

1
2
3
4
5
6
7
9
10

Toxicity of Local Natural Ingredients from East Java, Indonesia Yam Bean (*Pachyrhizus erosus*) and Avocado (*Persea americana Mill*) on the Liver and Kidney Structure of Sprague Dawley Rats

17
18
19
20
21
22

ABSTRACT

Aims: The aim of the research was to test the toxicity of jicama (*Pachyrhizus erosus*) and avocado (*Persea americana Mill*) on the structure of liver and kidney tissue in Sprague Dawley rats.

Study Design: Male Sprague Dawley rats aged 5 months, 24 animals. Treatment of synthetic and natural ingredients. 6 treatment groups were given daidzein (P1), 6 treatment groups were given niacin (P2), 6 treatment groups were given jicama (*Pachyrhizus erosus*) (P3) and 6 treatment groups were given avocado (*Persea americana Mill*) (P4). Each treatment was carried out for 28 days. On the 29th day, surgery was performed and the liver and kidneys were removed, followed by HE staining.

Result: The results showed that the structure of liver and kidney tissue given daidzein and niacin experienced necrosis, increased vascularization, hemorrhage and fatty degeneration.

Conclusion: The use of natural ingredients does not produce toxic (harmful) effects in the body compared to synthetic substances. The percentage of kidney necrosis with daidzein administration is 24%, and with niacin administration, it is 29%. The percentage of liver necrosis with daidzein administration is 20%, and with niacin administration, it is 24%.

23
24
25
26
27
28
29

Keywords: toxicity, natural ingredients, liver, kidney

1. INTRODUCTION

30 Biodiversity is a wealth of Indonesia's natural resources and contributes to various aspects of
31 life. Various plant species in each region originate from different countries or are native to
32 Indonesia, thriving well in this tropical country. One aspect of biodiversity is medicinal plants.
33 To date, medicinal plants have not been cultivated or utilized optimally. The utilization of
34 medicinal plants as a potential local wisdom is declining due to the dominance of
35 pharmaceutical developments in the health sector. Synthetic substances have dominated the
36 pharmaceutical industry, leading to the extinction of local plants with medicinal potential due
37 to underutilization.

38 Modern society views treatment with medicinal plants as unscientific and unreliable in terms
39 of safety. People prefer treatment with synthetic substances over natural ingredients. The
40 pharmaceutical field has significantly advanced with the production of synthetic compounds
41 considered more effective and efficient for treatment. Modern society prefers treatment using
42 synthetic compound formulations over medicinal plants.

43 Medicinal plants have very complex chemical compounds that interact with each other [1], [2],
44 [3]. The use of medicinal plants as natural ingredients with multi-component compounds differs
45 from the use of synthetic substances [2], [4]. This is because synthetic substances are single
46 compounds with one activity and one target [5], [6]. Natural ingredients as medicines usually
47 consist of one or more mixtures processed together, making the compounds more complex
48 [7], [8]. Additionally, their pharmacokinetics and pharmacodynamics become more complex
49 due to interactions between multi-component compounds within the biological system of the
50 body [9], [10].

51 There is a growing trend of lifestyle changes towards 'back to nature,' utilizing natural
52 ingredients as therapy for various diseases. *Pachyrhizus erosus* (yam bean) is one local plant
53 that grows very well in tropical climates like Indonesia. In Indonesia, yam bean is usually
54 consumed fresh in salads, fruit salads, and juices. Its development has reached the cosmetic
55 industry. However, its utilization in the health sector in Indonesia has not yet been realized.
56 Research findings indicate that yam bean tubers contain phytoestrogen compounds, such as
57 daidzein and genistein, which are isoflavone compounds with a chemical structure similar to
58 estrogen hormone [11], [12], [13]. These compounds can be used for estrogen replacement
59 therapy [14], [15], [16].

