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Seed and bark extracts of *Acacia catechu* protects liver from acetaminophen induced hepatotoxicity by modulating oxidative stress, antioxidant enzymes and liver function enzymes in Wistar rat model

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ABSTRACT

In this study we investigated the hepatoprotective effects and possible mechanism of *Acacia catechu* in acetaminophen (APAP) induced hepatotoxicity using female Wistar rat model. Hepatotoxicity was induced by oral administration of acetaminophen (750 mg/kg body weight) for 24 h. The seed (400 mg/kg body weight) and bark (400 mg/kg body weight) extract's treated groups exhibited hepatoprotective effects and was compared with well-known clinical anti-dote N-acetylcysteine (NAC). When groups treated with acetaminophen, significant increase of liver weight/body weight ratio, liver function enzymes such as alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) and decrease of antioxidant enzymes such as glutathione (GSH) and superoxide dismutase (SOD) were observed. The histopathology of APAP treated groups also showed moderate degree of sinusoidal congestion, centrilobular necrosis with polymorph nuclear cells infiltration, marked vacuolations and congestion. However, pretreatment with seed or bark extract groups decreased LPO accumulation, reduced the liver function enzymes and increased antioxidant defense enzymes. Moreover, histopathology of seed extract treated groups showed normal architecture whereas bark extract treated groups exhibited mild degree of vacuolations in the hepatocytes with minimal sinusoidal congestion. Taken together, our study concludes that *A. catechu* seed extract to be a more promising agent for protecting liver from APAP induced hepatotoxicity.

1. Introduction

The liver is the vital organ that performs essential functions including detoxification of deleterious materials. It regulates numerous metabolic functions and maintains body homeostasis [1]. Therefore, maintaining liver in healthy condition is essential for normal physiological function. Drugs are an important cause of hepatic injury and a significant clinical problem worldwide. Approximately 10% of all cases of acute hepatitis, 5% of all hospital admissions, and 50% of all cases of acute liver failures are due to drug induced liver injury. It is also documented that more than 75% of idiosyncratic drug reactions result in liver transplantation or liver death [2,3]. Most drug-induced liver injury and acute liver failure occur due to either accidental or intentional overdosage of acetaminophen also known as (*N*-acetyl-p-aminophenol, paracetamol, APAP) [4].

APAP is an anti-pyretic and analgesic drug used widely in clinical practice. When using at therapeutic doses, APAP is metabolized by gluconidation or sulfation by cytochrome P450 system into the reactive metabolite *N*-acetyl-p-benzoquinone imine (NAPQI) [5]. Under normal condition, NAPI is bio-converted into non-toxic metabolites by the enzyme glutathione (GSH). However, at high doses, NAPQI levels drastically increase and react with hepatic proteins and cause liver injury [6]. APAP-induced hepatotoxicity has been studied for several years due to their detrimental effects on health.

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In various traditional medical systems herbal medicine are used as hepatoprotective agents [7]. Several herbal medicines are used as hepatoprotective agents in ayurveda, unani, siddha and homeopathy medicinal systems. Herbal extracts are tested *in vitro* and *in vivo* to prove its hepatoprotective efficacy across the globe. Many polyherbal formulations were developed which is more effective than the known drugs as hepatoprotective agent [8].

Acacia catechu exhibited various pharmacological properties including anti-microbial, anti-fungal, anti-inflammatory and tringent and has also used to treat obesity, wound, and diabetes and to maintain oral health hygiene [9]. It is rich in phytochemical constituents such as catechins, epicatechins, epigallocatechin, quercetin, rutin, gallic acid [10]. Although *A. catechu* various extracts have been widely used for hundreds of years in treating various diseases, hepatoprotective effect of *A. catechu* seed extracts were poorly studied. In the present study, we hypothesized that *A. catechu* pharmacological effects could reduce acetaminophen induced liver injury by decreasing reactive oxygen species (ROS), liver function enzymes and increasing the antioxidant defensive system.

2. Materials & methods

2.1. Chemicals

Hydroxy propyl cellulose, acetaminophen (99% purity) and isoflurane were purchased from Sigma-Aldrich (USA), All the other chemicals used were of analytical grade.

2.2. Animals

Wistar female rats (130–170 g) that were randomly bred and maintained in the Animal Facility of the Centre for Laboratory and Animal Research (CLAR, Saveetha University) were used for the study. The animals were housed in polypropylene cages (5 per cage) containing sterilized paddy husk as bedding material and were provided with pellet diet (commercial feed) and water *ad libitum*. Animals were cared for and maintained as per the approved guidelines of the "Committee for Control and Supervision of Experiments on Animals" (CPCSEA, India) and the protocol was approved by the Institutional Animal Ethical Committee, Saveetha University (SU/BRULAC /RD/001–2015 Dated 13/03/2015).

2.3. Collection of plant material and extract preparation

Acacia catechu (L.f.) Wild., were collected from fields in Hosur (Tamilnadu, India) and were identified and authenticated by National Institute of Science Communication and Information Resources (NISCAIR -New Delhi)/ Raw Materials Herbarium and Museum Delhi (RHMD) with specimen voucher number- #NC/ASE/9003. Seeds were shade dried for a week and made into fine powder in a mixer grinder. The powder was passed through 100-mesh sieve and stored in sealed polythene bags. About 2.5 kg of *A. catechu* seed powder was mixed with 10 L of ethanol in a 20 L round bottom flask attached with Graham condenser and heated for 1 h at 65 °C. The condenser was cooled with circulating chilled water. After 1 h of extraction the flask was cooled to room temp and the extract was filtered through Whatman 1 filter paper and the filtrate was collected. The residue was extracted repeatedly with 10 L of ethanol, twice. All the filtrates were pooled. Under reduced pressure in a Buchi Rotary Evaporator (Switzerland) at 65 °C the pooled extract was subjected to dryness. About 150 g of dry powder was obtained per batch (6% yield). This extraction procedure was carried out in Green Chem Herbal Extractions and Formulations R and D (Bengaluru), India.

