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# Protective Effect of *Annona muricata* Linn Fruit Pulp Lyophilized Powder against Paracetamol-Induced Redox Imbalance and Hepatotoxicity in Rats

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**Abstract:** In the current investigation, *Annona muricata* Linn. lyophilized fruit pulp powder was evaluated for its hepatoprotective activity induced by paracetamol or acetaminophen (APAP). Male Sprague Dawley rats were orally pre-treated for 15 days with *A. muricata* lyophilized fruit pulp powder at low (1 g/kg b.wt) and high doses (2 g/kg b.wt). Silymarin (100 mg/kg) was administered as the standard drug. Hepatotoxicity was induced using APAP, in a single oral administration of 2.5 g/kg body weight dosage on the 15th day. Aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) were elevated in the APAP group but were found to be significantly reduced in the pre-treated groups in a dose-dependent manner. APAP administration brought down the serum total protein and albumin levels significantly. The activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase were reduced in the APAP administration; further, the reduced glutathione pool in the tissue was also diminished significantly. However, with the administration of Annona lyophilized fruit pulp powder, the level of antioxidant parameters was near normal. A significant increase in lipid peroxidation was observed in the APAP group, while the silymarin, AML, and AMH groups exhibited resistance to lipid peroxidation (LPO), as evident from lower levels of LPO generated. Histopathological examination also revealed considerable tissue damage in the APAP alone treatment group, which was not devastating in the silymarin, AML, and AMH groups. Altogether, the study concludes that the lyophilized fruit pulp of *A. muricata* is protective against APAP-induced liver injury in rats by modulating the hepatic redox systems.

**Keywords:** *Annona muricata*; paracetamol; hepatotoxicity; antioxidant level; oxidative stress



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## 1. Introduction

Several toxic compounds that are used in various industries are known to induce toxic responses mediated through inflammation and oxidative damage in hepatocytes [1,2]. Among the various hepatotoxic compounds, pharmacological drugs are important. Several clinically used drugs are known to have secondary ill effects on hepatocytes that induce toxic effects. Paracetamol (N-acetyl-para-aminophenol (APAP) or acetaminophen), the widely used antipyretic and analgesic drug, is also found to be hepatotoxic at chronic exposure [3–5]. APAP is activated through the hepatic cytochrome P450 enzymes [6,7]. Further, recent reports have indicated that hepatocyte damage induced by APAP is also mediated through cytochrome P450-independent mechanisms [8]. In the study, Miyakawa,

Albee, Letzig, Lehner, Scott, Buchweitz, James, Ganey, and Roth [8] indicated that at lower doses, APAP is activated through CYP450-mediated mechanisms. However, at concentrations higher than 5 mM, significant damage occurs even after the administration of 1-aminobenzotriazole (an inhibitor of CYP450 enzymes), which is suggestive of a CYP-independent method of APAP activation. Both these mechanisms produce the compound *p*-aminophenol in hepatocytes which in turn causes apoptotic cell death [9]. Further, the administration of APAP has been observed to induce significant redox imbalance in liver tissues [10]. It has been observed that APAP reduced the intracellular glutathione pool and also brought down the antioxidant enzyme activities [11,12]. This drug-mediated hepatotoxicity often progresses to drug-induced fatty liver disease and this can later cause complications including hepatocellular carcinoma [13–15].