60 *Persea americana* Mill (avocado) is another local plant that thrives in tropical climates. In
61 Indonesia, avocados are usually consumed fresh in desserts and as a bread topping. The
62 development of avocados in the cosmetic industry includes hand lotions, moisturizers, and
63 face masks. Research findings indicate that avocados contain complex compounds beneficial
64 for lowering cholesterol levels [17], [18]. Avocados contain several active ingredients
65 suspected to lower cholesterol levels, including pantethine, niacin (vitamin B3), beta-sitosterol,
66 vitamin C, vitamin E, vitamin A (beta carotene), pantothenic acid, oleic acid, folic acid,
67 selenium, amino acids, and fiber [18], [19].

68 Medicinal plants have complex compounds that can interact within the body's system [1], [20].
69 The interaction of complex compounds in medicinal plants can provide physiological potential
70 within the body's system. Long-term use of medicinal plants does not pose adverse effects on
71 the body [21], [22], [23]. Medicinal plants contain various bioactive compounds that enhance
72 physiological activity within the body, providing better effects [24], [25], [26].

73 The metabolism of plant compounds occurs in the kidneys and liver. One of the kidney's
74 functions is to cleanse the body of waste products from digestion or metabolism [27], [28]. The
75 liver also functions as a detoxification organ [29], [30]. Therefore, the effectiveness and safety

76 of medicinal plants for health applications require toxicity testing. Toxicity tests are conducted
77 to analyze the safety of treatments concerning liver and kidney necrosis. The purpose of this
78 study is to examine the preclinical effects of yam bean and avocado consumption on test
79 animals by observing changes in the histological structure of the kidneys and liver.

80

81 **2. MATERIAL AND METHODS**

82 **Materials**

83 Yam bean tubers were obtained from the plantations on the slopes of Mount Wilis in Madiun
84 Regency, East Java, Indonesia. Avocado fruits were obtained from plantations in Dolopo,
85 Madiun Regency, East Java, Indonesia. The yam bean tubers and avocado fruits were
86 harvested when ripe. Male Sprague Dawley rats, 5 months old, 24 in total, were used. Rat
87 feed included pellet milk A, 10% formalin, liquid paraffin, xylene, xylol, Hematoxylin-Eosin
88 (HE), 70% alcohol, 80% alcohol, 96% alcohol, paraffin, physiological NaCl 0.9%, 50% alcohol,
89 70% alcohol, absolute alcohol, xylol, a mixture of xylol-alcohol with xylol ratios of 1:3, 2:2, and
90 3:1, Li₂CO₃ solution, 1% HCl, PBS, eosin, 3% formalin and haupt, 25 mg/kg daidzein, 10
91 mg/kg niacin, 1.5 ml yam bean tuber juice, and 1.5 ml avocado juice.

92 **Method**

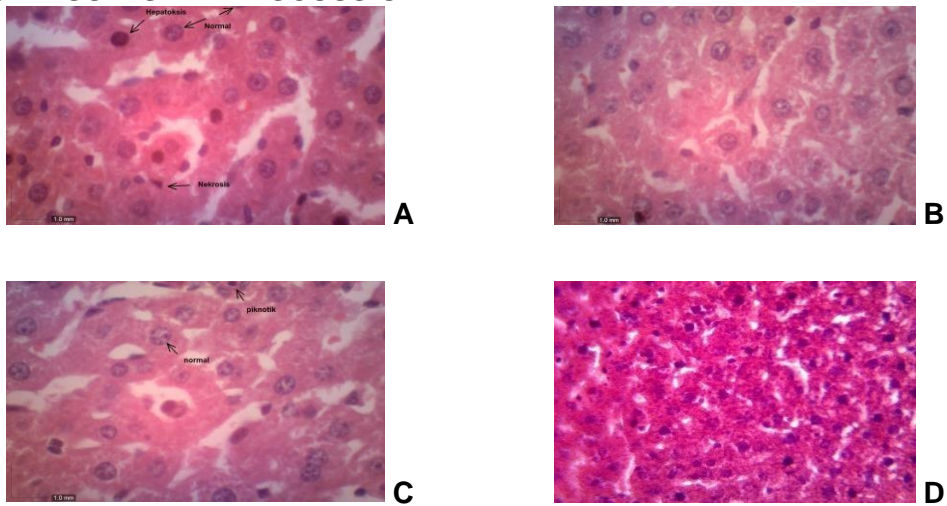
93 **Maintenance and Treatment of Test Animals**