2.4. Experimental design

At first, sub-acute toxicity studies of both *A. catechu* bark (ACBE) and seed (ACSE) were carried out by treating various concentration namely 250, 400 and 1000 mg/kg respectively for 28 consecutive days and results suggest that the dose of 400 mg/kg did not produce any significant changes hence it can be taken as NOAEL (no-observed–adverse- effect-level) (an unpublished data) and used for hepatoprotective study. The *in vivo* hepatoprotective activity of ACBE and ACSE were evaluated against acetaminophen (APAP) also known as paracetomol–induced hepatotoxicity in female wistar rats according to the method with minor modifications [11,12]. The animals (n = 5) were randomized into 5 experimental groups and the dosing regimen as follows.

Group A – Normal Control (0.5% hydroxy propyl cellulose)

Group B – APAP (750 mg/kg body weight)

Group C – APAP + N – acetyl cysteine (NAC) (200 mg/kg)

Group D – APAP + ACBE (400 mg/kg)

Group E – APAP + ACSE (400 mg/kg)

The animals were fasted for 24 h prior to experiment under standard laboratory conditions but allowed free access to food and water *ad libitum*. After 24 h, the experimental groups were orally administered with respective drugs of different concentration. After 1 h of the drug treatment, except Group A, all the other groups received oral administration of APAP 750 mg/kg body weight. After 24 h of hepatic toxicity induction, the animals were anaesthetized with isoflurane; blood was collected by retro orbital puncture into heparinized vacuotainers. Later, the blood was centrifuged at 3500 rpm for 10 min; plasma was collected and stored for liver function tests. The animals were then sacrificed by cervical dislocation and liver tissue was removed, weighed and stored at −80 °C for further biochemical and histological analysis.

2.5. Liver enzymes assessment

2.5.1. Plasma and liver assays

Plasma and liver tissue were used for the determination of liver enzyme levels *viz.*, alanine aminotransferase (ALT), alkaline phosphate (ALP), and aspartate aminotransferase (AST). The estimations were performed by using the standard procedures of commercial diagnostic kit (Accurex, Mumbai) on bio systems semi auto analyzer.

2.5.2. Biochemical analysis

10% liver tissue homogenate was prepared with ice cold 10% KCl and centrifuged at 1000 rpm for 15 min. The supernatant was used as the source of enzyme. The total protein content was estimated [13].

2.5.3. Estimation of lipid peroxides (LPO)

The amount of lipid peroxides was estimated as described previously [14]. Briefly, 0.2 mL of tissue homogenate was made up to 1 mL with normal saline, 0.5 mL of BHT (0.05%) and 3.5 mL TBA (0.8%) reagent was added and heated for 90 min in a boiling water bath. After cooling, the solution was centrifuged at 3500 rpm for 10 min and the precipitate obtained was discarded. The absorbance of the supernatant was determined at 532 nm using multimode reader (Perkin Elmer, U.S.A). The level of lipid peroxidation was expressed in terms of micrograms of malondialdehyde (MDA) equivalents/g of tissue.

2.5.4. Estimation of reduced glutathione (GSH)

The assay was performed as per the earlier methods [14]. Briefly, 0.25 mL of supernatant was added to equal volume of ice cold 5% TCA to precipitate the protein present in the tissue. The precipitate was removed by centrifugation at 4000 rpm for 10 min. To 1 mL aliquot of

supernatant, 0.25 mL of phosphate buffer (pH 8.0) and 0.5 mL of DTNB (0.6 mM) was added and mixed well. The absorbance was read at 412 nm using multimode reader (Perkin Elmer, U.S.A). The results were expressed in micromoles of GSH/g tissue.

2.5.5. Estimation of superoxide dismutase

Super oxide dismutase was assayed by the method [16]. Briefly, to 0.05 mL of supernatant, 0.3 mL of sodium pyrophosphate buffer (0.025 M), 0.025 mL of PMS (186 μM) and 0.075 mL of NBT (300 μM) was added. The reaction was started by addition of 0.075 mL of NADH (780 μM). After incubation at 30°C for 90 s, the reaction was immediately stopped by addition of 0.25 mL glacial acetic acid. Then the reaction mixture was stirred vigorously and shaken with 2.0 mL of n-butanol. The mixture was allowed to stand for 10 min and centrifuged. The color intensity in the butanol layer was read at 560 nm (Multiskan, Thermo Scientific, U.S.A). The activity of SOD was expressed as units/min/mg of protein. One unit of SOD activity is defined as the enzyme reaction, which gives 50% inhibition of NBT reduction in one minute under the specified assay conditions.

2.5.6. Analysis of histopathological changes

The liver bits of around 5 mm thickness were dehydrated with serial grades of ethanol solutions and embedded in paraffin. Sections of 4 microns were made using rotary microtome and staining was done using Hematoxylin and Eosin (H&E).

2.5.7. Statistical analysis

Data were expressed as mean ± S.E.M and analyzed by one-way analysis of variance followed by Students Newman kewl's multi-comparision test to determine the significance of differences between groups. A *p*-value lower than 0.05, considered as significant change.

3. Results

3.1. Effect of A. catechu bark and seed extracts against APAP induced hepatotoxicity on body weight, liver weight and liver weight/body weight ratio

Administration of APAP (750 mg/kg body weight) has significantly increased the body weight when compared to normal control (initial body weight was 140 ± 5). Significant differences were observed in the body weight of NAC 200 mg/kg body weight, *A. catechu* bark 400 mg/kg body weight, and *A. catechu* seed 400 mg/kg body weight were observed when compared to APAP group (Fig. 1A). Similarly, alteration in weight of liver tissue were also observed. Significant increase in the liver weight was observed in APAP induced group when compared to control groups. Whereas, significant decrease in the weight of liver tissue in all treated groups namely, NAC, bark and seed respectively (Fig. 1B).

