EFFECTS OF AQUEOUS SEED EXTRACT OF RIPE *Carica papaya* ON TOTAL BILIRUBIN AND ASPARTATE AMINOTRANSFERASE (AST) IN RATS TREATED WITH MONOSODIUM GLUTAMATE (MSG)

ABSTRACT

This study investigated the effects of aqueous seed extract of ripe *Carica papaya* on total bilirubin (TB) and aspartate aminotransferase (AST) levels in rats treated with monosodium glutamate (MSG). Twenty rats were divided into four groups, with five rats per group. The normal control group received water orally, the negative control group received MSG orally, the positive control group received ascorbic acid and MSG orally, and the test group received the aqueous seed extract of ripe Carica papaya and MSG orally. The experimental period was 28 days. The findings revealed that rats given MSG alone had higher levels of TB and AST compared to the normal control group, which suggests liver dysfunction. However, when the aqueous extract of ripe Carica papaya seeds was administered alongside MSG, it significantly reduced these increases, indicating a protective effect on liver health. Similarly, the positive control group that received both ascorbic acid and MSG showed lower TB and AST levels compared to the negative control group, demonstrating the liver-protective properties of ascorbic acid. These results imply that Carica papaya seed extract could offer therapeutic potential in preventing or alleviating liver damage linked to MSG intake.

Key words: Monosodium glutamate (MSG), *carica papaya*, total bilirubin (TB) and aspartateaminotransferase (AST)

1 INTRODUCTION

In recent years, there has been increasing interest in the health benefits of plant-based natural remedies. Among these, Carica papaya, or papaya, has garnered attention due to its abundant phytochemicals and its long-standing use in traditional medicine (Sharma and Khodaskar, 2014). Monosodium glutamate (MSG), a common flavor enhancer in the food industry, has been linked to negative

health impacts, particularly on liver function. Research suggests that excessive MSG intake can result in hepatotoxicity, as evidenced by elevated liver enzymes like AST and increased bilirubin levels. AST, an enzyme mainly present in the liver, heart, and skeletal muscles, becomes elevated in the blood when liver damage occurs (Anbazhagan and Devaki, 2010). Bilirubin, a yellow pigment formed from hemoglobin breakdown and processed by the liver, also rises with impaired liver function (Oboh *et al.*, 2012). Carica papaya, a tropical fruit originating from Central America, has been traditionally used for its medicinal benefits across various cultures. Different parts of the papaya plant—such as the fruit, leaves, seeds, and latex—are valued for their therapeutic properties, including anti-inflammatory, antioxidant, antimicrobial, and liver-protective effects. Notably, the seeds of papaya are of interest for their potentialliver-protective (hepatoprotective) benefits, due to their bioactive compounds like flavonoids, alkaloids, phenolic acids, and antioxidant enzymes (Ajiboye *et al.*, 2013).

Several preclinical studies have explored the hepatoprotective potential of papaya seed extract in animal models with liver damage caused by various toxins, including carbon tetrachloride (CCl4), acetaminophen, and ethanol. These studies have shown that papaya seed extract helps reduce liver damage by lowering oxidative stress, inflammation, and lipid peroxidation, while boosting antioxidant defenses and supporting liver repair (Igbinosa *et al*., 2014).

Despite these encouraging results, research on the hepatoprotective effects of Carica papaya seed extract in the context of MSG-induced liver damage is limited. Given the widespread use of MSG in

processed foods and its potential harmful impact on liver health, there is a need for natural remedies that could counteract MSG-induced liver toxicity. This study seeks to investigate the impact of aqueous extract from ripe Carica papaya seeds on bilirubin and AST levels in rats exposed to MSG, aiming to assess its potential in protecting against MSG-induced liver injury.

2 Sample Materials and Reagents

2.1 Materials

Fresh ripe *C. papaya* were purchased at Ekpoma market, Esan West Local Government Area. Sodium dihydrogen phosphate, Disodium hydrogen phosphate, Sodium chloride, Chloroform, quercetin, sodium chloride were acquired from BDH Chemical Ltd, Poole England. The bioassay test kits were purchased from Randox Laboratories Ltd, Crumlin, UK and Spectrum diagnostic, Cairo, Egypt. Distilled water was purchased from Megacorp scientific, Ekpoma, Edo State. Deionized water was prepared at the Laboratory of the Department of Biochemistry, Ambrose Alli University, Ekpoma, Edo State. All other chemicals were of analytical grade.

Equipment used were: Digital pH meter (pH 7110) from inolab; Analytical weighing balance (TX323L) from SHIMADZU; Digital water bath (HH.W21) from Metlab instruments; hot plate; L7 Double beam UV-vis spectrophotometer and centrifuge from SEARCHTECH instruments, British standard and other routine apparatus.

