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Protective roles of the red-dragon fruit peels (*Hylocereus costaricensis)* against the cigarette-smoke harmful effect in Wistar rats

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ABSTRACT

Background: Many people are exposed to cigarette smoke unintentionally in numerous places worldwide. Cigarette smoke contains carbon monoxide, nicotine, and polycyclic aromatic hydrocarbons, which are toxic and can trigger the production of free radicals in the body.

Objective: To study the impact of cigarette-smoke exposure twice daily for 30 days on 4-5 weeks Rattus norvegicus L. without or with a daily intake of the juice or ethanol extract of the red-dragon fruit peels H. costaricensis.

Materials and Methods: Twenty-eight 4-5 weeks old male Wistar rats were randomly allocated into Control (not exposed to cigarette-smoke), exposed to cigarette-smoke only (C_{smoke}), exposed to cigarette-smoke and H. Costaricensis-peel juice (Jc_{Hc}), exposed to cigarette-smoke and had H. Costaricensis-peel extract (Ex_{Hc}). Cigarette-smoke exposure was given twice daily. The juice (3g/mL) and extract (3.15g/mL) were given for 30 days ad libitum. Feed and drink intake, body weight, and serum biochemistry (MDA, bilirubin, ALT and AST) were determined. Data were analyzed by ANOVA.

Results: The positive control group with cigarette-smoke exposure (C_{smoke}) had a significant elevation in serum malondialdehyde (MDA), alanine-transaminase (ALT), and aspartate-transaminase (AST) and drinking water intake ($p<0.05$) but reduced feed intake and body weight ($p<0.05$). The Jc_{Hc} and Ex_{Hc} groups had reduced serum MDA, ALT, and AST and higher body weight and feed intake than the $C_{smoke,}$ and the extract had a better reduction than the juice ($p<0.05$). Furthermore, the extract had a lower biochemical profile than the Control group ($p<0.05$).

Conclusion: The disturbance in serum MDA, ALT, AST, water and feed intake, and body weight by cigarette smoke was ameliorated by H. costaricensis peel juice or extract daily for 30 days. H. costaricensis peel juice or extract can be used to prevent the adverse effects of cigarette smoke exposure and has the potential to be developed into valuable products.

Keywords*: antioxidants; free radicals; MDA; smoking; tobacco*

BACKGROUND

Many people of all ages are exposed to cigarette smoke unintentionally or involuntarily in various places and across the globe.[1-3] These exposures threaten public health due to the increased mortality and morbidity of various non-communicable diseases at the population level.[4,5] In 2019, data from 204 countries suggested that 7.69 million deaths were attributable to smoking tobacco use, which accounts for 13.6% of deaths worldwide.[6] Encumbrance of this magnitude also negatively impacted healthcare and economic costs.[7]

Meanwhile, cigarette smoke contains more than 7,000 toxic chemicals, some of which are carbon monoxide (CO), nicotine, benzo (a) pyrene, nitrous oxide (NO), and polycyclic aromatic hydrocarbons (PAH), which are dangerous free radicals.[8] Radicals containing cigarette smoke are pro-oxidants, and a high amount of exposure to free radicals that enter the body reduces antioxidant enzymes such as Superoxide Dismutase (SOD), catalase, Glutathione Peroxidase (GSH-PX), and antioxidants such as glutathione, coenzyme Q10, and melatonin.[9-12] Moreover, cigarette smoke also causes lipid peroxidation, which damages the normal cell membrane of the liver, causing cell leakage that leads to an increase in serum alanine transaminase (ALT) and aspartate transaminase (AST).[13] Therefore, supplementary antioxidant intake possesses the potential to neutralise such radicals containing cigarette smoke exposure.

Fruits and vegetables are natural sources rich in antioxidants. Numerous studies have explored and reviewed the role of antioxidants acquired from daily intake in combating oxidative stress, with results suggesting some positive effects.[14-16] The red-purple dragon fruit (*H. Costaricensis*) has a high antioxidant content from the natural pigment's anthocyanins and phenolic compounds. The peels of *H. costaricensis* fruit retain the deep red-purple, which signifies its high pigment content and antioxidants. The total phenolic, antioxidant and antiproliferative activity of *H. costaricensis* fruit peels is better than the flesh, with no toxic compounds.[17] Ethanol extract of *H. costaricensis* peels has an average total anthocyanin 58.0720 ±

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0.0001mg/L and betacyanin 186.90 mg/100g dry weight, with antioxidant activity (IC50) 96.95 mg/L within 5 minutes incubation.[18] Another study also showed that red and white dragon peels have stronger antioxidant activity than pulps.[19] One mg/ml dragon fruit *H. polyrhizus* extract can inhibit free radicals 83.48 ± 1.02%, while the fruit flesh inhibits only 27.45 ± 5.03%.[19] Despite its potential as an antioxidant source, fruit peels have been neglected and wasted.