The average liver weight and body weights ratio was also calculated. There is significant increase in the ratio was observed in APAP induced rat model when compared to that of normal control. The liver and body weight ratio in the pre-treated groups of *A. catechu* bark and seed extracts were found to be significantly decreased compared to APAP. The groups pretreated with seed extracts showed similar liver weight and body weight ratio as that of standard NAC group (Fig. 1A).

3.2. Effect of Acacia catechu bark and seed extracts against APAP induced hepatotoxicity on liver function tests in plasma

The results showed significant increase of ALT, AST and ALP liver enzymes in APAP group compared to that of control. The *A. catechu* bark and seed treated groups exhibited significant decrease in these liver enzyme levels, as compared to APAP induction group (Fig. 2A-C). However, compared to bark extract treated group, seed extract showed significant reduction of liver enzymes.

3.3. Effect of Acacia catechu bark and seed against APAP induced hepatotoxicity on liver function tests in Liver tissue

Effect of *A. catechu* bark and seed extract against APAP induced hepatotoxicity were observed by determining oxidative stress marker, lipid peroxidation (LPO) and antioxidants (GSH & SOD) in liver tissue. There is significant upregulation in the levels of oxidative stress marker, LPO was observed in APAP group, compared to that of normal control. The elevated levels of LPO by APAP induction was substantially decreased in the *Acacia* bark and seed extract treated groups compared to APAP (Fig. 3A).

Concomitant decrease in the antioxidants, reduced glutathione (GSH) and superoxide dismutase enzyme (SOD) was observed in APAP induced group. Amelioration in the levels of GSH and SOD was exhibited by both *A. catechu bark* and seed extracts pre-treated groups, besides NAC in a significant manner when compared to APAP (Fig. 3B-C).

The study exhibited significant increase of ALT, AST and ALP liver enzymes in APAP treated group as compared to untreated control. The *A. catechu* bark and seed extract pre-treated groups exhibited significant decrease in these enzyme levels when compared to APAP treated groups and groups pre-treated with seed extracts showed significant reduction in enzyme levels compared to bark extract (Fig. 4).

3.4. Histopathological observations

Histological profile of normal control group revealed normal histology of liver with central vein, hepatocytes and portal triads (Fig. 5A). APAP induced group revealed marked degree of necrosis along with polymorphonuclear cells infiltration, severe vacuolations in the hepatocytes and sinusoidal congestion (Fig. 5B). Pretreatment with NAC reduced APAP induced microscopic changes and showed marked beneficial effects in the liver architecture (Fig. 5C).

Pretreatment with *A. catechu* bark extract showed moderate degree of necrosis, mild degree of vacuolations in the hepatocytes with minimal sinusoidal congestion (Fig. 5D). However, *A. catechu* seed extract administration revealed regeneration of hepatocytes to normal architecture, absence of sinusoidal congestion and vacuolations in the hepatocytes (Fig.5E).

4. Discussion

Liver failures are rising every year in the United States, and drugs are the major cause of it. Drug induced hepatoxicity depends on various factors including age, gender, lifestyle factors, obesity, nutritional status, genetic background, dose, and exposure of drugs [17,18]. A high use of acetaminophen was either due to accidental intake or suicidal practice resulted in liver failures in many countries [19–21]. Acetaminophen liver injury cause functional suppression of immune cells and result in generation of reactive oxygen species (ROS) and increased lipid peroxidation thus resulting in the depletion of tissue GSH (an antioxidant) and thus cause alteration in membrane fluidity and permeability, enhanced rates of protein degradation and cell death.

During oxidative stress, there is an imbalance between reactive oxygen and nitrogen species (ROS/RNS) and the antioxidant that plays a significant role in various pathophysiological conditions [22,23]. In this case, there is increase production of ROS/RNS and decrease of antioxidants in a cell resulted due to an inability of endogenous antioxidants to counteract the oxidative damage on biomolecules [24]. Similarly, upon APAP overdose there is critical imbalance between ROS and the antioxidant defenses. Thus, attention has paid on antioxidant capacity of natural products to counteract free radicals. In addition, single *in vitro* model is not enough to evaluate and compare their antioxidant properties because *in vitro* model does not consider relevant parameters involved in biological environments such as lipophilicity and bioavailability. Therefore, use of *in vivo* model is often

