Indian Journal of Experimental Biology
Vol. 61, November 2023, pp. 852-860
DOI: 10.56042/ijeb.v61i11.1326

Abelmoschus esculentus (L.) Moench seed extract alleviates acute acetaminophen induced liver damage in rats

Huseyin Kocabey[1], Mervan Bekdas[1]*, Ayhan Cetinkaya[2], Selma Erdogan Duzcu[3], Murat Alisik[4], Gokce Kaya Dincel[1] & Idris Turel[5]

[1]Department of Pediatrics; [2]Department of Physiology; [3]Department of Pathology; [4]Department of Medical Biochemistry; [5]Department of Pharmacology, Abant Izzet Baysal University Medical Faculty, Bolu, Turkey

Received 26 December 2022; *Revised 10 May 2023*

Acetaminophen (N-acetyl-p-aminophenol, APAP), commonly called the 'paracetamol', is one of the most regularly utilized medicines, particularly in children. When administered at the recommended doses, it is a safe medication. However, oxidative stress and inflammation caused by exposure to toxic doses lead to centrilobular hepatic necrosis. N-acetylcysteine (NAC) is utilized in the therapy, however it has potential adverse effects. On the other hand, it is known that the seeds of the common vegetable Okra, *Abelmoschus esculentus* (AE), a herbal product, possess antioxidant and anti-inflammatory qualities. In the present study, we explored whether AE can be used as an alternative to standard NAC therapy without any adverse effect in the treatment of acute APAP induced liver injury. Forty male Wistar rats were placed into five groups: Control, AE, APAP, APAP+AE and APAP+AE+NAC groups. Antioxidants such as native thiol and total thiol were found rised in the APAP group by adding AE (p =0.043 and p =0.028, respectively). Anti-inflammatory indicator IL-10 was also found increased, while marker ALT, which is a sign of hepatotoxicity, got decreased (P=0.005 and P <0.001, respectively). Histologically, AE has been shown to improve worsened congestion (P =0.003), cytoplasmic vacuolization (P =0.01), sinusoidal dilatation (P =0.001), Kupffer cell proliferation (P <0.001) and inflammation (P <0.001). These results suggest that the okra seeds may be a potential therapeutic agent for paracetamol induced hepatotoxicity and it can be attributed to the antioxidant and anti-inflammatory properties.

Keywords: N-acetyl cysteine (NAC), Hepatic injury, Interleukin-10, Okra, Paracetamol, Reactive oxygen species (ROS), Thiol

Acetaminophen (N-acetyl-p-aminophenol, APAP), commonly known as 'paracetamol', is one of the most common medicines used in childhood. It is a safe medicine when taken at recommended doses, but more than 200 mg/kg in a single dose or more than 90 mg/kg/day for consecutive days is considered as a toxic dose[1,2]. The liver conjugates the bulk of ingested APAP; 5% is eliminated unaltered in the urine; and 5% is transformed to N-acetyl p-benzoquinonimine (NAPQI). NAPQI is eliminated by the kidneys after being transformed to mercapturic acid by glutathione. When therapeutic amounts of APAP are applied, the resultant NAPQI is detoxified by glutathione peptide. However, when taken in dangerous levels, it can cause cetrilobular liver necrosis. Acute liver injury caused by APAP consumption has been seen in the third stage of intoxication, between 72 and 96 h[2]. In addition to NAPQI, oxidative stress caused by increased reactive oxygen species (ROS) also contributes to necrosis[3].

Initial signs of APAP intoxication are nonspecific or nonexistent. If a hazardous dose of ingestion is suspected, serum APAP should be tested after 4 h at the earliest. The serum APAP level obtained during the first 4 h cannot be utilized to predict toxicity. The treatment decision in these patients should be based on where the serum APAP falls on the Rumack-Matthew nomogram. This nomogram is used to evaluate the risk of hepatotoxicity; nonetheless, it is advised to wait at least 4 h before beginning antidote medication in accordance with this nomogram[2,4].

By boosting NAPQI detoxification and avoiding hepatotoxicity, N-acetyl cysteine (NAC), a precursor to cysteine, refills the liver's depleted glutathione pool[4] NAC, on the other hand, can cause nausea, vomiting, rash, fever, headache, hypotension, and anaphylactoid reactions[2,5,6].

Abelmoschus esculentus (AE) has a lot of vitamins, minerals, and fiber. It has been already reported that AE, among other things, possesses antioxidant and

*Correspondence:
Telefax: +90 374 2534656
E-Mail: merbek14@yahoo.com
ORCID: 0000-0003-2469-9509)

anti-inflammatory properties[7]. In this study, we have explored whether AE can be used as an alternative to standart NAC therapy in the treatment of acute APAP induced liver injury without any adverse effects.

Material and Methods

Preparation of AE seed extract

The Aydın region, which has a pleasant, temperate temperature, is where AE was raised. The fruit was picked when it was 3.5-7 cm long, and each hectare produced 10.000 kg. This study made use of AE seed. AE seed was extracted using the maceration process and 95% ethanol as the solvent[8]. Then, 100 g of AE seeds that had been pulverized were put in a tight container. The container was filled with 600 mL of ethanol, and it was left to sit at 25°C for two days in the dark. The extract combination was filtered two days later, and the liquid component (ethanol) was put into a flask with a circular bottom. An oily golden yellow AE extract was produced in the flask following solvent evaporation at 40°C, and it was kept there at 4°C until it was tested.