A study of total oxidant status and oxidative stress index levels of active, passive, and non-smoker university students found that smoking reduces the plasma total antioxidant capacity and status.[20] Another study showed that single cigarette smoking significantly decreased plasma antioxidant concentration.[21,22] Therefore, the underutilised peels of *H. costaricensis* fruit were studied to prevent the adverse effect of cigarette smoke exposure on serum biochemistry, i.e., MDA (oxidative stress indicator), bilirubin, ALT, and AST levels in Wistar rats, including body weight, food and drink intake. The Wistar rats were chosen as the standard and ideal laboratory animal for mammalian study.[23] The Wistar rats enable researchers to control variables such as temperature, humidity, light, diet, and the tested intervention, i.e., cigarette exposure and *H. costaricensis* administration in this study, which is otherwise problematic in humans.

MATERIALS AND METHODS

Preparation of juice or ethanol extract of *H. costaricensis* peels

H. costaricensis was collected from agro-tourism in Sleman Regency, Central Java. *H. costaricensis* fruit peels were obtained by peeling the fruit's skin. The peels were blanched using water steam for 2 minutes, and then, after cooled, it was immediately packed in plastic and stored in the freezer until used. The peel juice was prepared by blending 100 g of the peels in 50 ml of drinking water. The blends were filtered using a tea filter, and the juice was stored in a refrigerator. The juice was given at 3 g/mL drinking water. The juice drink has a pinkish colour, indicating more anthocyanin content.

The peel extract was obtained by weighing 1000 g of the peels and extracted by wet maceration technique in 1000 mL of 96% ethanol mixed with 1% HCl with a volume ratio of 9 to 1. The 96% or absolute ethanol was used as a fruit extraction solvent, which is the safest after water.[24] While water extracts more polar compounds, 96% ethanol extracts more of less polar compounds. After 24 hours of maceration, the filtrate was collected by filtration. The filtrate was evaporated using a rotary vacuum evaporator to obtain a thick ethanol extract. The resulting filtrate was collected in a beaker glass, while the pulp was extracted again similarly. Extraction was repeated until all anthocyanins in the peels were extracted completely (the solvent was clear/colourless). All extracts were combined and then centrifuged for 15 minutes at 2000 rpm to separate the sediment and the supernatant. The supernatant obtained is then concentrated using a rotary vacuum evaporator at 40°C to get 30 ml of concentrated extract from the peels of the red-purple dragon fruit. The concentration of concentrated ethanol extract given to mice is 3.15 gr/ml drinking water. The extract drink is brownish, indicating higher levels of polyphenolic compounds.

Animal Experimental Design

Twenty-eight four to five weeks old male Wistar rats were randomly selected and divided into four groups, namely: control groups that were not exposed to cigarette smoke (Control), exposed to cigarette smoke (Csmoke), exposed to cigarette smoke and had Hylocereus costaricensis juice (JcHc), exposed to cigarette smoke and had *H. costaricensis* peels extract (ExHc). Each group consisted of seven rats. Each experimental rat was housed in individual hollow aluminium cages of 42x21x20 cm (length x wide, height). Wistar rats were kept in animal rooms with automatic room temperature control of 25 ± 20C, 70-90% relative humidity and a dark cycle of 12 hours per day.

For cigarette smoke exposure daily for 30 days, the experimental rats in the cage were placed in an enclosure made of glass. The bottom of the enclosure was a wire screen. The enclosure has two spaces: the lower for a cigarette and the upper for smoke exposure. The cigarette was positioned in a cigarette holder in the lower space of the enclosure (Figure 1).