2.3 Methods

2.3.1 Preparation aqueous extract of ripe *C. papaya* seed

After taxonomical identification, the *C. papaya* seedswere air dried and extracts of the seed were prepared according to the method described by Akinnibosun (2009). The dried seed of ripe *C. papaya* were

weighed and aseptically blended into powder. The dried powder was solubilized in distilled water as solvent, hermetically sealed and then allowed to stand for 24 hours with intermittent stiring. The solubilized powder was filtered using muslin cloth and the solvents removed by re-circulation. Concentration of supernatants were done by exposure to air in a container with wide surface and then stored at -4 °C until further analysis.

2.4. Animal Studies

2.4.1 Experimental animals

A total of twenty (20) adult male Wistar rats (10 weeks old, 160 ± 10g) were obtained from the Animal House, College of Medicine, Ambrose Alli University, Ekpoma, Edo State. The animals were hygienically housed in plastic cages placed in a well-ventilated vivarium with natural photoperiod of 12-hours light and dark cycle. They were fed with rat chow and given drinking water *ad libitum* during one week of acclimatization before the commencement of the experiment. All the animals received human care as indicated in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science (NAS) and published by the National Institute of Health (National Research Council, 2011).

2.4.2 Induction of MSG and experimental protocol

Rats were over dosed with MSG at 1000 mg/kg body weight every day for 28 days to cause hepatocyte injury (Xu *et al.*, 2021). The following regimen was used for treatment: group 1 (Test group) received ripe *C. papaya* aqueous seed extract (100mg/kg/day) and MSG (1000 mg/kg/day) orally for 28 consecutive days; group 2 (Negative control) received MSG (1000 mg/kg/day) orally for 28 consecutive days; group 3 (Normal control) received distilled water only (3ml/kg/day) for 28 consecutive days and

groups 4 (Positive control) received Vitamin C (100 mg/kg/day) and MSG (1000 mg/kg/day) orally for 28 consecutive days. The design for the animal study is illustrated in

Table 1: Experimental Design of Animal Study

Test	Test group	Control group		
		Normal		Negative
			Positive	
Test group: extract (100mg/kg) MSG (1000 mg/kg/day) *orally*	5	-	-	-
Negative control: MSG (1000 mg/kg/day) *orally*	-	-	3	-
*Normal control:*water (3mL/kg/day) *orally*	-	3	-	-
Positive Control: ascorbic acid (100mg/kg) MSG (1000 mg/kg/day) *orally*	-	-	-	3

Calculation of dosage administration

Extracts of *C. papaya* seed and MSGwere administered according to the method of Omemu *et al.*, (2006) based on the corresponding weight of animal, and calculated as:

$$\text{Volume (mL)} = \frac{Weight\ of\ animal\ (g) \times dosage\ (mg)}{Concentration\ (mgmL^{-1}) \times 1000\ g}$$

2.4.3Collection of organ samples

Animals were anaesthetized with chloroform and blood was collected through cardiac puncture. The liver was excised immediately and homogenized with phosphate buffer saline (1%, w/v). The homogenate was subsequently centrifuged at 3,000 rpm for 15 minutes.

Comment [A1]: Numbers of animal in one group is described 5

Comment [A2]: What is role of blood collection in the study, is it further used in the study for any data collection

The homogenate/supernatant was kept in the freezer at -10^{o}C until further analysis.

2.5 Biochemical Analyses

The liver homogenate was used for evaluation of total bilirubin level and AST activity using spectrophotometer.

2.5.1 Determination of total bilirubin Concentration in liver homogenate

A total bilirubin test kit (Randox Laboratories Ltd, Crumlin, UK), was used for the estimation of serum total bilirubin. Colorimetric method based on that described by Jendrassik and Grof (1938). Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue color complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diaotized sulphanilic acid. The content of the test kit is R1 (sulphanilic acid and hydrochloric acid), R2 (sodium nitrite), R3 (caffeine and sodium benzoate) and R4 (tartarate and sodium hydroxide). The reaction mixture contained 0.02 ml R1, 0.05ml R2, 1.0ml R3 and 0.02 ml of serum for sample's test tubes, while blank test tube contained 0.02 ml R1, 1.0ml R3 and 0.02 ml of serum. The mixture was incubated for 10minutes at 25^{o}C. After incubation, 1.0ml of R4 was added to each tube. The setup was mixed and allowed to stand for 10minutes at 25^{o}C. The absorbance of samples A_{sample} were measured against reagent blank (A_{TB})at 578nm and total bilirubin concentration is calculated as follows:

Total Bilirubin concentration (mg/ml) = 10.8 x A_{TB} (578nm)

2.5.2 Assay of Serum Aspartate Aminotransferase (AST) Activity

Aspartate aminotransferase Spectrum test kit (Spectrum diagnostic, Cairo, Egypt) was used for this assessment. The

principle of this assessment was based on the following equations:

$$\text{2 –oxoglutarate + L-aspartate} \xrightarrow{\text{AST}} \text{L – glutamate + Oxaloacetae}$$

$$\text{Oxaloacetate + NADH + H}^{+} \xrightarrow{\text{malate dehydrogenase}} \text{L – Malate + NAD}^{+}$$

$$\text{Sample pyruvate + NADH + H}^{+} \xrightarrow{\text{Lactate dehydrogenase}} \text{L – Lactate + NAD}^{+}$$

Procedure;

1ml of working solution was pipetted in a test tube and 0.01ml of liver homogenate was added, mixed and incubated at room temperature. After exactly 30 seconds, initial absorbance was read. The absorbance increase was thereafter measured every 60 seconds for 3minutes and change in absorbance per minutes was determined.