Animals

The Bolu Abant Izzet Baysal University Experimental Animals Application and Research Center sold male Wistar rats weighing 200-250 g. Rats were fed a conventional meal and given unlimited access to water in a standard laboratory setting. Temperature, humidity, and lighting were all set at a constant 19±2°C (humidity at 50-70%) with a 12 h light/dark cycle. All surgical operations and the care of the rats were designed in accordance with the Universal Declaration on the Rights of Animals.

Experimental design and protocols

Five equal groups made up of the 40 rats were created randomly. The following experimental model was developed: Control group (C): An identical volume of saline was administered by gavage for 11 days; AE group (AE): A single dosage of 600 mg/kg/day of AE extract was administered by gavage for 11 days[9]; APAP group (APAP): On the eighth day of the trial, a single intraperitoneal (IP) dosage of APAP 1 g/kg (Parol 10 mg/mL Atabay®, Istanbul, Turkey) was given. (The supplied dosage of APAP is regarded as hepatotoxic[10]); AE+APAP group (AE+APAP): 600 mg/kg/day single dose of AE extract was given by gavage for one week. On the eighth day, 1.5 h after the AE extract was given, a single dose of 1 g/kg APAP was given IP. AE extract was continued to be given by gavage for 3 days after the procedure day; and AE+APAP+NAC group (AE+APAP+NAC): One week before to the procedure day, a single dosage of 600 mg/kg/day of AE extract was began to be administered by gavage. On the eighth day, 1 g/kg APAP was provided as a single dose IP 1.5 h after the same dosage of AE extract, and 1.5 h after APAP, 300 mg/kg NAC (Asist 300 mg/3 mL Bilim Pharmaceuticals®, Istanbul, Turkey) was given as a single dose IP[11]. For three days following the day of the treatment, AE extract was still administered by gavage.

On the 11th day, all rats were intramuscularly sedated with 90/10 mg/kg xylazine and ketamine before being sacrified. Biochemical analysis required the collection of blood samples. The blood samples were maintained in Eppendorf tubes at −80°C until they were examined after being centrifuged at 4000 rpm for 10 min. The rats' abdomens were opened, and the livers were taken out. After that, they were embedded in paraffin blocks and preserved in 10% buffered formaldehyde for histological analysis. In a sterile laboratory setting, all surgical operations were carried out.

Measurement of biochemical parameters

For signs of liver injury, serum AST and ALT levels were checked[12]. We acquired rat AST and ALT ELISA kits from SinoGeneClon Biotech Co. Ltd. in China.

Serum samples were examined for levels of the pro-inflammatory cytokine TNF-α and the anti-inflammatory cytokine IL-10[13]. We obtained rat cytokine ELISA kits from SinoGeneClon Biotech Co. Ltd. in China.

The colorimetric technique established by Erel & Neselioglu[14] was used to measure the levels of serum thiol-disulfide, including native thiol, total thiol, and disulfide, as an indication of oxidant-antioxidant status.

Histopathological assessment of liver specimens

Hematoxylin-eosin dye was used to stain paraffin blocks with slices of 3 m thickness cut from animal liver for histological analysis. A skilled pathologist examined the sections with an LEICA DM 2000 LED light microscope. These significant indications of liver tissue injury, including vascular congestion, localized hepatocyte necrosis, inflammation, bile duct hyperplasia, cytoplasmic vacuolization, sinusoid dilatation, and Kuppfer cell proliferation, were scored semi-quantitatively. According to test results, a score of 0 indicates no change, a score of 1 indicates minor

focal changes, a score of 2 indicates moderate multifocal changes, a score of 3 indicates noticeable multifocal changes, and a score of 4 indicates common substantial alterations[15].

Statistical analysis

For statistical analysis and assessment, the SPSS-23 software was employed. The mean and standard deviation were used to express the data. The examination of the groups' fit to a normal distribution and variance homogeneity was conducted using the Kolmogorov-Smirnov test. Tukey tests were used to compare data from different experimental groups after one-way analysis of variance (ANOVA) was provided. The difference in histopathology scores was assessed using a Kruskal-Wallis analysis The Mann-Whitney U test was used to determine if the mean differences between groups Awere significant. A value of P <0.05 was considered significantly different.

Results

Biochemical analysis

The groups' natural thiol levels were found to be significantly different from one another (P <0.001). Although the native thiol levels in the APAP group were found to be lower than in the C group, this difference was not statistically significant (P =0.65). Despite being higher than in the APAP group, the native thiol levels in the prophylactic group with specified AE were not significantly different from those in the APAP group (P =0.59). Comparing the APAP group to the APAP+AE and APAP+AE+NAC groups, statistically significant increases in native thiol levels were observed (P <0.05 and P <0.001, respectively). When it came to raising native thiol levels in rats exposed to APAP, there was no discernible difference between APAP+AE and APAP+AE+NAC combinations (P =0.99) (Table 1).