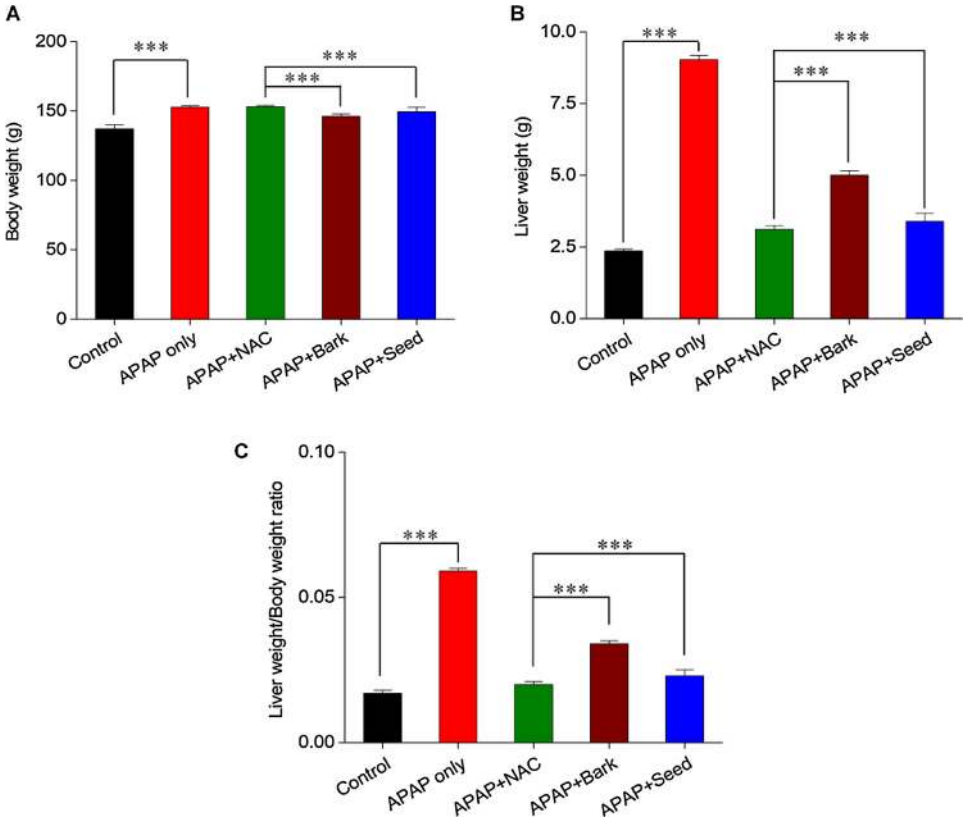

Fig. 1. Effect of Acacia bark and Seed against APAP induced hepatotoxicity on body weight, liver weight and liver weight/ body weight ratio. A – Control; B – APAP (750 mg/kg b. wt); C – APAP + Acacia bark (400 mg/kg b.wt); D – APAP + Acacia Seed (400 mg/kg b. wt); E – APAP + NAC (200 mg/ kg b. wt). Data were presented as mean ± SEM (n = 5). *P < 0.05, Students Newman kewl's multi comparison test.

recommended to determine the antioxidant potential and therapeutic properties of any drug to reciprocate nature environment conditions.

Several studies have been conducted to understand the mechanism of hepatotoxicity. The important role of generation of reactive oxygen species (ROS) in the cellular damage are widely investigated and indicated that covalent binding of ROS and reactive intermediates to macromolecules are responsible for the severe harmful drug reactions [25,26]. Several studies reported that hepatotoxicity results in

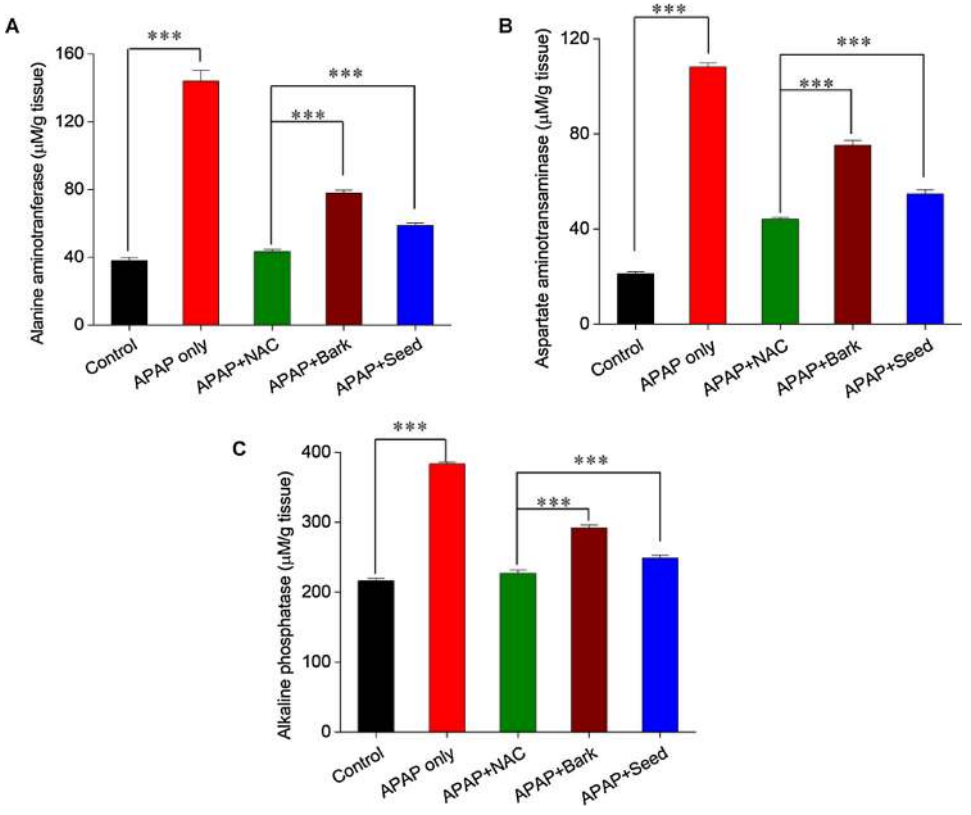

Fig. 2. Effect of *A. catechu* bark and seed extracts against APAP induced hepatotoxicity on liver ALT, AST and ALP enzyme levels. A – Control; B – APAP (750 mg/kg b. wt); C – APAP + NAC (200 mg/kg b. wt); D – APAP + bark (400 mg/kg b.wt); E – APAP + Seed (400 mg/kg b. wt); Data were presented as mean ± SEM (n = 5). *P < 0.05, Students Newman kewl's multi comparison test.