94 Male Sprague Dawley rats, 5 months old, were obtained from LPPT Gadjah Mada University.
95 A total of 24 rats, weighing 250-350 grams, were kept in group cages and acclimatized for 7
96 days. The rats were divided into 6 treatment groups: daidzein administration (P1), niacin
97 administration (P2), yam bean tuber juice administration (*Pachyrhizus erosus*) (P3), and
98 avocado juice administration (*Persea americana* Mill) (P4). The treatment lasted for 28 days,
99 and on the 29th day, surgery and organ (liver and kidney) removal were performed, followed
100 by HE staining.

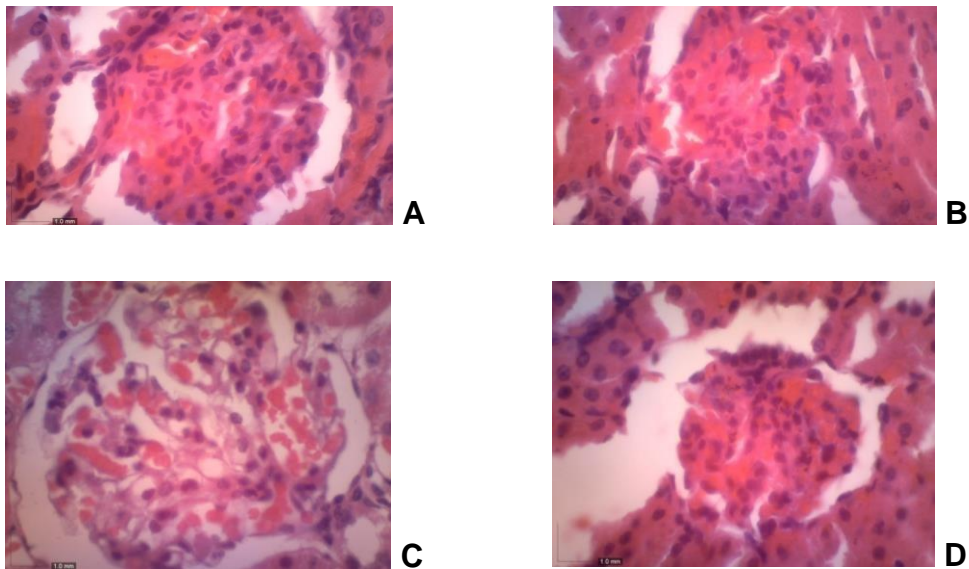
101 **Data Analysis**

102 Descriptive data analysis was conducted using an Optilab microscope to observe changes in
103 the structure of hepatocyte and glomerular cells. The percentage of necrotic cells in the
104 hepatocytes and glomeruli was calculated.

105 **3. RESULTS AND DISCUSSION**



106 Figure 1. HE staining rat kidney, 400X A) Necrosis and sinusoidal dilation in hepatocytes
 107 treated with daidzein, B) Normal hepatocytes in yam bean tuber treatment, C) Necrosis and
 108 sinusoidal dilation, pyknosis of nuclei in hepatocytes treated with niacin, D) Normal
 109 hepatocytes in avocado treatment.