The total thiol levels for the groups showed a statistically significant difference (P <0.001). Although the total thiol levels in the APAP group were lower than those in the C group, this difference was not statistically significant (P =0.64). The prophylactic group with provided AE had higher total thiol levels than the APAP group, although this difference was not statistically significant (P =0.57). Comparing the APAP group to the APAP+AE and APAP+AE+NAC groups revealed statistically significant increases in total thiol levels (P =0.028 and P =0.001, respectively). There was no significant difference between APAP+AE and APAP+AE+NAC combinations increasing total thiol levels in rats exposed to APAP (P =0.91) (Table 1).

The AST levels of the groups were observed to differ significantly (p=0.022). Although there was a higher concentration of AST in the APAP group compared to the C group, this difference was not statistically significant (P =0.57). Although the AST readings were lower in the group that received AE as a preventive measure than in the APAP group, this difference was not statistically significant (P =0.97). The difference in AST values between the APAP+AE and APAP groups was statistically insignificant (P =0.072), whereas the difference between the APAP and the APAP+AE+NAC groups was statistically significant (P =0.044). In rats exposed to APAP, there was no discernible difference in the lowering of AST levels between APAP+AE and APAP+AE+NAC combinations (P =0.99) (Table 1).

In terms of ALT levels, there was a significant difference between the groups (P <0.001). In comparison to the C group, the APAP group had higher ALT readings (P =0.022). ALT readings were lower in the group that received AE as a preventative measure than in the APAP group, although this difference was not statistically significant (P =0.054). When compared to the APAP group, the ALT levels in the APAP+AE and APAP+AE+NAC groups were statistically substantially lower (P <0.001 and P =0.001, respectively). In rats exposed to APAP,

Table 1 — Comparison of biochemical data between the groups

	C	APAP	AE	AE+APAP	AE+APAP+NAC	*P*
NThiol (µmol/L)	138.8±20	121.1±15.1	140.1±36.5	162.6±35.2	206±16.1	<0.001
TThiol (µmol/L)	206.2±24.8	186.7±16	207.9±34.3	237.1±23.6	250.2±31.1	0.001
Dis (µmol/L)	30±12.1	28.4±3.4	34.6±3.3	34.9±4.3	36±2.6	0.058
AST (IU/L)	160.1±33.2	191.7±78.4	179±21	132.1±20.9	127.2±16.8	0.022
ALT (IU/L)	65.5±9.4	82.2±7.4	67±12.4	51.7±4.7	58.8±12	<0.001
TNF-α (pg/mL)	168±37.8	173.3±63.4	151.9±17.2	139.5±17.7	134.6±22.4	0.16
IL-10 (pg/mL)	62.2±1.1	61.9±1.3	63.6±3.7	70±5.7	71±5.6	<0.001

[APAP, Acetaminophen; AE, AbelmoschusEsculentus; NAC, N-acetylcysteine; NThiol, Nativethiol; TThiol, Totalthiol; Dis, Disulfide]

there was no discernible difference in the lowering of ALT levels between APAP+AE and APAP+AE+NAC combinations (P =0.63) (Table 1).

Regarding IL-10 levels, there was a considerable variation between the groups (P <0.001). In terms of IL-10 readings, there was no discernible difference between the APAP group and the C group (P =0.99). IL-10 levels were lower in the group that received AE as a preventative measure than they were in the APAP group, although this difference was not statistically significant (P =0.93). When compared to the APAP group, the IL-10 levels in the APAP+AE and APAP+AE+NAC groups were statistically substantially higher (P =0.005 and P=0.002, respectively). In rats exposed to APAP, there was no discernible difference between APAP+AE alone and APAP+AE+NAC combos in terms of raising IL-10 levels (P = 0.98). (Table 1).

There was no discernible difference between groups in terms of disulfide or TNF-α levels (P =0.058 and P =0.18, respectively) (Table 1).

Histopathological analysis

There was a substantial difference between the groups' vascular congestion scores (P <0.001). In comparison to the C group, the APAP group had greater congestion scores (P =0.001). (Fig. 1 A-C). Congestion scores were statistically substantially lower in the group that received AE as a preventative measure than in the APAP group (P =0.012). The congestion scores in the APAP+AE and APAP+AE+NAC groups were statistically substantially lower than those in the APAP group (P =0.003 and P =0.003, respectively) (Table 2).

Regarding inflammatory scores, a substantial difference between the groups was discovered (P <0.001). When compared to the C group, the APAP group had greater inflammation scores (P <0.001). (Fig. 1 A, B and D). The group that received AE as a preventative measure had statistically substantially lower inflammation scores than the APAP group (P <0.001) (Fig. 1 E & F). The inflammatory scores in the APAP+AE and APAP+AE+NAC groups were considerably lower than those in the APAP group (P <0.001 and P <0.001, respectively) (Table 2) (Fig. 1 G, H, J & K).