Plant products are widely used as hepatoprotective agents against various oxidative stress inducers including drugs [16,17]. Freitag et al. [18] indicated the protective effect of the bioactive compounds silymarin and silibinin, isolated from *Silybum marianum*, against APAP-induced hepatotoxicity. Likewise, the plants belonging to the Annonaceae family are widely known for their protective effects against different toxicants in multiple organs [19–21]. *Annona muricata*, commonly called soursop, owing to the sweet–sour flavor of the large fruit, is a lowland tropical fruit-bearing tree. It is widely cultivated in tropical countries worldwide, including south Florida and Southeast Asia, for its edible fruit. Many active compounds have been found in the plant, and most of the research on *A. muricata* focuses on a novel set of compounds called annonaceous acetogenins [22]. Various parts of the plant, such as its leaves, fruits, roots, seeds, and bark, are attributed to traditional medicinal properties. Leaves are used as diuretics, antiarthritics, and antiparasitics, while seeds are used as carminatives and antiparasitics in different parts of the world. Fruits are given orally to reduce fever and diarrhea [23]. *A. muricata* leaves have been shown to reduce the hepatotoxic effect of APAP and carbon tetrachloride [24]. Likewise, the stem bark extract of *A. muricata* was also found to inhibit CCl4-induced hepatotoxicity by restoring the levels of hepatotoxicity markers [25]. Further, the leaf extract was also effective in preventing the hepatotoxic effect of thioacetamide in rats [26]. The commercially available Graviola dry extract was also effective in preventing hepatotoxicity induced by monosodium glutamate, a flavor enhancer [27]. Likewise, the dried leaf extract of *A. muricata* prevents diabetes-associated hepatic dysfunction in rats [28].

The fruits of *Annona muricata* are highly consumed in different parts of the world. Fruits are highly nutritive in terms of macronutrients, fiber, trace elements, and vitamin contents, hence making them healthy for dietary consumption [29]. Extracts of fruit pulp were found to inhibit the proliferation of breast cancer cells [30]. A report by Gyesi et al. [31] indicated that the fruits of *A. muricata* contain large quantities of phenolic compounds and subsequently possess strong antioxidant activities. A preliminary in vitro study by Adefegha et al. [32] indicated the antidiabetic and antihypertensive effect of the fruit pulp. Later, an animal model study indicated the potential of the fruit pulp extracts in preventing etoposide-induced damage to gastrointestinal tracts [33]. *A. muricata* pulp is usually consumed raw or it should be preserved using lyophilization for long-term use. Previous studies evaluated the biological properties of *A. muricata* fruits using chemical solvent extracts. Hence, the present study is significant as lyophilized powder of *A. muricata* fruit pulp was used, which can be stored for the long term. The present study, therefore, analyzed the protective effect of *A. muricata* lyophilized fruit pulp powder against APAP (2.5 g/kg)-induced hepatotoxicity in male Sprague Dawley rats.

## 2. Materials and Methods

### 2.1. Chemicals

Aluminum chloride, Folin–Ciocalteu's reagent, sodium carbonate, sodium hydroxide, phosphate buffer, and EDTA were purchased from SRL Chemicals (Mumbai, India). Pure compounds such as gallic acid, quercetin, paracetamol, and silymarin were procured from

Sigma-Aldrich (St. Luis, MO, USA). All other reagents and chemicals used in the present study were of reagent grade and procured from HiMedia (Chennai, India).

### 2.2. Collection, Extraction, and Lyophilization of Fruit Pulp

The *Annona muricata* fruits were collected from the outskirts of Thrissur District, Kerala (10.5506° N, 76.2836° E). The fruit pulp (500 g) of *Annona muricata* was prepared by homogenizing the fruits using a mixer grinder after the removal of the pericarp and seeds. The pulp (500 g) was freeze-dried at a temperature of −55 °C, the vacuum pressure was set at 0.015 bar for 1 week using a lyophilizer (SGS Lab Instruments, Chennai, India), and the powder was dissolved in water (100 mg/mL *w*/*v*) for further analysis.

### 2.3. Quantitative Phytochemical Content Analysis

The total phenolic content of *A. muricata* was spectrophotometrically determined as per the methods described by Nolasco–González et al. [34]. Briefly, the phenolics reacted with Folin–Ciocalteu's reagent and sodium bicarbonate, and the resulting color was measured at 720 nm. The contents of flavonoids in the lyophilized powder were estimated according to the methods of Justino et al. [35]; briefly, the flavonoids reacted with aluminum chloride (5%) and the resulting color was measured at 420 nm using a spectrophotometer. The phenol content was expressed as gallic acid equivalents, whereas the flavonoid content was expressed as quercetin equivalents.