A local commercial cigarette was ignited to produce the cigarette smoke. The smoke entered the upper space where the rats were placed. The lit cigarette was left burning till it went out. One cigarette smoke was given twice daily at 09.00 a.m. and 03.00 p.m. Feed (AIN - 93G) was delivered every morning at 08.00 *ad libitum* before cigarette smoke exposure.[25] For the JcHc and ExHc groups, red-dragon fruit peel juice and extract, respectively, were provided via a drinking bottle that was freely accessible *(ad libitum).* The juice group received juice drinks only. The extract group received extract drinks only. No plain water bottle was given. Each group has only one type of drinking bottle according to the treatment (Figure 2). The volume of

the juice and extract in the drinking bottles for each rat was the same (50 mL). For the Control and C_{smoke} groups, a regular drinking bottle with plain water was given and replaced with a fresh one daily. The remaining feed and drink of individual rats in each group were measured and recorded daily before the new replacement.

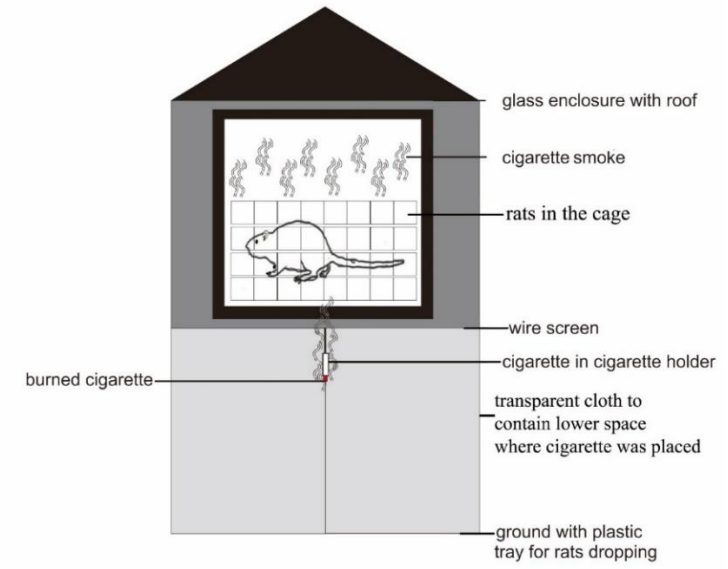

Figure 1. Cigarette smoke exposure method

The administration of *H. costaricensis* lasted for 30 days, considering our previous studies utilizing fruits and botanicals have shown positive effects.[26-28] After 30 days of treatment, blood sampling was carried out by administering ketamine anaesthesia at 50 mg/kg, and blood was withdrawn via the sinus orbitalis. Serum was collected and stored frozen until serum MDA, bilirubin, ALT and AST were determined. This study had ethical clearance from the preclinical health research ethics commission of the Integrated Research and Testing Laboratory, No.00054/04/LPPT/V/2018 Gadjah Mada University, where the animal study was conducted.

Figure 2. Animal Experimental Design. The grey square represents one rat housed individually

The coloured bottle represents the juice (pinkish) and the extract (brownish) drinks. The Control and Csmoke groups received plain water. All bottles were filled with 50 mL drinks.

Serum Malondialdehyde (MDA) as a marker of oxidative stress

Serum MDA was determined to assess oxidative stress using the TBRS (2-thiobarbituric acid reactive substance) approach. MDA in the serum combines with TBRS in an acidic medium for 60 minutes at a temperature of 95°C to produce a reactive thiobarbituric acid product that can be detected spectrophotometrically at 534 nm.[26]

Serum Bilirubin

Serum bilirubin was measured using a DiaSys kit (Diagnostic system GmBh) following the instruction manual. Diazotized Sulphanilic Acid (DSA) and bilirubin combine to generate a red-azo product that may be detected spectrophotometrically at 546 nm.

Serum ALT and AST

The serum alanine transaminase (ALT) and aspartate transaminase (AST) (previously designated as glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were analysed following the instruction manual of DiaSys kit.[27-30]

Statistical Analysis

All data were analysed using Microsoft Excel 2019 version 2306 and SPSS Statistics 25 for Windows (Microsoft Corporation Redmond, WA, USA). The normality test was done using the Saphiro-Wilk test. Normal distribution was followed with one-way ANOVA and further analysed with a posthoc test when statistical significance was reached. When the data were not normally distributed, Kruskall-Wallis was used; and if the result was significant, further analysis with the Mann-Whitney test was performed.

RESULTS

In this experiment, cigarette smoke exposure to Wistar rats went well, as it could be observed from their behaviour, who were attracted to and approached cigarette smoke, sniffing and quietly enjoying it. The serum MDA, bilirubin, SGOT, AST, feed and drink intake, and body weight results are presented in Table 1.