In terms of cytoplasmic vacuolization scores, there was a substantial difference between the groups (P <0.001). In comparison to the C group, the APAP group had higher scores for cytoplasmic vacuolization (P =0.001). Cytoplasmic vacuolization scores were

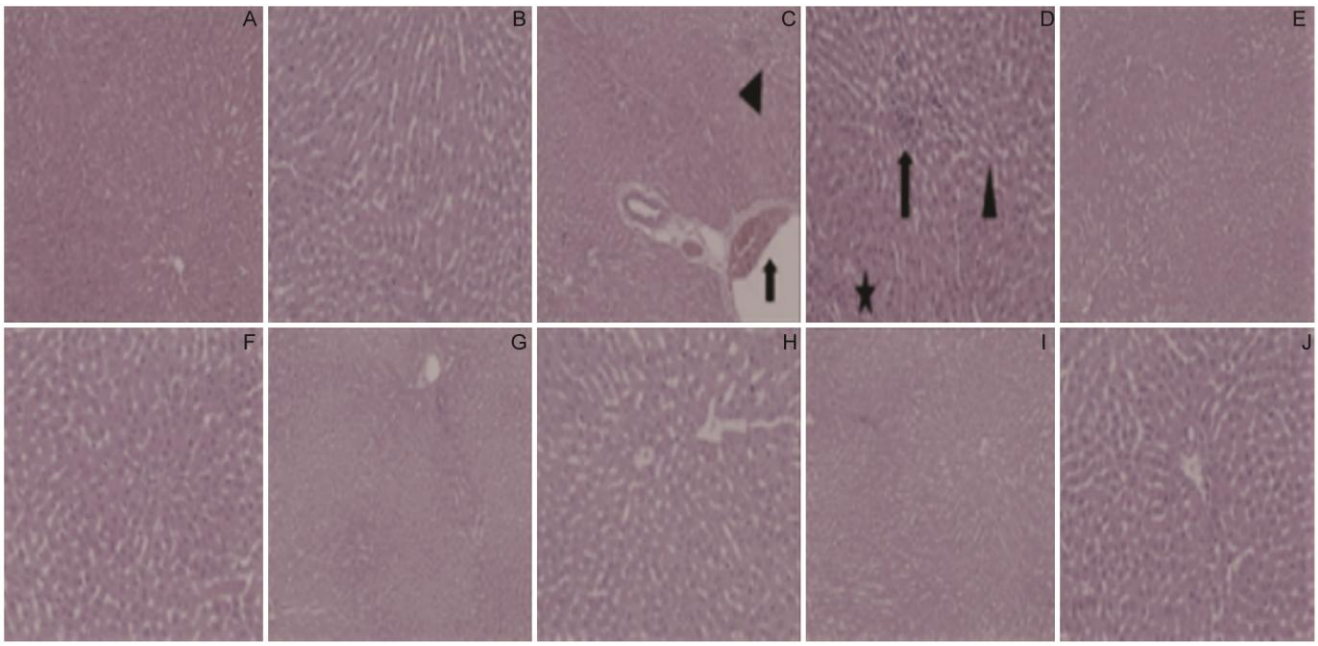

Fig. 1 — Histological changes of AE in APAP-induced hepatotoxicity. (A) Control group (HEX100); (B) Control group (HEX200); (C) APAP group vascular congestion (arrow), sinusoidal dilatation (arrow head) (HEX100); (D) APAP group focal hepatocyte necrosis, inflammation (arrow), sinusoidal dilatation (arrow head), Kupffer cell proliferation (asterisk) (HEX200); (E) AE group (HEX100); (F) AE group (HEX200); (G) APAP+AE group (HEX100); (H) APAP+AE group (HEX200); (I) APAP+AE+NAC group (HEX100); and (J) APAP+AE+NAC group (HEX200). [APAP Acetaminophen, AE *Abelmoschus esculentus*, NAC N-acetylcysteine, HEX Hematoxylin-eosin stain]

Table 2 — Comparison of histological scores between the groups

	C	APAP	AE	AE+APAP	AE+APAP+NAC	*P*
Vascular congestion	0.7±0.4	2.6±0.7	1.5±0.5	1.2±0.4	1.2±0.4	<0.001
Focal hepatocytenecrosis	0.2±0.4	1.1±0.8	0.8±0.3	0.6±0.5	0.6±0.5	0.088
Inflammation	0.7±0.4	2.1±0.3	0.8±0.3	0.7±0.4	1±0	<0.001
Bile ductus hyperplasia	0.2±0.4	0.2±0.4	0.2±0.7	0.2±0.4	0.1±0.3	0.96
Cytoplasmic vacuolization	0.6±0.5	2.5±0.5	0±0	1.6±0.5	1±0	<0.001
Sinusoid dilatation	1.1±0.3	2.2±0.4	0.8±0.3	0.8±0.6	1.2±0.4	<0.001
Kupffer proliferation	0.6±0.5	2.2±0.4	0.5±0.5	0.8±0.3	0.7±0.7	<0.001

[APAP, Acetaminophen; AE, *Abelmoschus esculentus*; NAC, N-acetylcysteine]

statistically substantially lower in the group administered AE as a preventative measure than in the APAP group (P <0.001). Cytoplasmic vacuolization scores were statistically substantially lower in the APAP+AE and APAP+AE+ NAC groups compared to the APAP group (P =0.01 and P <0.001, respectively) (Table 2).

Between the groups, there was a substantial difference in sinusoid dilatation scores (P <0.001). In comparison to the C group, the APAP group had greater sinusoid dilatation scores (P =0.001) (Fig. 1 A-D). Sinusoid dilatation scores were statistically substantially lower in the group that received AE as a preventative measure than in the APAP group (P =0.001). Sinusoid dilatation ratings were statistically substantially lower in the APAP+AE and APAP+AE+NAC groups than in the APAP group (P =0.001 and P =0.003, respectively) (Table 2).