### 2.4. In Vitro Antioxidant Activity

The in vitro radical quenching potentials were measured using DPPH, ABTS, and FRAP assays. Previously described methods were used for DPPH [36], ABTS [37] scavenging, and ferric-reducing potentials [38]. The final result was represented as the $IC_{50}$ values for DPPH and ABTS, whereas $EC_{50}$ value was used for FRAP assay.

### 2.5. Animal Use and Experimentation

A total of 30 male Sprague Dawley rats were purchased from the Small Animal Breeding Station, Kerala Veterinary and Animal Science University, Thrissur, India. The animals were kept in the animal house for two weeks for acclimatization. All the animal experiments were performed with Institutional Animal Ethics Committee approval (Approval No. ACRC/IAEC/15/02-(01)). The animal groups comprised six animals (having body weight 188.6 ± 7.3 g) each in the following manner:

Group I: Normal rats (Control).

Group II: Paracetamol (APAP) alone treatment group (2.5 g/kg) (negative control) (dose was selected as per the report of Okokon et al. [39]).

Group III: Silymarin (100 mg/kg) (dose selected as per the study of Saidurrahman et al. [40]) + APAP (2.5 g/kg) (positive control).

Group IV: *Annona muricata* low-dose (AML) (lyophilized powder 1 g/kg) + APAP (2.5 g/kg).

Group V: *Annona muricata* high-dose (AMH) (lyophilized powder 2 g/kg) + APAP (2.5 g/kg).

All the compounds, including APAP, silymarin, and *Annona muricata* fruit pulp, were dissolved in water at a concentration of 100 mg/mL and administered orally as described above.

The animals were initially treated orally by respective drug regimens for 14 days (without APAP) and on the morning of the 15th day, all animals except group I received 2.5 g/kg APAP (oral). The animals were euthanized after 24 h and the liver and blood were collected for biochemical and histological analysis. The serum was separated by centrifuging at 1500× *g* for 15 min.

### 2.6. Biochemical Parameters of Hepatotoxicity and Histological Analysis

The serum was analyzed for aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin, protein, and albumin contents using Span Diagnostic Kits, according to the prescribed methods. A portion of liver tissue was homogenized (25%) in tris buffer (0.1 M). The clear supernatant was prepared by centrifugation at 4 °C for 15 min at 12,000× *g* and it was used for the assay of reduced glutathione (GSH) content [41] and activities of glutathione peroxidase (GPx) [42], catalase [43], and superoxide dismutase (SOD) [44]. The advanced state of lipid peroxidation was quantified following the method by Buchner et al. [45]. The remaining tissue was fixed in buffered formalin and assessed for histological change using hematoxylin and eosin staining.

### 2.7. Statistical Analysis

Results were tabulated using Microsoft Excel and the values are indicated in the format mean ± SD. The significance of variation was analyzed using one-way ANOVA followed by Tukey HSD test using GraphPad Prism ver. 7.0. The statistical notations used were * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ in comparison with the normal group, whereas # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$ vs. APAP group. The complete statistical analyses are given in Supplementary Tables S1 and S2.

## 3. Results

### 3.1. Estimation of Total Flavonoids and Total Phenols

The total flavonoid content was estimated to be 0.837 ± 0.011 mg equivalent of quercetin/gm of *Annona muricata* fruit pulp and the total phenol content was 0.179 ± 0.003 mg equivalent of gallic acid/gm of fruit pulp (Table 1).

**Table 1.** The quantitative phytochemicals of *A. muricata* fruit pulp.

| Phytochemical | Quantity (mg Equivalent/g Lyophilized Fruit Pulp) |
|---|---|
| Total polyphenol content | 0.837 ± 0.011 |
| Total flavonoid content | 0.179 ± 0.003 |

### 3.2. In Vitro Radical Quenching Properties

The in vitro radical quenching ability is indicated in Table 2. The DPPH radical quenching was reported with an IC50 value of 19.17 ± 0.34 µg/mL. The ABTS scavenging potential was indicated to have an IC50 value of 16.64 ± 0.41 µg/mL. The ferric reducing antioxidant power was expressed as EC50 value at 6.11 ± 0.23 µg/mL.