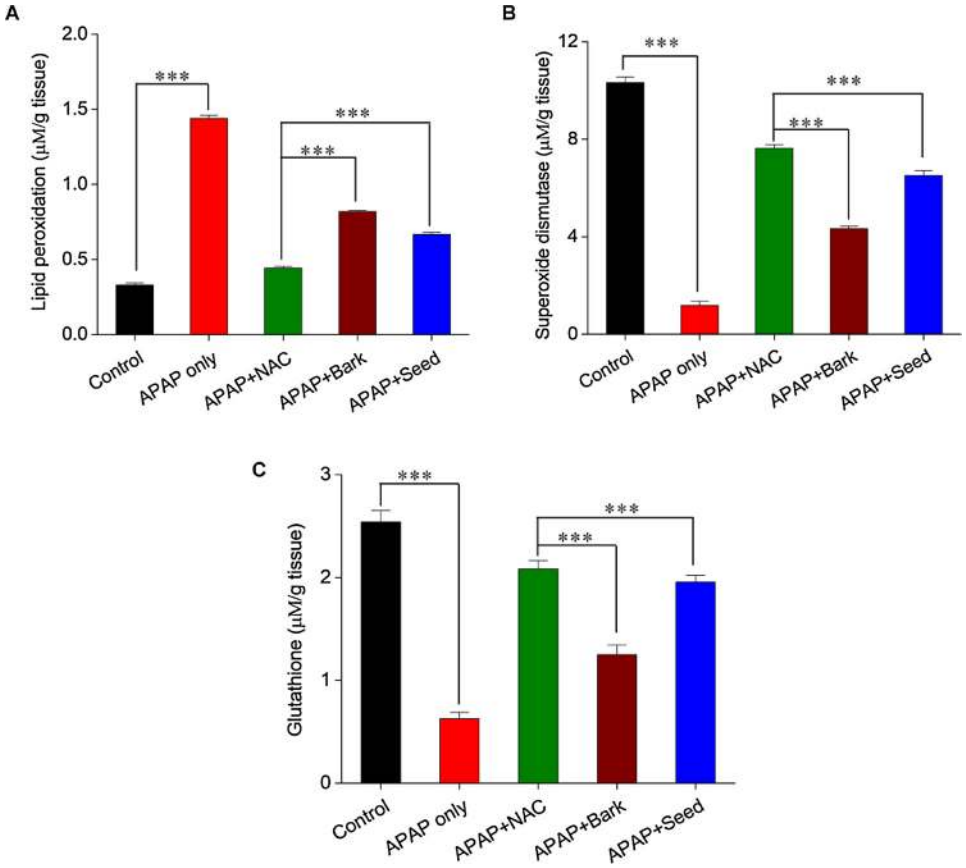

Fig. 3. Effect of *A. catechu* bark and seed extracts against APAP induced hepatotoxicity on oxidative stress marker and antioxidants in Liver tissue. A – Control; B – APAP (750 mg/kg b. wt); C – APAP + NAC (200 mg/kg b. wt); D – APAP + bark (400 mg/kg b. wt); E – APAP + Seed (400 mg/kg b.wt). Data were presented as mean ± SEM (n = 5). *P < 0.05, Students Newman kewl's multi comparison test.

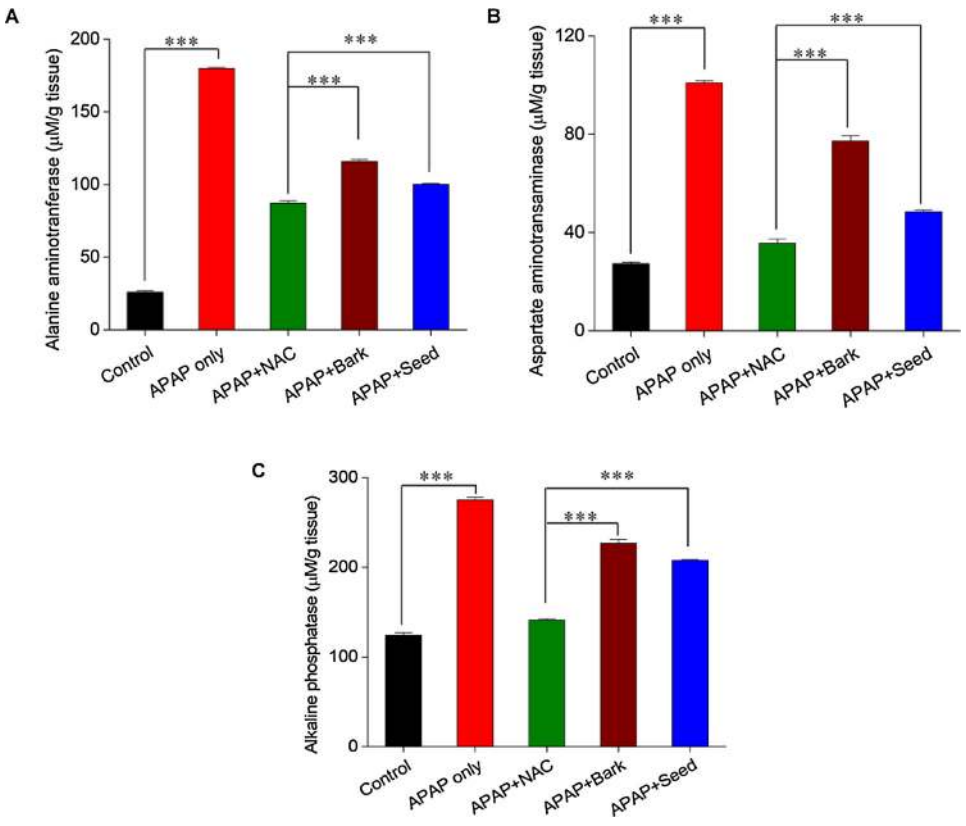

Fig. 4. Effect of *A. catechu* bark and seed against APAP induced hepatotoxicity on Liver function enzymes in Plasma. A – Control; B – APAP (750 mg/kg b. wt); C – APAP + bark (400 mg/kg b. wt); D – APAP + seed (400 mg/kg b. wt); E – APAP + NAC (200 mg/kg b. wt). *P < 0.05, Students Newman kewl's multi comparison test.