The groups' Kupffer proliferation scores significantly differed from one another (P <0.001). In the APAP group, Kupffer proliferation scores were greater than in the C group (P <0.001). (Fig. 1 A, B and D). Kupffer proliferation scores were statistically substantially lower in the group receiving AE as a preventative measure than in the APAP group (P =0.001). The Kupffer proliferation scores in the APAP+AE and APAP+AE+NAC groups were statistically substantially lower than those in the APAP group (P <0.001 and P =0.001, respectively) (Table 2).

The scores for localized hepatocyte necrosis and bile duct hyperplasia did not significantly differ between the groups (P =0.088 and P =0.96, respectively) (Fig. 1 A-D) (Table 2).

Discussion

Acetoaminophen (APAP), an antipyretic and analgesic, is used frequently worldwide despite the fact that high dosages are thought to be hepatotoxic. Our current study has shown that *Abelmoschus esculentus* (AE), an antioxidant and anti-inflammatory, can be utilized to treat acute hepatotoxicity brought on by APAP's harmful side effects.

Certain patient features could overly raise the risk of hepatotoxicity. The most significant of them is the long-term use of various drugs and some diseases that induce the synthesis of CYP2E1, the enzyme responsible for the generation of N-acetyl *p*-benzo-quinonimine (NAPQI), or lengthen its half-life[16]. The depletion of glutathione reserves brought on by febrile illnesses or the interruption of oral intake brought on by gastroenteritis are additional variables that raise the risk of hepatotoxicity. All of these factors combined promote NAPQI production, hence the patient's anamnesis should be scrutinized for these characteristics. Hepatotoxicity caused by APAP is more likely to occur in regions with a relatively high concentration of the CYP2E1 enzyme in hepatocytes. This results in the APAP-specific centrilobular development of hepatocellular necrosis[17].

Hepatotoxicity is brought on by the buildup of NAPQI, an intermediary molecule that glutathione can detoxify. The APAP-protein adducts that cause APAP-induced hepatotoxicity bond covalently to the cysteine groups of the protein when the glutathione depositions in the cell are diminished. It is feasible that these interactions will cause the cell's mitochondria to dysfunction[18].

When triggered by antigens, defense-related cells such neutrophils, monocytes, and macrophages release ROS[19]. It is well known that liver injury results from inflammation brought on by oxidative stress brought on by an increase in ROS and a reduction in antioxidants[20]. Oxidative stress impairs cell membrane permeability, enzyme activities and DNA structure[21]. By raising ROS and lowering antioxidants, APAP is known to promote cell hepatotoxicity[22].

Native thiols, usually referred to as thiols, are crucial antioxidants that contain sulfhydryl groups.

Thiols are crucial in the destruction of ROS. Thiols provide hydrogen to create disulfide bonds under oxidative stress. The disulfides are reduced to their natural thiols when the oxidative stress has been eliminated. Total thiol consists of the sum of native thiols and disulfides. Normally, the ratio of thiols to disulfides is balanced. Thiol levels drop whereas disulfide levels rise with oxidative stress[23]. Oxidative stress was suggested as the cause of the drop in thiols following APAP exposure.

Glutathione, intracellular thiol, is one of the most important of the non-enzymatic antioxidants. Protecting cells from oxidative stress is the primary purpose of glutathione, which is a component of glutamic acid, cysteine, and glycine. The glutathione peroxidase enzyme breaks down one mole of hydrogen peroxide[24]. By replenishing glutathione deposition and scavenging ROS, NAC discovered that boosting the non-toxic sulfate conjugation of APAP in liver cells also has a powerful antioxidant activity. The APAP-related mortality is known to be reduced with the use of NAC in the treatment of patients[4]. It should be emphasized that NAC is a chemical compound with potential side effects for patients, in contradiction to the claimed function. Intriguingly, our findings revealed that using AE, without NAC, increased the antioxidant activity of the cell and would, thus have a therapeutic impact against the hepatotoxicity produced by APAP. AE is also well recognized for being high in antioxidative phenolic substances, including derivatives of quercetin, catechin oligomers, and derivatives of hydroxy-cinnamic acid. On the other hand, it is known that by boosting the synthesis of endogenous antioxidants, such as superoxide dismutase, catalase and glutathione peroxidase, AE exhibits its antioxidant action in hepatotoxicity[9].

One of the main mediators of liver injury is the proinflammatory cytokine TNF-α, which is largely generated by macrophages in the liver[25]. According to some research, APAP exposure increases the production of cytokines like TNF-α, which in turn induces hepatotoxicity. Conversely, reducing TNF-α expression can lessen hepatic injury[26]. On the other hand, it has been suggested that TNF-α can stimulate liver regeneration by increasing the sensitivity of hepatocytes to growth factors[27]. In our investigation, APAP induction resulted in a rise in TNF-α levels and AE administration resulted in a decrease in TNF-α levels, although these changes were not statistically significant. This result was attributed to the quality and storage conditions of the ELISA kit since inflammation identified histologically.