**Table 2.** The radical quenching abilities of *A. muricata* fruit pulp lyophilized powder.

| Assay | Value (µg/mL) |
|---|---|
| DPPH assay | 19.17 ± 0.34 |
| ABTS assay | 16.64 ± 0.41 |
| FRAP assay | 6.11 ± 0.23 |

### 3.3. Aspartate Transaminase Activity

The AST activity was found to be 64.74 ± 8.4 U/L in the untreated rats. As a response to APAP treatment, the APAP-treated animals had elevated AST activities (101.65 ± 9.2 U/L). In pre-treated groups fed with fruit pulp, there was a reduction in the activities of AST to 75.42 ± 3.3 U/L and 78.66 ± 5.3 U/L, respectively, in the AML and AMH groups. The silymarin administration resulted in a significant reduction in AST activity to 70.42 ± 4.5 U/L in these animals (Table 3).

**Table 3.** The hepatotoxicity markers of rats exposed to APAP-induced toxicity and the protective effect of different treatments.

| Parameter | Treatment Groups | | | | |
|---|---|---|---|---|---|
| | **Normal** | **APAP Alone** | **APAP+ Silymarin** | **APAP+ AML** | **APAP+ AMH** |
| AST (IU/L) | 64.74 ± 8.4 | 101.65 ± 9.2 *** | 70.42 ± 4.5 ### | 75.42 ± 3.3 ***, ### | 78.66 ± 5.3 ***, ### |
| ALT (IU/L) | 67.51 ± 4.5 | 113.59 ± 5.9 *** | 91.19 ± 3.9 ***, ### | 80.33 ± 6.2 ***, ### | 73.31 ± 5.0 ### |
| ALP (IU/L) | 48.9 ± 5.1 I | 72.12 ± 6.2 *** | 53.22 ± 1.98 ### | 64.13 ± 3.7 ***, ## | 58.98 ± 5.1 ***, ### |
| Total protein (g/dL) | 7.08 ± 0.42 | 5.84 ± 0.21 $^{ns}$ | 6.98 ± 0.45 $^{ns}$ | 6.72 ± 0.36 $^{ns}$ | 5.48 ± 0.39 $^{ns}$ |
| Total bilirubin (mg/dL) | 0.29 ± 0.08 | 1.34 ± 0.11 $^{ns}$ | 0.68 ± 0.10 $^{ns}$ | 0.77 ± 0.09 $^{ns}$ | 0.71 ± 0.14 $^{ns}$ |
| Albumin (g/dL) | 5.26 ± 0.29 | 1.19 ± 0.12 $^{ns}$ | 5.21 ± 0.13 $^{ns}$ | 5.03 ± 0.24 $^{ns}$ | 5.24 ± 0.23 $^{ns}$ |

(*** $p < 0.001$ vs. control (normal) group; ## $p < 0.01$ and ### $p < 0.001$ vs. APAP group, $^{ns}$ not significant).

### *3.4. Alanine Transaminase Activity*

The activity of ALT in the normal rats was estimated to be 67.51 ± 4.5 IU/L (Table 3). However, treatment with APAP induced a marked elevation in the values to 113.59 ± 5.9 IU/L. Further, the administration of low (AML) and high doses (AMH) of lyophilized fruit powder restored the values of ALT to 91.19 ± 3.9 IU/L and 80.33 ± 6.2 IU/L, respectively. This reduction was further augmented in the silymarin treatment (73.31 ± 5 IU/L).

### *3.5. Alkaline Phosphatase Activity*

The concentration of ALP had a significant rise in the APAP group (72.12 ± 6.2 IU/L) when compared to the normal group (48.9 ± 5.1 IU/L). Pre-treatment with silymarin lowered ALP activity (53.22 ± 1.98 IU/L), while the AML and AMH groups produced a decrease in ALP activities of 58.98 ± 5.1 IU/L and 64.13 ± 3.7 IU/L, respectively (Table 3), showing moderate restoration towards the level of normal rats (48.9 ± 5.1 IU/L).