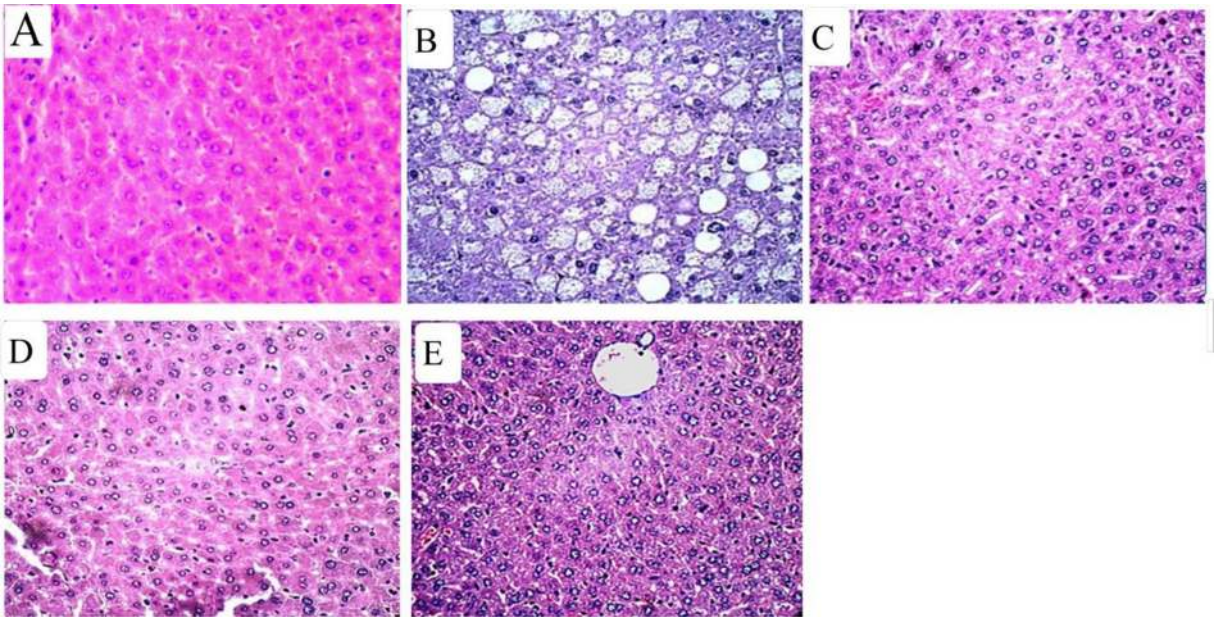

Fig. 5. Protective effects of *A. catechu* bark, seed and NAC against the effect of APAP induced liver damage using H& E staining. A) Control- Liver section showing normal architecture with central vein, hepatocytes radiating from the central veins and the portal triads. B) APAP- APAP induced liver section showing moderate degree of sinusoidal congestion, centrilobular necrosis with polymorph nuclear cells infiltration and marked vacuolations in the hepatocytes. C) APAP + NAC- Liver section showing apparently normal architecture of the hepatocytes, absence of hepatic vacuolations and congestion. D) APAP + ACBE- Liver section showing mild degree of vacuolations in the hepatocytes with minimal sinusoidal congestion. E) APAP + ACSE- Liver section showing apparently normal architecture of the hepatocytes, absence of hepatic vacuolations and congestion. (Magnification, 40×).

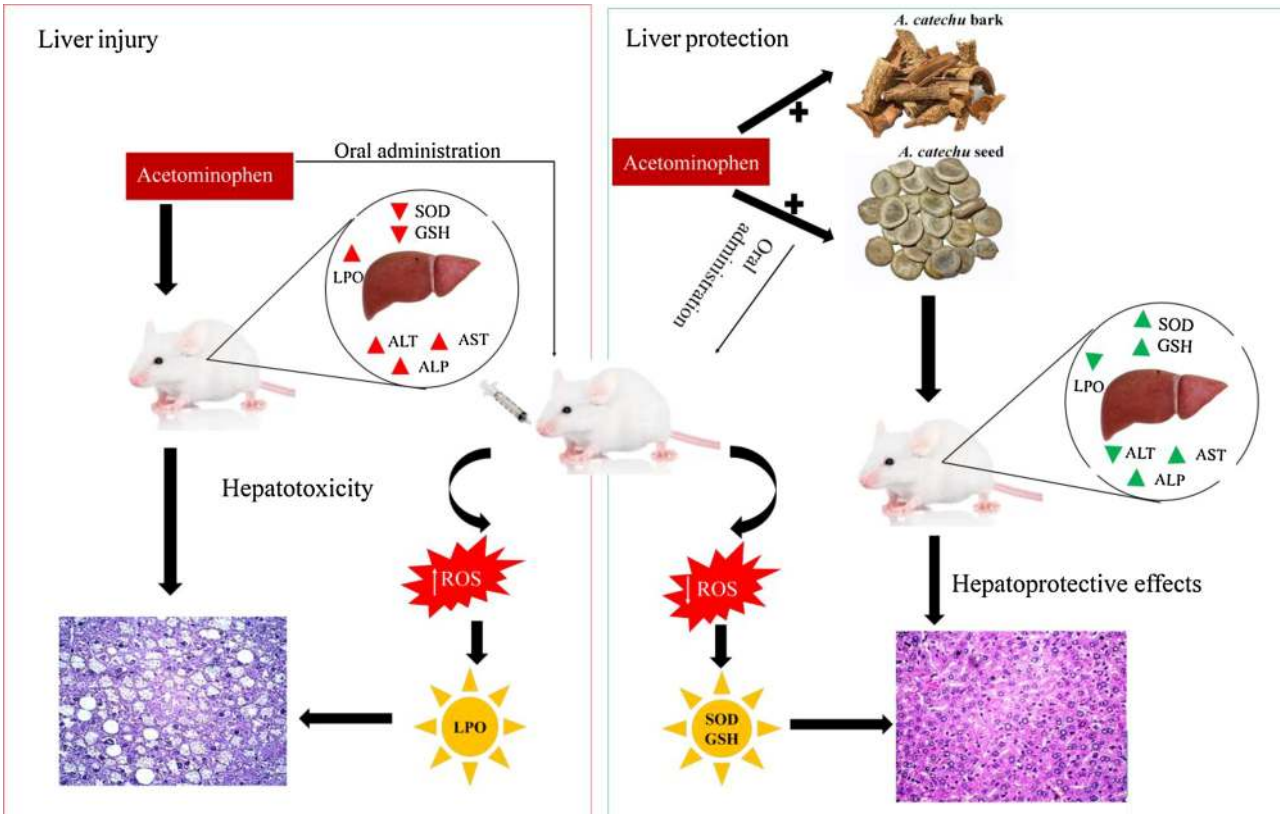

Fig. 6. Schematic representation of hepatoprotective effects of *A. catechu* seed and bark extracts. Acetaminophen treated groups showed significant increase of liver function enzymes such as alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) and decrease of antioxidant enzymes such as glutathione (GSH) and superoxide dismutase (SOD) and thus resulted in hepatotoxicity effects. Whereas, groups treated with *A. catechu* seed or bark extract exhibit decrease of liver function enzymes such as ALT, ALP and AST and increase of antioxidant enzymes such as GSH, SOD and thus showing hepato-protective effects.