Interleukin (IL)-10, an anti-inflammatory and immunosuppressive cytokine released by diverse innate and adaptive immune cells, is another cytokine engaged in the inflammation. The majority of IL-10 in the peripheral blood circulation is produced by macrophages[28]. The anti-inflammatory function is activated with the induction of heme oxygenase-1 in the macrophages. IL-10's anti-inflammatory effects are mediated via heme oxygenase-1. As a result, during inflammation, the manufacturing of proinflammatory cytokines is inhibited, and antiapoptotic pathways are activated, which regulate tissue homeostasis[29]. It is widely acknowledged that hepatocyte injury increases the release of anti-inflammatory cytokines like IL-10, whose function is to preserve the liver[30]. AE seed also possesses anti-inflammatory properties in addition to its antioxidant properties. It is generally established that AE causes an increase in IL-10 secretion[13]. In our study, it has been demonstrated that just AE as well as AE+NAC raise IL-10 levels, an indicative of diminished anti-inflammatory effectiveness with APAP.

The liver sinusoid contains kupffer cells, which are the primary resident macrophages in the liver. Major sources of proinflammatory cytokines, which can harm the liver, include Kupffer cells[31]. Kupffer cells increased following APAP exposure, however in our research they were decreased by AE+NAC as well as AE alone. This change in Kupffer cells was evaluated as the therapeutic effect of AE. Kupffer cells that sensing to the danger signals release proinflammatory cytokines at an early stage[32]. After the first 24 h of the response, there are less Kupffer cells in the circulation, which leads to the release of anti-inflammatory cytokines rather than pro-inflammatory cytokines[33]. However, the number of them rises after 72 h[34]. Along with the aforementioned function, Kupffer cells also play a role in tissue healing and hepatic blood vessel repair by producing hypoxia-inducible factor-α[35].

The largest and most significant metabolic organ is the liver. Although there is no particular biomarker that can be used to quantify drug-induced liver injury, hepatotoxicity is often assessed by the serum AST and ALT levels[36]. Since hepatocytes contain the

majority of ALT, it is a more selective enzyme for liver injury[37]. The liver serves a variety of purposes, but it also plays a significant part in drug detoxification. As a function test for liver injury, biochemical parameters such as AST and ALT are employed in the diagnosis of several illnesses. It is generally established that elevated serum ALT and AST values are linked to liver tissue inflammation and injury. Administration of APAP to rats resulted in a rise in TNF-α, a reduction in IL-10, and an increase in liver enzymes. ALT has a longer half-life than AST, so AST gets eliminated from the bloodstream more quickly than ALT[37]. After APAP exposure, serum ALT values, one of the indications of hepatotoxicity, increased, but we found that when AE seed was administered to these rats alone, ALT values dropped significantly. When viewed in terms of ALT levels in hepatotoxicity, this data suggests that AE seed alone may be adequate to heal hepatic injury. We suggest that this could be due to the high concentration of polyphenolic chemicals found in AE seed.

One of the types of tissue stains used to identify the pathological alterations is hematoxylin-eosin. The rise in ALT levels in our study, together with liver injury signs such cytoplasmic vacuolization with indications of early hepatocyte death[38], sinusoid dilatation[39], the proliferation of Kupffer cells, and inflammation and congestion, showed that the APAP group had hepatotoxicity. Our findings were compatible with the literature[40]. It is said that AE has therapeutic effects against hepatotoxicity, a condition brought on by the many toxins[41]. In this investigation, the severity of histological liver degeneration, as measured by cytoplasmic vacuolization, sinusoid dilatation, Kuppfer proliferation, inflammation, and congestion, can be reduced by administering just AE as opposed to AE+NAC or NAC for treatment of APAP exposure. It is obvious that ALT lowering has a therapeutic effect in acute APAP hepatotoxicity and is linked with only AE without NAC.

Since AE is a herbal medication, it can be used as a therapy option even in the first four hours after APAP exposed, whether or not there has been intoxication. As a result, it is not necessary to check serum APAP levels or use the Rumack-Matthew nomogram.

Conclusion

The results of this study have demonstrated the benefits of okra (*Abelmoschus esculentus*) seed extract against acute acetaminophen (APAP) induced liver injury. Inflammation and oxidative stress have a substantial impact on the liver injury in such cases. It shows that the okra seed extract reduces the acute APAP induced oxidative and inflammatory liver tissue injury, and helps to maintain proper liver function. Further, it matches with the therapeutic effects of N-acetylcysteine, a typical drug in such cases. This study reveals that N-acetylcysteine's side effects are not a concern when treating these individuals with a herbal medicine, Abelmoschus esculentus extract. However, further human investigations will be needed before these findings can be applied clinically.

Acknowledgement

The authors thank Dr. Arzu Birinci Yıldırım for preparing *Abelmoschus esculentus* seed extract. This research was supported by the scientific research fund from the Abant Izzet Baysal University (2020.08.23.1469).

Ethical clearance

Our work was carried out with the approval of Abant Izzet Baysal University Animal Studies Ethics Committee (2020/19). Care of the rats were designed in accordance with the Universal Declaration on the Rights of Animals.

Conflict of Interest

Authors declare no competing interests.

References

1 Chidiac AS, Buckley NA, Noghrehchi F & Cairns R, Paracetamol (acetaminophen) overdose and hepatotoxicity: mechanism, treatment, prevention measures, and estimates of burden of disease. *Expert Opin Drug Metab Toxicol*, 19 (2023) 297.