Table 1. Serum MDA, Bilirubin, ALT, and AST, Feed and Drink Intake, and Body Weight of Wistar Rats Exposed to Cigarette Smoke for 30 Days Without or With A Daily Intake of *Hylocereus costaricensis* Peel Juice and Ethanol Extract

Variables	Control	Csmoke	Jc_{Hc}	Ex_{Hc}	p
MDA (mg/mL)	38.69 ± 14.47^{b}	59.47 ± 15.76^{c}	28.56 ± 11.03^{b}	15.19 ± 8.65^{a}	0.000^{*}
Bilirubin (mg/dL)	0.44±0.06	0.43±0.05	0.44±0.09	0.51±0.07	0.101
ALT (U/L)	48.64 ± 8.06^{b}	55.16 ± 9.48^{c}	43.11 ± 4.55^{b}	34.63 ± 8.67^{a}	0.000^{*}
AST (U/L)	98.49 ± 14.04^{b}	133.33 ± 30.31^{b}	96.17 ± 33.97^{b}	77.90 ± 18.02^{a}	0.000^{*}
Feed Intake (g)	19.23 ± 0.15^{c}	16.90 ± 0.28^{a}	17.11 ± 0.40^{b}	17.46 ± 0.30^{b}	0.000^{*}
Drink Intake (mL)	8.97 ± 0.39^{a}	10.20 ± 0.35^{b}	8.53 ± 0.31^{a}	10.30 ± 0.30^{b}	0.000^{*}
Body Weight (g)	218.71 ± 7.39^{c}	156.71 ± 11.66^{a}	192.00 ± 7.55^{b}	197.14 ± 12.09^{b}	0.000^{*}

(Control): not exposed to cigarette smoke, (C_{smoke}): exposed to cigarette smoke only, (Jc_{Hc}): exposed to cigarette smoke with the intake of *H. costaricensis* peel juice, (Ex_{Hc}): exposed to cigarette smoke with the intake of *H. costaricensis* peels extract. Each data is the average of seven replicate rats ± SD. Different letters within the same row mean significant at $p<0.05$. *significant $p<0.05$.

The rats exposed to cigarette smoke alone (C_{smoke}) had significantly (<0.05) higher MDA, SGOT, and AST, drink intake but lower feed intake and body weight than the control rats not exposed to cigarette smoke (Control). The rats in the Jc_{Hc} group had serum MDA, ALT, and AST similar to those in the control group. However, the rats in the Ex_{Hc} group had significantly (<0.05) the lowest serum MDA, ALT and AST than Control, C_{smoke} and Jc_{Hc} groups. The drink intake of the Ex_{Hc} group was similar to the C_{smoke} group and was significantly higher than the Control group. The juice and extract groups had significantly higher body weight than the C_{smoke} group but were still lower than the Control group.

DISCUSSION

Table 1 shows that exposure to cigarette smoke increased MDA, ALT, and AST levels. Serum ALT and AST are biomarkers of hepatocyte integrity or cholestasis.[31] These enzymes catalyse reactions crucial for gluconeogenesis and urea formation.[32] Although their concentration is high in the liver, they are also present in the kidney, heart, skeletal muscle, and, to some extent, in the spleen, small intestine, and brain. The slightest disruption in hepatocyte membrane permeability can cause these enzymes to leak and be detectable, which may indicate necrosis or inflammation. It has been established that smoking creates a pro-inflammatory environment with the increase of cytokines, which then causes tissue inflammation and cell death.[33,34] The rise in liver enzymes could also be due to harmful free radicals of cigarette smoke, including carbon monoxide

(CO), nicotine, and polycyclic aromatic hydrocarbon.[8] Nicotine exposure has been shown to increase oxidative stress and hepatocellular apoptosis through multiple signalling pathways.[35,36]

The increase in MDA levels correlates to producing reactive oxygen species (ROS), thus causing oxidative stress.[36] Formation of MDA can occur when hydroxyl free radicals react with fatty acid components from cell membranes so that a chain reaction known as lipid peroxidation occurs. Lipid peroxidation will cause the breakdown of fatty acid chains into various compounds such as hydrocarbons (pentane, ethane) and aldehydes such as MDA. MDA is a dialdehyde compound that is the end product of lipid peroxidation in the body, which is toxic.[37] High MDA levels can portray the process of liver damage, which, through the cell membrane oxidation process, can damage the cell membranes and lead to elevated liver enzymes. [38]