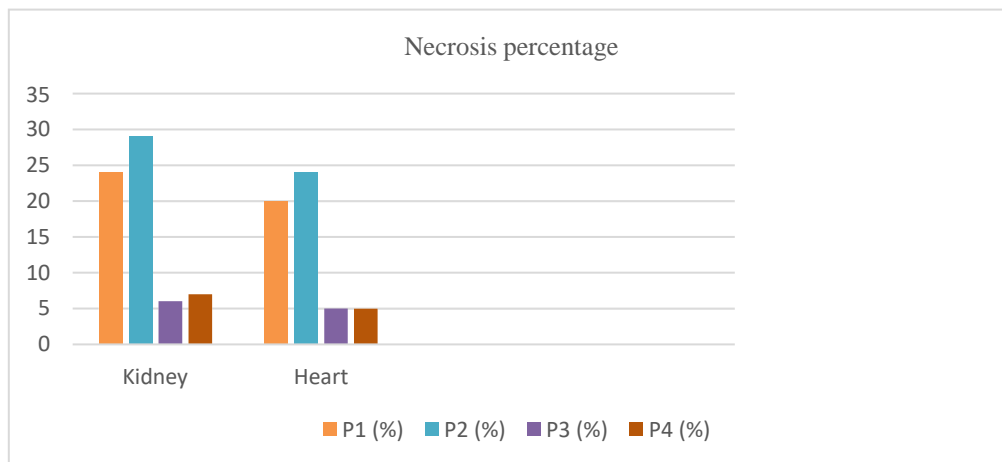


110 Figure 2. HE staining rat glomerulus, 400X A) Necrosis and vascularization in treatment with
 111 daidzein; B) normal glomerulus in yam bean tuber treatment; C) Necrosis, fatty degeneration
 112 and vascularization in treatment with niacin; D) normal glomerulus in avocado treatment

113 Table 1. Percentage kidney and glomerulus necrosis in rats

No	Organ terdeteksi	Sel nekrosis (%)			
		P1	P2	P3	P4
1	Kidney	24	29	6	7

114



115
116

Figure 3. Necrosis Percentage

117 The liver is an important organ in the body that functions to detoxify various substances
 118 ingested by the digestive tract. The functional unit of the liver is the liver lobule, which is
 119 cylindrical in shape, surrounding the central vein that flows into the hepatic vein, and then into
 120 the vena cava [31], [32]. The liver is crucial in metabolism, detoxification, storage, and
 121 excretion of xenobiotics and their metabolites, and is particularly susceptible to damage [33].
 122 Accumulation of toxic substances in the liver parenchyma can cause hepatocyte cell damage
 123 due to exposure. Histological changes vary depending on the dose, type, effects of the
 124 substance, or other diseases, and susceptibility [34]. Liver damage from toxic substances is
 125 influenced by several factors, such as the type of chemical involved, the dose given, and the
 126 duration of exposure [35], [36].

127 The basic functional unit of the kidney is the nephron, which regulates water and solutes,
 128 especially electrolytes in the body, by filtering the blood [37], [38]. It then reabsorbs fluids and
 129 molecules through the capillaries that are still needed by the body [39], [40]. Chemicals
 130 entering the body through various administration routes generally undergo absorption,
 131 distribution, metabolism, and excretion [41], [42]. The metabolism of medicinal plant
 132 compounds occurs in the kidneys [43], [44]. The kidney is highly susceptible to the toxic effects
 133 of drugs and chemicals. Examination of glomerular and tubular necrosis is one way to
 134 demonstrate kidney cell damage [45].

135 Various phytochemicals in yam bean tubers and avocado fruits can work complexly within the
 136 body. Natural chemical compounds are complex and non-toxic compared to synthetic or
 137 isolated single compounds [46], [47]. Daidzein in yam bean tubers, a compound similar to 17β-
 138 estradiol, has enantiomeric structures, including cis-tetrahydrodaidzein and trans-
 139 tetrahydrodaidzein, which have chiral structures [48]. This increases the number of daidzein-
 140 like compounds that can actively enter the bloodstream. Daidzein is followed into the
 141 bloodstream by genistein and quercetin, phytoestrogen compounds from yam bean tubers
 142 [49], [50]. Avocado fruits contain potassium, phosphorus, calcium, magnesium, niacin,
 143 pyridoxine, riboflavin, thiamine, and biotin [51]. Niacin is a chemical compound that can lower
 144 cholesterol [52], [53].

145 Consuming natural ingredients has better effects on the body than consuming isolated
 146 synthetic substances [54]. This is because natural ingredients have complex chemical

147 compounds that interact with each other, creating beneficial physiological effects [55], [56]. All
148 medicinal plants contain mixtures of active compounds with pharmacological activities [56].