2 Theobald J & Kostic M, Poisoning. In: *Nelson Textbook of Pediatrics.* (Eds. Kliegman R, Stanton B, St. Geme J & Schor N; Elsevier, Philadelphia), 2019, 2430.

3 Conde de la Rosa L, Goicoechea L, Torres S, Garcia-Ruiz C & Fernandez-Checa JC, Role of Oxidative Stress in Liver Disorders. *Livers*, 2 (2022) 283.

4 Licata A, Minissale MG, Stankevičiūtė S, Sanabria-Cabrera J, Lucena MI, Andrade RJ & Almasio PL, N-Acetylcysteine for Preventing Acetaminophen-Induced Liver Injury A Comprehensive Review. *Front Pharmacol*, 13 (2022) 828565.

5 Schwalfenberg GK, N-Acetylcysteine A Review of Clinical Usefulness (an Old Drug with New Tricks). *J Nutr Metab*, 2021 (2021) 1.

6 Šalamon Š, Kramar B, Marolt TP, Poljšak B & Milisav I, Medical and Dietary Uses of N-Acetylcysteine. *Antioxidants*, 8 (2019) 111.

7 Islam MT, Phytochemical information and pharmacological activities of Okra (*Abelmoschus esculentus*) A literature-based review. *Phytother Res*, 33 (2019) 72.

8 Tzanova M, Atanasov V, Yaneva Z, Ivanova D & Dinev T, Selectivity of Current Extraction Techniques for Flavonoids from Plant Materials. *Processes*, 8 (2020) 1222.

9 Xia F, Zhong Y, Li M, Chang Q, Liao Y, Liu X & Pan R, Antioxidant and Anti-Fatigue Constituents of Okra. *Nutrients*, 7 (2015) 8846.

10 Kouritas VK, Magkouta S, Zisis C, Psallidas I, Gourgoulianis KI & Kalomenidis I, Paracetamol and ibuprofen block hydrothorax absorption in mice. *Eur J Cardiothorac Surg*, 47 (2015) 426.

11 Nakhaee S, Dastjerdi M, Roumi H, Mehrpour O & Farrokhfall K, N-acetylcysteine dose-dependently improves the analgesic effect of acetaminophen on the rat hot plate test. *BMC Pharmacol Toxicol*, 22 (2021) 4.

12 Slika R, Acetaminophen. In: *Textbook of Clinical Pediatrics*, 2nd edn. (Eds. Elzouki A, Harfi H, Nazer H, Stapleton F, Oh W & Whitley R; Springer-Verlag, Germany), 2012, 2593.

13 Chen H, Jiao H, Cheng Y, Xu K, Jia X, Shi Q, Guo S, Wang M, Du L & Wang F, *In Vitro* and *In Vivo* Immunomodulatory Activity of Okra (*Abelmoschus esculentus* L.) Polysaccharides. *J Med Food*, 19 (2016) 253.

14 Erel O & Neselioglu S, A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem*, 47 (2014) 326.

15 Toledo-Pereyra LH, Rodrıguez FJ & Cejalvo D, Neutrophıl Infıltratıon As An Important Factor In Lıver Ischemıa And Reperfusıon Injury. *Transplantation*, 55 (1993) 1265.

16 Andrade RJ, Aithal GP, Björnsson ES, Kaplowitz N, Kullak-Ublick GA, Larrey D & Karlsen TH, EASL Clinical Practice Guidelines Drug-induced liver injury. *J Hepatol*, 70 (2019) 1222.

17 Guengerich FP, Cytochrome P450 2E1 and its roles in disease. *Chem Biol Interact*, 322 (2020) 109056.

18 Liao J, Lu Q, Li Z, Li J, Zhao Q & Li J, Acetaminophen-induced liver injury Molecular mechanism and treatments from natural products. *Front Pharmacol*, 14 (2023) 1122632.

19 Reshetnikov V, Hahn J, Maueröder C, Czegley C, Munoz LE, Herrmann M, Hoffmann MH & Mokhir A, Chemical Tools for Targeted Amplification of Reactive Oxygen Species in Neutrophils. *Front Immunol*, 9.(2018) 1827.

20 Ore A & Akinloye O, Oxidative Stress and Antioxidant Biomarkers in Clinical and Experimental Models of Non-Alcoholic Fatty Liver Disease. *Medicine*, 55 (2019) 26.

21 Hajam YA, Rani R, Ganie SY, Sheikh TA, Javaid D, Qadri SS, ramodh S, Alsulimani A, Alkhanani MF, Harakeh S, Hussain A, Haque S & Reshi MS, Oxidative Stress in Human Pathology and Aging Molecular Mechanisms and Perspectives. *Cells*, 11 (2022) 552.

22 Wong H-S, Dighe PA, Mezera V, Monternier P-A & Brand MD, Production of superoxide and hydrogen peroxide from specific mitochondrial sites under different bioenergetic conditions. *J Biol Chem*, 292 (2017) 16804.

23 Sener S, Akbas A, Kilinc F, Baran P, Erel O & Aktas A, Thiol/disulfide homeostasis as a marker of oxidative stress in rosacea a controlled spectrophotometric study. *Cutan Ocul Toxicol*, 38 (2019) 55.