Radicals of cigarette smoke inhaled into the lungs and the bloodstream will be distributed throughout the body and cause damage to the membranes of the cells of various tissues, one of which is cells of the liver, a processing center of drugs and toxicants.[39-41] Damage to the liver cell membrane causes the liver enzymes in cells to leak and enter the blood circulation, resulting in increased serum levels.[41-43] The serum and salivary liver enzyme levels in male smokers who smoked at least ten cigarettes per day for 15 years or more than 100 over a lifetime increased sharply compared to non-smokers.[13, 44] Microsomal enzymes in the liver bind chemicals such as tar in cigarette smoke, transporting nicotine to the lungs and can cause cancer.[41, 45] Cigarette smoking enhances lipid peroxidation, and this peroxidation is responsible for the observed fatty degeneration in the liver.[46]

The rats treated with juice (JcHc) or extract (ExHc) had lower MDA, ALT, and AST levels than the group exposed to cigarette smoke only (Csmoke) and even lower than the Control group. It showed that the antioxidant content of *H.costaricensis* peels in juice or extract could reduce MDA, ALT, and AST levels in Wistar rats exposed to cigarette smoke. MDA, ALT, and AST levels in the ExHc group were the lowest compared to the other three groups of rats. Such lower levels, presumably due to the active substance in the extracts, namely anthocyanin and polyphenolic compounds, which function as antioxidants, are higher than in juice and more effective in reducing MDA, ALT, and AST. A higher antioxidant in extracts neutralizes cigarette smoke radicals and can also likely regenerate liver cells damaged by free radicals; therefore, the serum ALT and AST decrease in this group is the highest (lowest level of ALT and AST). All groups did not have differences in bilirubin levels, and this is because bilirubin is not a sensitive indicator to show the effect of antioxidant administration.

The control rats not exposed to cigarette smoke had the highest feed intake, manifested in the highest body weight among all groups. In the Csmoke group, the feed consumption and body weight were lowest among all groups, which proved that exposure to cigarette smoke decreased appetite in Wistar rats, whereas, in the group of *H.costaricensis* peel juice (JcHc) and extract (ExHc), the feed intake and body weight are higher than the group exposed to cigarette smoke only (Csmoke) even though it is still not equal to Control group. It also proved that supplying *H.costaricensis* peel juice or extracts as antioxidants improved cigarette smoke-decreased appetite in Wistar rats. The group exposed to cigarette smoke only (Csmoke) consumed more drinks than the rats not exposed to cigarette smoke (Control). These results showed that exposure to cigarette smoke increases drinking water consumption, possibly due to higher water requirements to neutralize or metabolize radicals in inhaled cigarette smoke. Such a mechanism was supported by increasing serum ALT and AST levels in the Csmoke group. Exposure to cigarette smoke radicals in vivo causes lipid peroxidation, which causes damage to normal liver cell membranes. The damage can cause leakage of intra-cellular transaminase enzymes into the blood resulting in increased levels of AST and ALT.[47] The rats receiving *H.costaricensis* peel juice (JcHc) consumed less drinks than the group given *H.costaricensis* peels extract (ExHc), which is probably an indication that the intake of *H.costaricensis* peels extract (ExHc) requires more water consumption to metabolize higher levels of the active substance (anthocyanin and polyphenol). This mechanism is in line with the metabolism of polyphenolic compounds *in vivo* by conversion into a more hydrophilic form and enabling their excretion via bile or urine.[48, 49]

CONCLUSIONS

Exposure to one cigarette smoke twice daily caused an increase in oxidative stress as indicated by elevated MDA levels, liver cell damage characterized by a marked increase in serum AST and ALT levels, water consumption, decreased feed consumption, and weight loss. Daily intake of the juice or ethanol extract of *H. costaricensis* fruit peels can restore the disrupted MDA, AST and ALT, feed and drink intake, and weight loss. Therefore, the underutilized peels of *H. costaricensis* fruit can be developed into a valuable product to prevent cigarette smoke-induced oxidative stress and reduce food waste.