149 Consuming fresh natural ingredients, without processing, shows beneficial effects on the body.
150 This is because the compounds in plants have not been damaged during processing. Non-
151 processed food is considered to have a better chemical composition compared to ultra-
152 processed food, making the consumption of non-processed food in large quantities and over
153 a long period safe and non-toxic. The complexity of natural compounds allows them to work
154 in the body with the principle of balance metabolism, as some compounds enhance and others
155 reduce effects, making complex compounds non-toxic. Based on this analysis, natural
156 ingredients are more moderate compared to synthetic or isolated substances.

157

158 **4. CONCLUSION**

159

160 The use of natural ingredients does not produce toxic (harmful) effects in the body compared
161 to synthetic substances. The percentage of kidney necrosis with daidzein administration is
162 24%, and with niacin administration, it is 29%. The percentage of liver necrosis with daidzein
163 administration is 20%, and with niacin administration, it is 24%.

Ethical Approval

Animal Ethic committee approval has been collected and preserved by the author(s)

164

165 **ACKNOWLEDGEMENTS**

166

167 Thank you to the Institute for Research and Community Service at PGRI Madiun University
168 for providing financial support for this research. Thank you to Ardiana for assisting with the
169 preparation of histological slides and HE staining.

170

171 **AUTHORS' CONTRIBUTIONS**

172

173 Cicilia Novi Primiani designed the study, conceptualization, animal experiment, histology
174 analysis. Joko Widiyanto concept methodology, histology analysis. Rafaella Chandraseta
175 Megananda managed manuscript, literature study, food analysis application.

176

177 **REFERENCES**

178

179 [1] C. G. Awuchi, "Medicinal plants: the medical, food, and nutritional biochemistry and
180 uses," *Int. J. Adv. Acad. Res.*, vol. 5, no. 11, pp. 220-241, 2019.

181 [2] N. Vaou *et al.*, "Interactions between medical plant-derived bioactive compounds:
182 focus on antimicrobial combination effects," *Antibiotics*, vol. 11, no. 8, p. 1014, 2022.

183 [3] F. Noor, M. T. ul Qamar, U. A. Ashfaq, A. Albutti, A. S. Alwashmi, and M. A. Aljasir,
184 "Network pharmacology approach for medicinal plants: review and assessment,"
185 *Pharmaceuticals*, vol. 15, no. 5, p. 572, 2022.

186 [4] M. Bunse *et al.*, "Essential oils as multicomponent mixtures and their potential for
187 human health and well-being," *Front. Pharmacol.*, vol. 13, p. 956541, 2022.

188 [5] C. J. Gerry *et al.*, "Real-time biological annotation of synthetic compounds," *ournal Am.*
189 *Chem. Soc.*, pp. 8920-8927, 2016.

190 [6] G. Martelli and D. Giacomini, "Antibacterial and antioxidant activities for natural and
191 synthetic dual-active compounds," *Eur. J. Med. Chem.*, vol. 158, pp. 91-105, 2018.

192 [7] G. Joana Gil-Chávez *et al.*, "Technologies for extraction and production of bioactive
193 compounds to be used as nutraceuticals and food ingredients: An overview," *Compr.*
194 *Rev. food Sci. food Saf.*, vol. 12, no. 1, pp. 5-23, 2013.

195 [8] F. F. Y. Stéphane, Bankeu Kezetas Jean Jules, G. E. Batiha, Iftikhar Ali, and Lenta
196 Ndjakou Bruno, "Extraction of bioactive compounds from medicinal plants and herbs,"