### *3.6. Serum Total Protein*

In normal rats, total protein level was 7.08 ± 0.42 g/dL, while APAP alone treatment resulted in a low total protein content of 5.84 ± 0.21 g/dL. However, the treatment with AML and AMH resulted in protein levels of 6.72 ± 0.36 g/dL and 5.48 ± 0.39 g/dL. Likewise, the level was 6.98 ± 0.45 g/dL in the silymarin treatment group (Table 3).

### *3.7. Serum Bilirubin*

In normal rats, total bilirubin level was 0.29 ± 0.08 mg/dL, while APAP treatment resulted in a low total bilirubin level of 1.34 ± 0.11 mg/dL. However, the treatment with AML and AMH resulted in total bilirubin levels of 0.77 ± 0.09 mg/dL and 0.71 ± 0.14 mg/dL. Likewise, the level was 0.68 ± 0.10 mg/dL in the silymarin treatment group (Table 3).

### *3.8. Serum Albumin*

The normal group showed a serum albumin level of 5.26 ± 0.29 g/dL, and it was markedly low in the APAP alone treatment group (1.19 ± 0.12 g/dL). The animals administered with silymarin, as well as the AML and AMH group, showed improved levels of serum albumin (5.21 ± 0.13, 5.24 ± 0.23, and 5.03 ± 0.24 g/dL, respectively).

### *3.9. Effect of Annona Muricata Lyophilized Fruit Pulp Powder on Tissue Antioxidant Profile*

The indicators of liver tissue antioxidant status, including superoxide dismutase activity, level of non-enzymatic antioxidant glutathione, glutathione peroxidase level, and catalase level, were found to be reduced in the APAP group when compared to the normal animal group. Silymarin-treated groups also showed significantly high levels of these parameters. In response to drug treatment, the above parameters showed a significant increase in a dose-dependent manner (Figure 1).