generation of free radicals and reactive metabolites including [27,28]. Apart from this, depletion of glutathione (GSH) results in the membrane lipid peroxidation and resulting in the functional integrity [29,30]. The use of herbal medicines as an antioxidant have attracted recently due to their potential and efficacy against drug-induced liver toxicity [31]. The hepatoprotective activity of a drug against liver toxicity must significantly decrease oxidative stress, and liver function enzymes by elevating antioxidants. When Wistar rats was orally administered acetaminophen significantly increased body weight, liver weight, liver function enzymes and decreased antioxidant enzymes was observed in both plasma and liver tissues. Whereas, rats administrated with acetaminophen and *A. catechu* seed or bark extracts tend to decrease oxidative stress marker enzymes such as lipid peroxidase, liver enzymes including aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP), antioxidative enzymes such as glutathione (GSH), superoxide dismutase (SOD) and lipid peroxidation assay in liver tissues. These reports confirm previous findings regarding decrease of liver enzymes after the treatment of different plant extracts hepatoprotective effects [32–38].

The toxic effects of APAP were also observed histologically, showing moderate degree of sinusoidal congestion, centrilobular necrosis with polymorph nuclear cells infiltration and marked vacuolations in the hepatocytes. The liver histopathological analysis in groups pretreated with bark extracts showed mild degree of vacuolations in the hepatocytes with minimal sinusoidal congestion. However, groups pretreated with seed extracts showed apparently normal architecture of the hepatocytes and absence of hepatic vacuolations and congestion suggesting that *A. catechu* seed extract exhibited more prominent role against APAP induced hepatotoxicity as same as NAC treated groups. Overall view of the present study was described in Fig. 6. Oral administration of APAP induced liver toxicity by increase in accumulation of oxidative stress and caused liver injury. However, when *A. catechu* seed or bark extracts treatment recovered liver from APAP induced damage by reducing oxidative stress and by increasing antioxidant defense signals.

In conclusion, we have demonstrated that oral administration of *A. catechu* seed or bark extract recovered liver from hepatotoxicity. This protective effect of these extracts may be due inhibition of oxidative stress and hepatic biomarkers (ALT, AST, ALP), and by elevation of antioxidant defense mechanism thus resulted in regeneration of liver tissues. Further studies are needed to investigate the molecular targets that involved in hepatoprotective effect.

Author's contributions

L.T: Participated in the design of the study, treatment and maintenance of animals and doing experiments. S.R.B: critically interpreted, analyzed the data and wrote the manuscript. S.S, S.R: Participated in the analysis of data and interpreted histopathology data. H.P: Gave critical comments on the manuscript and drafted the manuscript. P.R. helped L.T to carry out the experiment and maintenance of animals. V.R: assisted all the authors for to carryout experiments.

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None.

Conflict of interest

The authors declare that they have no conflicts of interest with respect to research, authorship, nor publication of this article.