- 197 *Nat Med Plants*, 2021.
- 198 [9] A. Kumar, A. Adlakaha, and K. Mukherjee, "The effect of perceived security and
199 grievance redressal on continuance intention to use M-wallets in a developing country,"
200 *Int. J. Bank Mark.*, vol. 36, no. 7, pp. 1170-1189, 2018, doi: 10.1108/IJBM-04-2017-
201 0077.
- 202 [10] G. M. Husain, M. A. Khan, M. Urooj, and M. H. Kazmi, "Pharmacodynamic Evaluation:
203 Herbal Medicine," *Drug Discov. Eval. Methods Clin. Pharmacol.*, pp. 483-497, 2020.
- 204 [11] C. N. Primiani, "The phytoestrogenic potential of yam bean (*Pachyrhizus erosus*) on
205 ovarian and uterine tissue structure of premenopausal mice," *Biol. Med. Nat. Prod.*
206 *Chem.*, vol. 4, no. 1, pp. 5-9, 2015.
- 207 [12] P. Trisunuwati, "Efficacy of Water Clover Extract (*Marsilea crenata*) Against Blood
208 Estrogen Progesteron Balance, Blood Calcium Levels and Impact on Dense of Bone
209 Tissue of Rat (*Rattus norvegicus*)," *Res. J. Life Sci.*, vol. 4, no. 1, pp. 50-55, 2017.
- 210 [13] E. F. Hadiningsih *et al.*, "The effect of bengkuang (*Pachyrhizus erosus*) ethanol extract on
211 the number of ovarian follicles, amount of epithelium and endometrium stroma cells
212 in DMPA-treated *Rattus norvegicus*," in *AIP Conference Proceedings*, 2020.
- 213 [14] D. M. Țiț, A. Pallag, C. Iovan, G. Furău, C. Furău, and S. Bungău, "Somatic-vegetative
214 symptoms evolution in postmenopausal women treated with phytoestrogens and
215 hormone replacement therapy," *Iran. J. Public Health*, vol. 46, no. 11, p. 1528, 2017.
- 216 [15] D. M. Tit *et al.*, "Effects of the hormone replacement therapy and of soy isoflavones on
217 bone resorption in postmenopause," *J. Clin. Med.*, vol. 7, no. 10, p. 297, 2018.
- 218 [16] J. Li *et al.*, "Efficacy and safety of phytoestrogens in the treatment of perimenopausal
219 and postmenopausal depressive disorders: A systematic review and meta-analysis,"
220 *Int. J. Clin. Pract.*, vol. 75, no. 10, 2021.
- 221 [17] A. L. Ramos-Aguilar *et al.*, "The importance of the bioactive compounds of avocado
222 fruit (*Persea americana* Mill) on human health," *Biotecnia*, vol. 21, no. 3, pp. 154-162,
223 2019.
- 224 [18] P. Harnis, Y. A. Sari, and M. A. Rahman, "Segmentasi Citra Kue Tradisional
225 menggunakan Otsu Thresholding pada Ruang Warna CIE LAB," *Pengemb. Teknol.*
226 *Inf. Dan Ilmu Komput*, p. 2548, 2019.
- 227 [19] G. James-Martin, P. Brooker, G. Hendrie, and W. Stonehouse, "). A Review of the
228 Health Effects of Avocados.," 2022.
- 229 [20] A. Gurib-Fakim, "Medicinal plants: traditions of yesterday and drugs of tomorrow," *Mol.*
230 *Aspects Med.*, vol. 27, no. 1, pp. 1-93, 2006.
- 231 [21] S. Kumar, G. J. Dobos, and T. Rampp, "The significance of ayurvedic medicinal plants,"
232 *J. Evid. Based. Complementary Altern. Med.*, vol. 22, no. 1, pp. 494-501, 2017.
- 233 [22] Ćwieląg-Drabek *et al.*, "Health risks from consumption of medicinal plant dietary
234 supplements," *Food Sci. Nutr.*, vol. 8, no. 7, pp. 3535-3544, 2020.
- 235 [23] C. Meng *et al.*, "Ecological and health risk assessment of heavy metals in soil and
236 Chinese herbal medicines," *Environ. Geochem. Health*, pp. 1-12, 2022.
- 237 [24] G. Mustafa, R. Arif, A. Atta, S. Sharif, and A. Jamil, "Bioactive compounds from
238 medicinal plants and their importance in drug discovery in Pakistan," *Matrix Sci.*
239 *Pharma*, vol. 1, no. 1, pp. 17-26, 2017.
- 240 [25] S. Mickyaray, "Efficacy and mechanism of traditional medicinal plants and bioactive
241 compounds against clinically important pathogens," *Antibiotics*, vol. 8, no. 4, p. 257,
242 2019.
- 243 [26] L. Rathor, "Medicinal plants: A rich source of bioactive molecules used in drug
244 development." Evidence Based Validation of Traditional Medicines: A comprehensive
245 Approach," pp. 195-209, 2021.
- 246 [27] B. Meijers, R. Farré, S. Dejongh, M. Vicario, and P. Evenepoel, "Intestinal barrier
247 function in chronic kidney disease," *Evenepoel*, vol. 10, no. 7, p. 298, 2018.
- 248 [28] J. E. Hall and Michael E. Hall, *Guyton and Hall Textbook of Medical Physiology*. 2020.
- 249 [29] J. C. Ozougwu, "Physiology of the liver," *Int. J. Res. Pharm. Biosci.*, vol. 4, no. 8, pp.