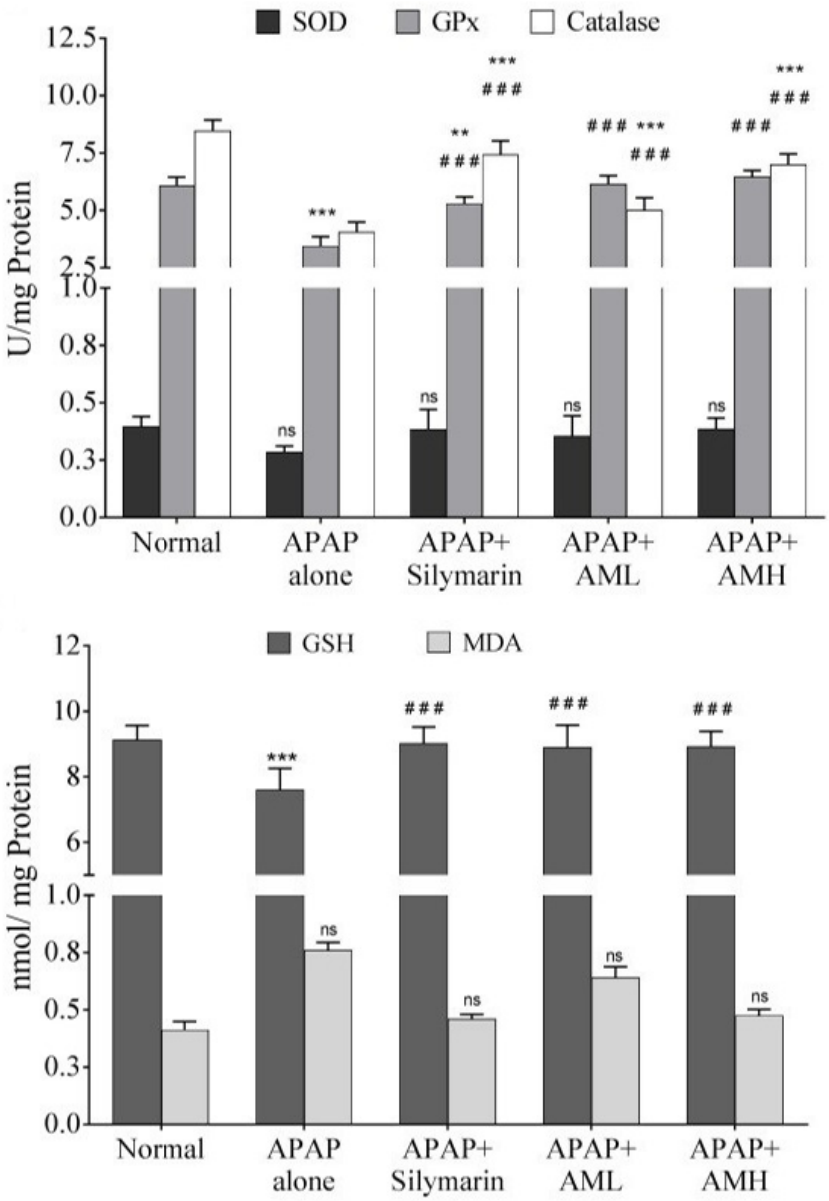


**Figure 1.** Effect of *A. muricata* lyophilized fruit pulp powder on liver antioxidant status. Values are expressed as mean ± SD for 6 animals (** $p < 0.01$ and *** $p < 0.001$ vs. control (normal) group; ### $p < 0.001$ vs. APAP group, ns indicate not significant).

The level of lipid peroxidation in the liver tissue was assessed in terms of MDA production. The level was high in the APAP alone treatment group (0.759 ± 0.036 U/L), in comparison to the normal group (0.412 ± 0.038 U/L), which indicated a high extent of tissue lipid peroxidation. The lyophilized fruit pulp powder pre-treatment reduced the extent of lipid peroxidation in liver tissue, in spite of APAP treatment, as evident from the MDA levels

formed in AML and AMH groups (0.474 ± 0.029 U/L and 0.640 ± 0.048 U/L, respectively). Results were comparable to that in the silymarin-treated animals (0.459 ± 0.022 U/L).

### *3.10. Histopathology*

As revealed from the histopathological studies, profound liver damage was noticed in the APAP alone group without any supplementary treatment. The liver architecture showed necrosis in several areas as well as ballooning of hepatocytes, as compared to the normal hepatic architecture of the normal group animals which is seen in Figure 2. However, AML and AMH groups seemed to withstand APAP-induced hepatic parenchyma damage, as evidenced by the histopathological figures showing fewer or none of such changes (Figure 2). The grading of histological changes was measured as indicated in Table 4 in accordance with the grading scheme proposed by Kleiner et al. [46] and modified by Liang et al. [47].