Calculation;

AST/GOT concentration (U/L) = ΔA/min X 1746

2.6 Statistical analysis and data presentation

The data obtained from these studies were analyzed with Statistical Package (Graphpad prism 8.0) using one-way analysis of variance (ANOVA) with Turkey's multiple comparisons post hoc tests which compared the level of significance between the control and test groups. All data were expressed as mean ± SEM. The test for statistical significance was carried out at the 95% confidence limit. The data were presented as table.

RESULTS

Table 2: Effect of Aqueous extract of ripe *C. papaya* seed on AST and total bilirubin in MSG-induced Rats following daily oral administration for 28days

Biochemical parameters

Comment [A3]: Is there any comparison done in between the groups. How benefiacial is one group from another.

Group/dose (mg/kg/day b.wt.)	TB (mg/mL)	AST (U/L)
*Normal control:*water (3mL/kg/day) *orally*	1.51 ± 0.06[ab]	78.57 ± 5.04[a]
***Negative control**:* MSG (1000) *orally*	1.84 ± 0.06[a]	191.48 ± 10.10[b]
Positive Control: ascorbic acid (100mg) + MSG (1000) *orally*	1.51 ± 0.25[ab]	102.43 ± 3.24[b]
Test group: extract (100mg) + MSG (1000) *orally*	1.19 ± 0.06[b]	98.52 ± 3.14[a]

n = 3; values expressed as mean ± SEM; data analyzed using one-way ANOVA followed by Tukey's multiple comparisons post hoc test. **TB – total bilirubin, AST – aspartate aminotransferase** and Values marked with different superscript are significantly different at $p < 0.05$.

Comment [A4]: The use of a, b and ab Abbreviation should be explained below the table with p value

4. DISCUSSION

This study investigated the potential hepatoprotective effects of the aqueous seed extract of ripe *Carica papaya* in rats treated with monosodium glutamate (MSG). Important biochemical parameters, total bilirubin (TB) and aspartate aminotransferase (AST), known

indicators of liver function were assessed. Rats treated with MSG alone exhibited elevated levels of TB and AST compared to the normal control group, indicating hepatic dysfunction. However, co-administration of the aqueous extract of ripe *Carica papaya* seed with MSG significantly attenuated these elevations, suggesting a protective effect on liver function.

Total bilirubin is a marker of hepatic bilirubin metabolism, and elevated levels are indicative of impaired liver function, biliary obstruction, or hemolysis. The observed decrease in TB levels in rats treated with the aqueous extract of ripe Carica papaya seed suggests an improvement in hepatic function, possibly through enhanced bilirubin metabolism or protection against hepatocellular damage.

Aspartate aminotransferase (AST) is an enzyme predominantly found in hepatocytes, and elevated serum levels are indicative of hepatocellular injury or necrosis (Chalasani *et al*., 2020). The significant reduction in AST levels in rats co-administered the aqueous extract of ripe *Carica papaya* seed with MSG suggests a protective effect against hepatocellular damage induced by MSG (Table 3).

The observed hepatoprotective effects of the aqueous seed extract of ripe *Carica papaya* could be attributed to its rich phytochemical composition, including flavonoids, phenolic compounds, and other bioactive constituents (Akhtar *et al*., 2022). These compounds possess antioxidant, anti-inflammatory, and cytoprotective properties, which may help mitigate oxidative stress, inflammation, and cellular damage in the liver induced by MSG (Yuniarti *et al*., 2020).

The positive control group treated with ascorbic acid and MSG also exhibited a reduction in TB and AST levels compared to the negative control group, indicating the hepatoprotective effects of ascorbic acid. This finding is consistent with previous studies demonstrating the antioxidant and cytoprotective properties of ascorbic acid in mitigating hepatic damage (Padma *et al.*, 2019).

5 Conclusion

In conclusion, the aqueous seed extract of ripe *Carica papaya* demonstrated significant hepatoprotective effects against MSG-induced liver injury in rats, as evidenced by the reduction in total bilirubin and aspartate aminotransferase levels. These findings suggest that *Carica papaya* seed extract may have potential therapeutic applications in preventing or ameliorating liver dysfunction associated with MSG consumption. Further studies are warranted to elucidate the underlying mechanisms of action and to evaluate the long-term safety and efficacy of *Carica papaya* seed extract as a hepatoprotective agent.

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