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REFERENCES

1. TSS Collaborative Group. A cross country comparison of exposure to secondhand smoke among youth. *Tob Control* 2006; 15 Suppl 2, ii4-19. DOI: 10.1136/tc.2006.015685
2. Brody DJ, Faust E, Tsai J. Secondhand smoke exposure am ong nonsmoking adults: United States, 2015-2018. NCHS Data Brief. 2021;(396):1-8
3. Hashemi-Aghdam MR, Shafiee G, Ebrahimi M, Ejtahed HS, Yaseri M, Motlagh ME, et al. Trend of passive smoking and associated factors in Iranian children and adolescents: the CASPIAN studies. BMC Public Health. 2022;22(1):603. DOI: 10.1186/s12889-022-13045-8
4. Pope CA, Burnett RT, Turner MC, Cohen A, Krewski D, Jerrett M. et al. Lung cancer and cardiovascular disease mortality associated with ambient air pollution and cigarette smoke: shape of the exposure–response relationships. Environ. Health Perspect. 2011; 119(11):1616-1621. DOI: 10.1289/ehp.1103639
5. Wang Y, Qi F, Jia X, Lin P, Liu H, Geng M. et al. Mortality and burden of disease attributable to cigarette smoking in Qingdao, China. Int. J. Environ. Res. Public Health. 2016; 13(9):898. DOI: 10.3390/ijerph13090898
6. GBD 2019 Tobacco Collaborators. Spatial, temporal, and demographic patterns in prevalence of smoking tobacco use and attributable disease burden in 204 countries and territories, 1990-2019: a systematic analysis from the Global Burden of Disease Study 2019. Lancet. 2021;397(10292):2337-2360. DOI: 10.1016/S0140-6736(21)01169-7
7. May SM, Li JT. Burden of chronic obstructive pulmonary disease: healthcare costs and beyond. Allergy and Asthma Proc. 2015; 36(1):4-10. DOI: 10.2500/aap.2015.36.3812
8. Talhout R, Schulz T, Florek E, Benthem JV, Wester P, Opperhuizen A. Hazardous compounds in tobacco smoke. Int. J. Environ. Res. Public Health. 2011; 8(2):613-628. DOI: 10.3390/ijerph8020613
9. Pasupathi P, Saravanan G, Chinnaswamy P, Bakthavathsalam G. Effect of chronic smoking on lipid peroxidation and antioxidant status in gastric carcinoma patients. Indian J. Gastroenterology. 2009; 28(2):65-67. DOI: 10.1007/s12664-009-0021-4
10. Padmavathi P, Raghu PS, Reddy VD, Bulle S, Maturu P, et al. Chronic cigarette smoking-induced oxidative/nitrosative stress in human erythrocytes and platelets. Mol. Cell. Toxicol. 2018; 14:27-34. DOI: 10.1007/s13273-018-0004-6
11. Aspera-Werz RH, Ehnert S, Heid D, Zhu S, Chen T, Barun B, et al. Nicotine and cotinine inhibit catalase and glutathione reductase activity contributing to the impaired osteogenesis of scp-1 cells exposed to cigarette smoke. Oxid. Med. Cell. Longev. 2018(3172480). DOI: 10.1155/2018/3172480
12. Naresh CK, Rao SM, Shetty PR, Ranganath V, Patil AS, Anu AJ. Salivary antioxidant enzymes and lipid peroxidation product malondialdehyde and sialic acid levels among smokers and non-smokers with chronic periodontitis-A clinico-biochemical study. Family Med. Prim. Care 2019; 8(9):2960-2964. DOI: 10.4103/jfmpc.jfmpc_438_19
13. Alsalhen KS, Abdalsalam RD. Effect of cigarette smoking on liver functions: a comparative study conducted among smokers and non-smokers male in El-beida City, Libya. Int. Curr. Pharm. J 2014;3(7):291-295. DOI: 10.3329/icpj.v3i7.19077
14. Kim SA, Shin S. Fruit and vegetable consumption and non-alcoholic fatty liver disease among Korean adults: a prospective cohort study. J. Epidemiol. Community Health. 2020; 74(12):1035-1042. DOI: 10.1136/jech-2020-214568
15. Lu W, Shi Y, Wang R, Su D, Tang M, Liu Y, et al. Antioxidant activity and healthy benefits of natural pigments in fruits: A Review. Int. J. Mol. Sci. 2021; 22(9):4945. DOI: 10.3390/ijms22094945
16. Akbari B, Baghaei-Yazdi N, Bahmaie M, Abhari FM. The role of plant-derived natural antioxidants in reduction of oxidative stress. BioFactors. 2022;48(3):611-633. DOI: 10.1002/bopf.1831
17. Wu LC, Hsu HW, Chen YC, Chiu CC, Lin YI, Ho JA. Antioxidant and antiproliferative activities of red pitaya. Food Chem. 2006; 95(2):319-327. DOI: 10.1016/j.foodchem.2005.01.002
18. Putri NKM, Gunawan IWG, Suarsa IW. Aktivitas antioksidan antosianin dalam ekstrak etanol kulit buah naga super merah (*Hylocereus costaricensis*) dan analisis kadar totalnya. J. Chem. 2015; 9(2):254-248. DOI: 10.24843/JCHEM.2015.v09.i02.p15