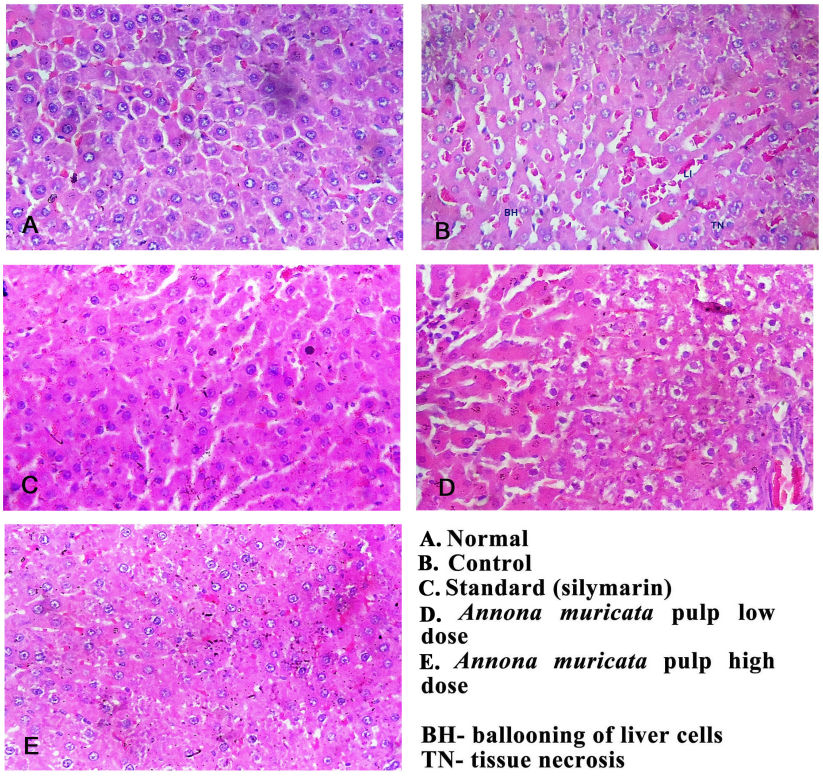


**Figure 2.** Effect of *A. muricata* lyophilized fruit pulp powder on histology of liver tissue in APAP-induced damage animals (the images were taken at a magnification of 250× using Magnus INVI microscope).

**Table 4.** Grading of liver histological changes in APAP and different treatment groups.

| Parameter | Normal | Paracetamol | Silymarin | AML | AMH |
|---|---|---|---|---|---|
| Hepatocellular ballooning | 0 | 2 | 1 | 1 | 1 |
| Macrovesicles | 0 | 3 | 1 | 2 | 1 |
| Microvecicles | 0 | 3 | 2 | 2 | 2 |
| Portal tract inflammation | 0 | 1 | 0 | 0 | 0 |

Grades 0, 1, 2, and 3 indicate, no damage, mild damage, moderate damage, and severe damage, respectively.

## 4. Discussion

Drug-induced non-alcoholic fatty liver diseases are a major threat to the management of hepatic health [48]. Among various drugs, paracetamol (acetaminophen) is a widely used analgesic and antipyretic drug [49,50]. Upon activation by the hepatic microscomal CYP-450 enzymes, acetaminophen is converted to *p*-aminophenol [51]. The compound induces oxidative insult and subsequently induces hepatic dysfunction. The use of APAP, therefore, is an issue in long-term treatment. However, studies have indicated that antioxidant compounds, especially plant-derived antioxidants, are highly useful in the prevention of drug-induced hepatic dysfunction. The compounds isolated from *Silibum marianum* such as silibinin and silymarin are well-known hepatoprotective agents [52]. Compared to general phytochemicals, functional foods are also gaining attention as hepatoprotective agents against drug-induced hepatic dysfunction [53]. The fruits belonging to the *Annonaceae* family are major functional foods and low-cost nutritive alternatives [21]. Hence, the present study evaluated the potential of *A. muricata* lyophilized fruit pulp powder against APAP-induced acute hepatic dysfunction. The results indicated the presence of polyphenol compounds and flavonoids in the lyophilized fruit pulp. Previous studies have confirmed the presence of bioactive metabolites such as acetogenins and flavonoids such as quercetin, kaempferol, myricetin, etc. in *A. muricata* fruit pulp [54,55].

In the present study, a significant elevation in the serum levels of AST and ALT was observed. These enzymes are markers of hepatic toxicity induced by various chemicals and toxicants. A report by Kim et al. [56] has indicated that the liver toxicity markers AST and ALT vary significantly in acute liver injury. According to their reports, AST rose rapidly with acute liver injury and the ALT level steadily increased over time, reaching similar levels to AST by 24–48 h. However, in chronic liver injury, the ALT level tends to rise and reach high AST/ALT ratios during fibrosis and cirrhosis [57,58]. Hence, the observed increase in AST and ALT values over 24 h of APAP administration in rats possibly indicates the development of acute hepatotoxicity. Likewise, the serum activity of alkaline phosphatase enzyme was also elevated in APAP administration; the ALP activities are reported to be elevated in acute hepatic damage and thereby act as an indicator of hepatic dysfunction [59]. Further, the treatment with the low and high doses of *A. muricata* lyophilized fruit pulp powder restricted these elevations in liver function markers in the corresponding animal groups. The reduced levels of AST, ALT, and ALP are therefore indicative of the prevention of APAP toxicity in rats. In agreement with the changes in liver function enzymes, there were increased levels of total bilirubin in APAP-treated rats.