24 Sarıkaya E & Doğan S, Glutathione Peroxidase in Health and Diseases. In: *Glutathione System and Oxidative Stress in Health and Disease*. (Ed. Bagatini MD; IntechOpen), 2020. https://www.intechopen.com/chapters/70955.

25 Binatti E, Gerussi A, Barisani D & Invernizzi P, The Role of Macrophages in Liver Fibrosis. New Therapeutic Opportunities. *Int J Mol Sci*, 23 (2022) 6649. doi: 10.3390/ijms23126649.

26 Ding Y, Li Q, Xu Y, Chen Y, Deng Y, Zhi F & Qian K, Attenuating Oxidative Stress by Paeonol Protected against Acetaminophen-Induced Hepatotoxicity in Mice. *PLoS ONE*, 11 (2016) e0154375.

27 Tao Y, Wang M, Chen E & Tang H, Liver Regeneration Analysis of the Main Relevant Signaling Molecules. *Mediators Inflamm*, 2017 (2017) 1.

28 Yeung ST, Ovando LJ, Russo AJ, Rathinam VA & Khanna KM, CD169+ macrophage intrinsic IL-10 production regulates immune homeostasis during sepsis. *Cell Rep*, 42 (2023) 112171.

29 Naito Y, Takagi T, Higashimura Y. Heme oxygenase-1 and anti-inflammatory M2 macrophages. *Arch Biochem Biophys*, 564 (2014) 83.

30 Gong L, Liao L, Dai X, Xue X, Peng C & Li Y, The dual role of immune response in acetaminophen hepatotoxicity Implication for immune pharmacological targets. *Toxicol Lett*, 351 (2021) 37.

31 Banerjee P, Gaddam N, Chandler V & Chakraborty S, Oxidative Stress–Induced Liver Damage and Remodeling of the Liver Vasculature. *Am J Pathol*, 193 (2023) 1400. doi 10.1016/j.ajpath.2023.06.002.

32 Laskin DL, Macrophages and Inflammatory Mediators in Chemical Toxicity A Battle of Forces. *Chem Res Toxicol*, 22 (2009) 1376.

33 Triantafyllou E, Pop OT, Possamai LA, Wilhelm A, Liaskou E, Singanayagam A, Bernsmeier C, Khamri W, Petts G, Dargue R, Davies SP, Tickle J, Yuksel M, Patel VC, Abeles RD, Stamataki Z, Curbishley SM, Ma Y, Wilson ID, Coen M, Woollard KJ, Quaglia A, Wendon J, Thursz MR, Adams DH, Weston CJ & Antoniades CG, MerTK expressing hepatic macrophages promote the resolution of inflammation in acute liver failure. *Gut*, 67 (2018) 333.

34 Graubardt N, Vugman M, Mouhadeb O, Caliari G, Pasmanik-Chor M, Reuveni D, Zigmond E, Brazowski E, David E, Chappell-Maor L, Jung S & Varol C, Ly6Chi Monocytes and Their Macrophage Descendants Regulate Neutrophil Function and Clearance in Acetaminophen-Induced Liver Injury. *Front Immunol*, 8.(2017) 626.

35 You Q, Holt M, Yin H, Li G, Hu CJ & Ju C, Role of hepatic resident and infiltrating macrophages in liver repair after acute injury. *Biochem Pharmacol*, 86 (2013) 836.

36 Kaur R, Kumar NR & Harjai K, Antioxidant potential and protective effects of bee pollen extract against Salmonella induced hepatic and renal toxicity in BALB/c mice. *Indian J Exp Biol*, 60 (2022) 432.

37 Chung J, Longo DM & Watkins PB, A Rapid Method to Estimate Hepatocyte Loss Due to Drug-Induced Liver Injury. *Clin Pharmacol Ther*, 105 (2019) 746.

38 Muhammad-Azam F, Nur-Fazila SH, Ain-Fatin R, Noordin MM & Yimer N, Histopathological changes of acetaminophen-induced liver injury and subsequent liver regeneration in BALB/C and ICR mice. *Vet World*, 12 (2019) 1682.

39 Rivera P, Pastor A, Arrabal S, Decara J, Vargas A, Sánchez-Marín L, Pavón FJ, Serrano A, Bautista D, Boronat A,

de la Torre R, Baixeras E, Lucena MI, de Fonseca FR & Suárez J, Acetaminophen-Induced Liver Injury Alters the Acyl Ethanolamine-Based Anti-Inflammatory Signaling System in Liver. *Front Pharmacol*, 8 (2017) 705.

40 Shalaby AS, Eid HH, El-Shiekh RA, Youssef FS, Al-Karmalawy AA, Farag NA, Sleem AA, Morsy FA, Ibrahim KM & Tadros SH, A Comparative GC/MS Analysis of Citrus Essential Oils Unveiling the Potential Benefits of Herb-Drug Interactions in Preventing Paracetamol-Induced Hepatotoxicity. *Chem Biodivers*, 9 (2023) e202300778.

41 Wahyuningsih SPA, Sajidah ES, Atika BND, Winarni D & Pramudya M, Hepatoprotective activity of okra (*Abelmoschus esculentus* L.) in sodium nitrite-induced hepatotoxicity. *Vet World*, 13 (2020) 1815.