19. Nurliyana R, Zahir IS, Suleiman KM, Aisyah MR, Rahim KK. Antioxidant study of pulps and peels of dragon fruits: a comparative study. Int. Food Res. J 2010; 17:367-375.
20. Ahmadkhaniha R, Yousefian F, Rastkari N. Impact of smoking on oxidant/antioxidant status and oxidative stress index levels in serum of the university students. J Environ Health Sci Eng. 2021;19(1):1043-1046. DOI: 10.1007/s40201-021-00669-y
21. Tsuchiya M, Asada A, Kasahara E, Sato EF, Shindo M, Inoue M. Smoking a single cigarette rapidly reduces combined concentrations of nitrate and nitrite and concentrations of antioxidants in plasma. Circulation. 2002;105(10):1155-7. DOI: 10.1161/hc1002.105935
22. Alberg AJ, Byers P. Cigarette Smoking and Endogenous Antioxidants. In: Laher, I. (eds) Systems Biology of Free Radicals and Antioxidants. 2014. DOI: 10.1007/978-3-642-30018-9_124
23. Clause BT. The Wistar rat as a right choice: establishing mammalian standards and the ideal of a standardized mammal. J Hist Biol. 1993;26:329–349. DOI: 10.1007/BF01061973
24. Plaskova A, Mlcek J. New insights of the application of water or ethanol-water plant extract rich in active compounds in food. Front Nutr. 2023;10:1118761. DOI: 10.3389/fnut.2023.1118761
25. Reeves PG, Nielsen FH, Fahey GC. AIN-93 Purified diets for laboratory rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. J Nut. 1993;123(11): 1939-1951. DOI: 10.1093/jn/123.11.1939
26. Rachma DE, Murwani R, Juniarto AZ. Dietary fresh and boiled mangkokan leaves (*Nothopanax scutellarius*) normalised body weight, serum lipid profile and malondialdehyde in metabolic syndrome rats. Curr Res Nutr Food Sci. 2020;8(3):889-902. DOI: 10.12944/CRNFSJ.8.3.19
27. Hanifah IDN, Murwani R, Juniarto AZ. *Etlingera elatior* (Jack) R.M, Sm Containing Diet Normalizes Some Metabolic Syndrome Markers due to High-fat High-fructose Diet in Wistar Rats. Current Nut Food Sci. 2021;17(7): 727-736. DOI: 10.2174/1573401316666201208101359
28. Murwani R, Kusumanti E, Naumova EN. *Areca catechu L.* and *Anredera cordifolia (Ten) Steenis* supplementation reduces faecal parasites and improves caecal histopathology in laying hens. Int J Vet Sci Med. 2022;10: 52-63. DOI: 10.1080/23144599.2022.2090732
29. Murwani R, Bayuardhi B. Broilers serum cholesterol and glutamic oxaloacetic transaminase and their relation to antibiotic in feed and medication programs in four broiler producers in Semarang region-Central Java, Indonesia. Int. J. Poult. Sci. 2007;6(4):266–270. DOI: 10.3923/IJPS.2007.266.270
30. Murwani R, Indriani A, Yuliana I, Wihardani K. Blood biochemical indices and productivity of broilers on diet supplemented with mannan oligosaccharide, baker yeast, or combined baker yeast and noni leaves extracts. Int. J. Poult. Sci. 2011;10(12):990–997. DOI: 10.3923/ijps.2011.991.998
31. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. Can. Med. Assoc. J. 2005;172(3):367-379. DOI: 10.1503/cmaj.1040752
32. Center SA. Interpretation of liver enzymes. Vet. Clin. North. Am. Small Anim. Pract. 2007;37(2):297-333. DOI: 10.1016/j.cvsm.2006.11.009
33. Malhi H, Gores GJ. Cellular and molecular mechanisms of liver injury. Gastroenterology, 2008;134(6):1641-1654. DOI: 10.1053/j.gastro.2008.03.002
34. 34.Altamiran J, Bataller R. Cigarette smoking and chronic liver diseases. Gut. 2010;59(9):1159-1162. DOI: 10.1136/gut.2008.162453
35. Marti-Aguado D, Clemente-Sanchez A, Bataller R. Cigarette smoking and liver diseases. J. Hepatol. 2022;77(1):191-205. DOI: 10.1016/j/jhep.2022.01.016
36. Khaled S, Makled MN, Nader MA. Tiron protects against nicotine-induced lung and liver injury through antioxidant and anti-inflammatory actions in rats in vivo. Life Sci. 2020;1(260). DOI: 10.1016/j.lfs.2020.118426
37. Yin H, Xu L, Porter NA. Free radical lipid peroxidation: Mechanisms and analysis. Chem. Rev. 2011; 111(10):5944-5972. DOI: 10.1021/cr200084z
38. Seriner R, Dağlıoğlu K, Coşkun G, Bilgin R. Examination of the effect of curcumin in experimental liver damage created by diethylnitrosamine in Swiss albino mice to superoxide dismutase and catalase activities and glutathione, malondialdehyde, and advanced oxidation protein products levels. Biotechnol. Appl. Biochem. 2022;69(3):1217-1225. DOI: 10.1002/bab.2198
39. Li L, Yang DC, Chen CH. Metabolic reprogramming: A driver of cigarette smoke-induced inflammatory lung diseases. Free Radic. Biol. and Med. 2021;163:392-401. DOI: 10.1016/j.freeradbiomed.2020.12.438