The rise in AST and ALT in the serum is also reported to be due to the necrotic damage of hepatic cells [60]. Further, hepatic oxidative stress and inflammatory cytokines drive the hepatocyte necrosis and subsequent release of these transaminases [61]. Hence, the study went on to evaluate the changes in hepatic redox balance markers including enzymatic and non-enzymatic antioxidants as well as lipid peroxidation. The results indicated a significant reduction in the level of reduced glutathione and enzymatic antioxidants in the APAP treatment. These results corroborate the previous studies of Zakaria et al. [62] and Hota et al. [63], where a similar reduction in enzymatic and non-enzymatic antioxidants was observed. However, the pre-treatment of *A. muricata* lyophilized fruit pulp powder significantly inhibited the redox imbalance induced by the acute exposure to APAP. The level of glutathione was restored to normal and activities of enzymatic antioxidants such as SOD, catalase, and GPx were also reinstated. Glutathione is the central antioxidant molecule that works in association with the dependent enzymes GPx and glutathione-s-transferase in detoxification of xenobiotic compounds and free radicals [64]. Likewise, superoxide dismutase reduces superoxide radicals to peroxides and catalase and GPx enzymes detoxify peroxide moieties to water molecules [65]. Corroborating the alterations in the antioxidant defense systems, elevation in the MDA levels was observed upon administration of APAP. This elevation in MDA levels was previously reported in APAP-induced hepatic dysfunction [66,67]. Overall, the restoration of these antioxidant parameters is thus indicative

of the restoration of redox balance and alleviation of APAP-induced acute liver injury in *A. muricata* pulp-administered rats.

Corroborating these changes in biochemical parameters, a significant change in the hepatic architecture of APAP-administered rats was observed. The predominant changes include hepatocellular ballooning and the formation of micro- and macrovesicles in the hepatocytes. However, pre-treatment with the different doses of *A. muricata* pulp powder significantly reduced these histological changes and thus confirmed the protective effect against APAP-induced acute liver injury. Histological changes in APAP treatment, including hepatocellular ballooning, vesicle formation, and inflammatory cell infiltration, were previously reported [68–70]. Hence, the prevention of the histological changes is thus suggestive of the protective effect of the treatment.

In connection with these results, a previous study on *Annona muricata* leaves showed a reduction of the hepatotoxic effect of APAP and carbon tetrachloride by effectively regulating the redox imbalance [24]. Likewise, the stem bark extract of *A. muricata* was also found to inhibit CCl4-induced hepatotoxicity by restoring the levels of hepatotoxicity markers [25]. Further, the leaf extract was also effective in preventing the hepatotoxic effect of thioacetamide in rats [26]. The commercially available Graviola dry extract was also effective in preventing hepatotoxicity induced by monosodium glutamate, a flavor enhancer [27]. Likewise, the dried leaf extract of *A. muricata* prevents diabetes-associated hepatic dysfunction in rats [28]. Besides these, a study by Oyebamiji et al. [71] has indicated that compounds of *Annona muricata* seeds can inhibit the cytochrome P450 enzymes associated with drug activation. Hence, it is also possible that the *A. muricata*-derived bioactive constituents may have inhibited the hepatic microsomal CYP450 enzymes and thereby resulted in reduced hepatotoxicity.

## 5. Conclusions

Overall, the results indicated a significant protective effect of the *Annona muricata* lyophilized fruit pulp powder against APAP-induced acute liver injury in rats. The effects are found to be mediated through the restoration of hepatic redox balance and subsequent maintenance of hepatic architecture in the treated rats. In addition, the possible ability of *A. muricata* fruit pulp in the modulation of cytochrome P450 enzymes is also expected. Hence, it is concluded that the lyophilized powder of *A. muricata* fruit pulp can be an effective functional food for the management of drug-induced hepatotoxicity and it can be suitable for storing for a longer period.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr11010276/s1, Material S1: Details of animal experimentation; Table S1: Statistical comparison details of Table 3; Table S2. Statistical analysis details of Figure 1 (antioxidant parameters).

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Centre (Approval No. ACRC/IAEC/15/02-(01)) and strictly followed the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division, Government of India.

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