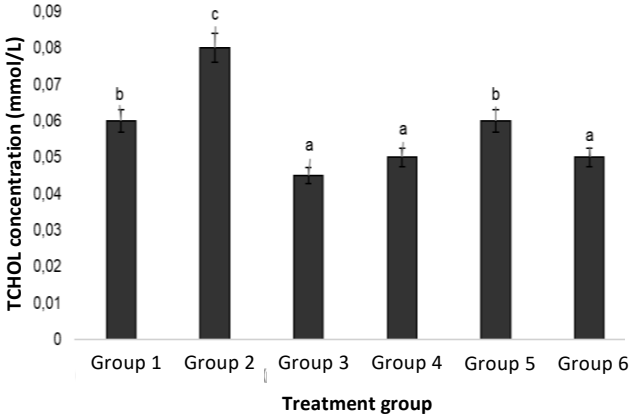

Figure 2. Effect of the administration of the polyherbal extract (*Curcuma longa*, *Carica papaya*, *Allium sativum*, and *Azadirachta indica)* on the Total Cholesterol in Wistar albino rats. N = 5, Result presented as mean ± standard deviation. The same alphabets indicate no significance level, while different alphabets indicate the significance level

Table 1. Effect of polyherbal extracts (*Curcuma longa*, *Carica papaya*, *Allium sativum*, and *Azadirachta indica*) on liver function

Parameter	AST (U/I)	ALT (U/I)	ALP (U/I)	TB (mg/dL)	TP (mg/dL)	ALB(mg/dL)
Group 1	304.82±2.44^{d}	222.14±34.32^{c}	72.57±5.23^{a}	12.11±3.75^{c}	29.16±2.55^{c}	43.97±3.25^{c}
Group 2	364.23±4.49^{e}	339.60±7.00^{d}	348.03±7.20^{f}	21.48±1.56^{d}	19.89±0.33^{b}	34.32±7.93^{b}
Group 3	147.65±7.55^{a}	134.94±6.78^{a}	194.54±10.90^{c}	13.95±3.94^{c}	30.15±1.26^{c}	45.42±0.95^{d}
Group 4	235.30±4.60^{c}	152.67±2.60^{b}	299.54±2.08^{e}	11.62±2.01^{c}	29.36±0.57^{c}	43.60±0.02^{c}
Group 5	178.12±3.89^{b}	156.40±7.87^{b}	162.56±6.55^{b}	7.84±1.32^{b}	29.71±1.61^{c}	44.04±0.88^{c}
Group 6	164.71±5.67^{b}	237.07±6.45^{c}	233.55±7.56^{d}	4.46±0.55^{a}	18.44±1.24^{a}	24.43±1.54^{a}

Note: No = 5; Results are presented as mean ± standard deviation. Results with the same superscripts in the same column indicate no significant difference, while different superscripts indicate significant differences

The increased level of liver enzymes in the bloodstream due to the acetaminophen may be associated with central/submissive liver necrosis, which causes severe hepatic injury (Contreras-Zentella and Hernández-Muñoz 2015). On the other hand, the increased serum ALP may be due to increased synthesis or cholestatic disturbance to the free flow of biliary tract contents due to increased biliary pressure (Wallace 2004). Similarly, increased enzyme levels in rats caused by acetaminophen have been previously reported by Awodele et al. (2016); it was attributed to the damage to the structural integrity of the liver from autolytic breakdown or cellular necrosis.

However, this mixed polyherbal aqueous extract treatment significantly ($p<0.05$) lowered the concentrations of these enzymes as compared to the normal rats and standard drug (silymarin) groups. This finding is similar to Beerendra et al. (2012), in which a polyherbal formulation showed good hepatoprotective activity by lowering the levels of SGOT, alkaline phosphatase, bilirubin parameters, and lipid profiles-cholesterol, triglyceride, and LDL.

Consequently, the hepatoprotective effect of the polyherbal extract may be due to the Phyto-constituents like polyphenols that exhibit varying antioxidant capacities levels (Akachi et al. 2010). Furthermore, the in vitro free radical scavenging activity, such as DPPH, superoxide, and hydroxyl radical scavengings of the polyherbal mixture of various components, suggest the ability of the extract to reduce biological oxidative stress (Madhukiran and Ganga 2011). Hence, the hepatoprotective effect of the extract may be affected by its scavenging free radical activity (Ravikumar and Gnanadesiga 2011).

Bilirubin was significantly increased in the Group 2 experiment (acetaminophen-induced but not treated with extract); however, the high level of bilirubin was lowered significantly ($p<0.05$) in the extract-treated rats (Groups 4, 5 and 6) compared to Group 2 (acetaminophen-induced but not treated). Meanwhile, the bilirubin reduction in Group 4 experiment was comparable with the Groups 1 and 3 experiments that served as normal and standard drug groups respectively while that of Groups 5 and 6 were lower compared to Groups 1 and 3 experiments. Bilirubin is the conventional indicator of liver diseases (Achliya et al. 2004). The bilirubin reduction concentration close to normal in the extract-treated groups may be due to the inhibitory effects on cytochrome P450 or/and the promotion of its glucuronidation (Cavin et al. 2001). On the other hand, it showed that the administration of acetaminophen decreased Total Protein and Albumin levels and increased Total Cholesterol and triglycerides. However, these parameters (total protein, Albumin, Total Cholesterol and triglycerides) were maintained close to normal levels in the extract-treated animals (Groups 4, 5 and 6). Therefore, the aqueous extract of polyherbal treatment indicates inhibitory activity against the adverse effect of acetaminophen. It may result from interfering with cytochrome P450, so the formation of hepatotoxic free radicals could be inhibited. The site-specific oxidative damage in some susceptible amino acids of proteins is now regarded as the primary cause of metabolic dysfunction during pathogenesis which alters the concentration of metabolic products (Bhattacharyya et al. 2014). The normalization of protein, cholesterol, and triglyceride levels in the acetaminophen-induced rats, followed by extract-treated rats, confirms the hepatoprotective effect of the plant extract.

A previous study showed that various parts of the plants (*C. papaya*, *A. sativum*, *C. longa*, and *A. indica*) used in the herbal mixture have potent antioxidant and hepatoprotective properties against paracetamol-induced rats (Chattopadhyay 2003; Somanawat et al. 2013; Awodele et al. 2016; Ghobadi et al. 2019). The findings in this study showed the hepatoprotective effect of these plant extracts, which was observed from the liver enzyme levels approaching normal after plat extract treatment.

In conclusion, the polyherbal used in this study has ameliorative potency against hepatotoxicity-induced rats; therefore, the herbal mixture extract can manage hepatic disorders. The exact mechanism of the hepatoprotection and antioxidation of the polyherbal should be investigated further.

ACKNOWLEDGEMENTS

Michael Sunday Abu designed and supervised the work, assisted in the lab work, prepared the manuscript, and provided technical support; Rukaiyat Lawal Mashi carried out data analysis and interpretation; Jamila Yahaya Lawal carried out manuscript proofreading and editing; and Oyebisi Sunday Samuel funded the study and carried out the lab work.

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