40. Premkumar M, Anand AC. Tobacco, cigarettes, and the liver: the smoking gun. J Clin Exp Hepatol. 2021;11(6):700-712. DOI: 10.1016/j.jceh.2021.07.016
41. Jabbar DK, Abdul-Hassan HK. Cigarette Smoking and Serum Liver Enzymes. J. University of Babylon for Pure and Appl Sci. 2017;25(3):1111-1117.
42. Al-Awaida W, Akash M, Aburubaiha Z, Talib WH, Shehadeh H. Chinese green tea consumption reduces oxidative stress, inflammation and tissues damage in smoke exposed rats. Iran J Basic Med Sci. 2014;17(10):740-6. DOI: 10.22038/IJBMS.2014.3445
43. Napierala M, Olszewski J, Miechowicz I, Jablecka A, Czarnywojtek A, Malinger S, et al. The influence of tobacco smoke exposure on selected markers of oxidative stress, kidneys and liver function in the serum of rats with streptozotocin-induced diabetes. Pharmacol. Reports. 2019;71:1293-1298. DOI: 10.1016/j.pharep.2019.07.012
44. Patil AS, Ranganath V, Kumar CN, Naik R, John AA, Pharande SB. Evaluation of salivary biomarkers of periodontitis among smokers and non-smokers: A novel study. J Family Med Prim Care. 2020;9(2):1136-1142. DOI: 10.4103/jfmpc.jfmpc_937_19
45. Jain D, Chaudhary P, Varshney N, Razzak KSB, Verma D, Zahra TRK, et al. Tobacco smoking and liver cancer risk: potential avenues for carcinogenesis. J. Oncol 2021;10(2021):1- 11. DOI: 10.1155/2021/5905357
46. Kumar S, Mahat RK, Batra J. Evaluation of lipid parameters, liver function test, CRP and MDA (as a marker of lipid peroxidation) in chronic cigarette smokers. Int. J. Biomed and Adv. Res. 2015; 6(02):115-119. DOI: 10.7439/IJBAR.V6I2.1636
47. Contreras-Zentelle ML, Hernández-Muñoz R. Is Liver Enzyme Release Really Associated with Cell Necrosis Induced by Oxidant Stress? Oxid. Med. Cell. Longev. 2016;(3529149). DOI: 10.1155/2016/3529149
48. Bešlo D, Golubić N, Rastija V, Agić D, Karnaš M, Šubarić D, Lučić B. Antioxidant Activity, Metabolism, and Bioavailability of Polyphenols in the Diet of Animals. Antioxidants. 2023; 12(6):1141. DOI: 10.3390/antiox12061141
49. Stromsnes K, Lagzdina R, Olaso-Gonzalez G, Gimeno-Mallench L, Gambini J. Pharmacological Properties of Polyphenols: Bioavailability, Mechanisms of Action, and Biological Effects in In Vitro Studies, Animal Models, and Humans. Biomed. 2021; 9(8):1074. DOI: 10.3390/biomedicines9081074