References

[1] C. Mayuren, V.V. Reddy, S.V. Priya, V.A. Devi, Protective effect of Livactine against CCl4 and paracetamol induced hepatotoxicity in adult Wistar rats, N. Am. J. Med. Sci. (Boston) 2 (2010) 491.
[2] G. Ostapowicz, R.J. Fontana, F.V. Schiødt, A. Larson, T.J. Davern, H. Han, et al., Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States, Ann. Intern. Med. 137 (2002) 947–954.
[3] A. Pandit, T. Sachdeva, P. Bafna, Drug-induced hepatotoxicity: a review, Int. J. Appl. Pharm. Sci. Res. 02 (2012) 233–243.
[4] B.L. Woolbright, H. Jaeschke, Role of the inflammasome in acetaminophen-induced liver injury and acute liver failure, J. Hepatol. 66 (4) (2017) 836–848.
[5] S. Agarwal, B. Khazaeni, Toxicity, Acetominophen.Treasure Island (FL), StatPearls Publishing, 2018.
[6] L. Coles, P. Wilson, J.A. Wardman, S.D. Hinson, B. Nelson, B. Ketterer, The spontaneous and enzymatic reaction of n-acetyl-p-benzoquinonimine with glutathione: a stopped-flow kinetic study, Arch. Biochem. Biophys. 264 (1988) 253–260.
[7] Y.K. Sharma, H. Singh, B.L. Mehera, Hepatoprotective effect of few herbs in patients receiving antituberculus treatment, Indian J. Trad. Knowledge 3 (2004) 391–396.
[8] B. Ahmed, T. Alam, M. Varshney, S.A. Khan, Hepatoprotective activity of two plants belonging to the *apiaceae* and the *euphorbiaceae* family, J. Ethnopharmacol. 79 (2002) 313–316.
[9] S.J. Stohs, D. Bagchi, Antioxidant, Anti-inflammatory and Chemoprotective properties of*Acacia catechu* Heartwood extracts, Phytother. Res. 29 (2015) 818–824.
[10] M. Rahmatullah, M. Hossain, A. Mahmud, Antihyperglycemic and antinociceptive activity evaluation of "khoyer" prepared from boiling the wood of *Acacia catechu* in water, Afr. J. Tradit. Complement. Altern. Med. 10 (2013) 1–5.
[11] P.R. Tulio, J.A. Correa, J.V. Beatriz, M.F. Aarestrup, V. Orlando de Sousa, A. Ademar, Filho da Silva, Protective effects of *Baccharis dracuncufolia* leaves extract against carbon tetra choloride and Acetominophen –induced hepatotoxicity in experimental animals, Molecules 19 (2014) 9257–9272.
[12] G. Kuvandik, M. Duru, Y.Z. Nacar, R. Helvaci, S. Sogut, Effects of Erdosteine on acetaminophen induced hepatotoxicity in rats, Exp. Toxicol. Pathol. 36 (2008) 714–719.
[13] R. Parameswari, K. Mano, M. Aruna, A.S. Vickram, M. Rameshpathy, T.B. Sridharan, Human sperm DNA damage inhibition and antioxidant activity of *Tarjuna* bark an *in vitro* study, Biotech 7 (2017) 188.
[14] K.H. Han, N. Hashimoto, K. Shimada, M. Sekikawa, T. Noda, H. Yamauchi, M. Hashimoto, H. Chiji, D. Topping, M. Fukushima, Hepatoprotective effect of purple potato extract against D galactosamine induced liver injury in rats, Biosci. Biotechnol. Biochem. 70 (2006) 1432–1437.
[16] K.M. Surapaneni, G.R. Venkata, Lipid peroxidation and antioxidant status in patients with carcinoma of prostate, Indian J. Physiol. Pharmacol. 50 (2016) 350–354.
[17] Y. Yamazaki, S. Kakizaki, N. Horiguchi, H. Takagi, M. Mori, M. Negishi, Role of nuclear receptor CAR in carbontetrachloride induced hepatotoxicity, World J. Gastroenterol. 11 (2005) 5966–5972.
[18] U.A. Boelsterli, H.K. Ho, S. Zhou, K.Y. Leow, Bioactivation and hepatotoxicity of nitro aromatic drugs, Curr. Drug Metab. 7 (2006) 715–727.
[19] A.M. Larson, J. Polson, R.J. Fontana, T.J. Davern, E. Lalani, L.S. Hynan, et al., Acute liver failure study group. Acetaminophen-induced acute liver failure: results of a United Sattes multicenter prospective study, Hepatology 42 (2005) 1364–1372.
[20] M. Mitka, FDA asks physicians to stop prescribing high-dose acetaminophen products, JAMA 311 (2014) 563.
[21] Y. Wang, Y. Jiang, X. Fan, H. Tan, H. Zeng, Y. Wang, et al., Hepato-protective effect of resveratrol against acetaminophen-induced liver injury is associated with inhibition of CYP-mediated bioactivation and regulation of SIRT1–p53signaling pathways, Toxicol. Lett. 236 (2015) 82–89.
[22] J. Flora, Role of free radicals and antioxidants in health and disease, Cell. Mol. Biol. 53 (2007) 1–2.
[23] B. Halliwell, M.C. Gutteridge, Free Radical in Biology, 4th ed, Oxford University Press, Newyork, 2007.
[24] H. Sies, Oxidative Stress: Introductory Remarks, Academic Press, London, 1985.
[25] R.O. Racknagel, E.J.R. Glende, J.A. Dolak, R.I. Waller, Mechanism of carbon tetra chloride- induced hepatotoxicity. Hepatocellular damage by reactive carbon tetrachloride metabolites, Z. Naturforsch. C 56 (7-8) (1989) 649–659.
[26] N. Masubuchi, C. Makino, N. Murayama, Prediction of in vivo potential for metabolic activation of drug into chemically reactive intermediate: correlation of invitro and *in vivo* generation of reactive intermediates and *in vitro* glutathione conjugate formation in rats and humans, Chem. Res. Toxicol. 20 (2007) 455–464.
[27] B.K. Park, N.R. Kitteringham, J.L. Maggs, M. Pirmohamed, D.P. Williams, The role of metabolic activation in drug-induced hepatotoxicity, Ann. Rev. Pharmacol. Toxicol. 45 (2005) 177–202.
[28] R.O. Racknagel, E.J.R. Glende, J.A. Dolak, R.I. Waller, Mechanism of carbon tetra chloride toxicity, Pharmacol. Ther. 43 (1989) 130–154.
[29] D. Ross, Glutathione free radicals and chemotherapeutic agents, Pharmacol. Ther. 37 (1988) 231–249.
[30] A. Guven, M. Gulmez, The effect of kefir on the activities of GSH, Px, GST, CAT, GSH and LPO levels in carbon tetrachloride-induced mice tissues, J. Vet. Med. B Infect. Dis. Vet. Public Health 50 (2003) 412–416.
[31] D. Singh, W.C. Cho, G. Upadhayay, Drug-Induced liver toxicity and prevention by herbal antioxidants: an overview, Front Pharmacol. 6 (2016) 363.
[32] S.S. Alkiyumi, M.A. Abdullah, A.S. Alrashdi, S.M. Salama, S.I. Abdelwahab, A.H.A. Hadi, *Ipomoea aquatica* extract shows protective action against thioacetamide-induced hepatotoxicity, Molecules 17 (5) (2012) 6146–6155.
[33] L.L. Bell, R. Vuppalanchi, P.B. Watkins, et al., Serum proteomic profiling in patients with drug-induced liver injury, Aliment. Pharmacol. Ther. 35 (5) (2012) 600–612.
[34] T.J. Green, M.L.A. Sivilotti, C. Langmann, et al., When do the aminotransferases rise after acute acetaminophen overdose, Clin. Toxicol. 48 (8) (2010) 787–792.
[35] M. Mroueh, C. Daher, M.E.I. Sibai, C. Tenkarian, Antioxidant and hepatoprotective activity of *Tragopogon porrifolius* methanolic extract, Planta Med. (2011) 77–PF72.
[36] P. Govind, Medicinal plants against liver diseases, Int. Res. J. Pharm. Pharmacol. 2 (2011) 115–121.
[37] F. Ozturk, M. Gul, B. Ates, I.C. Ozturk, A. Centin, N. Vardi, et al., Protective effect of apricot (*Prunus armeniaca L.*) on hepatic steatosis and damage induced by carbon tetrachloride in Wistar rats, Br. J. Nutr. 102 (2009) 1767–1775.
[38] S. Kang, C. Lee, H. Koo, D. Ahn, H. Choi, J. Lee, et al., Hepatoprotective effects of aqueous extract from aerial part of agrimony, Korea J. Pharmacogn. 37 (2006) 28–32.