- 250 13-24, 2017.
- 251 [30] M. Hassani, "Liver Structure, Function and its Interrelationships with Other Organs," *Int*
- 252 *J Dent Med Sci Res*, vol. 4, no. 1, pp. 88-92, 2022.
- 253 [31] J. Bruslé and G. G. i Anadon, "The structure and function of fish liver," *Fish Morphol.*,
- 254 pp. 77-93, 2017.
- 255 [32] S. S. Usanov, "Anatomical and Histological Parameters of the Liver of White Nonbored
- 256 Rats in Normal," *BARQARORLIK VA YETAKCHI TADQIQOTLAR ONLAYN ILMiy*
- 257 *JURNALI*, vol. 2, no. 1, pp. 123-128, 2022.
- 258 [33] M. . Brzoska, J. Moniuszko-Jakoniuk, B. iłat-Marcinkiewicz, and B. Sawicki, "Liver and
- 259 kidney function and hystology in rats exposed to cadmium and ethanol," *Alcohol*
- 260 *Alcohol.*, vol. 38, no. 1, pp. 2-10, 2003.
- 261 [34] Cramer K, T. Charrois, and S. Vohra, "Valerian practical management of adverse
- 262 effects and drug interactions," *Can. Pharm. J.*, vol. 139, no. 3, pp. 39-41, 2006.
- 263 [35] D. E. Hinton, H. Segner, and T. Braunbeck, "Toxic responses of the liver," *Target organ*
- 264 *Toxic. Mar. Freshw. teleosts*, pp. 224-268, 2017.
- 265 [36] "The liver as a target organ for toxicity," *Timbrell, John A.* , pp. 145-173, 2020.
- 266 [37] D. . Eaton and J. . Pooler, "Vander's renal physiology. Mc Graw Hil Medicall.," 2009.
- 267 [38] M. Nair, "The renal system," *Fundam. Anat. Physiol. Nurs. Healthc. Students.*, 2016.
- 268 [39] O. Nakonechna, S. Stetsenko, and A. Bondareva, *Water-salt and mineral metabolism.*
- 269 *Urinary function of kidney.* 2020.
- 270 [40] Л. М. Ростока, А. Д. Сіткар, and Я. Ю. Бурмістрова, "Functional biochemistry of
- 271 blood, liver and kidneys.," 2021.
- 272 [41] T. A. Mace *et al.*, "Bioactive compounds or metabolites from black raspberries
- 273 modulate T lymphocyte proliferation, myeloid cell differentiation and Jak/STAT
- 274 signaling," *Cancer Immunol Immunother*, vol. 63, no. 9, pp. 889-900, 2014, doi:
- 275 10.1007/s00262-014-1564-5.Bioactive.
- 276 [42] J. W. Osborn, R. Tyshynsky, and L. Vulchanova, "). Function of renal nerves in kidney
- 277 physiology and pathophysiology," *Annu. Rev. Physiol.*, vol. 83, pp. 429-450, 2021.
- 278 [43] R. . Yudhani, R. . Pesik, S. Azzahro, A. . Anisa, and R. Hendriyani, "Renal function
- 279 parameter on acute toxicity test of kapulaga (*Amomum cardamom*) seed extract in
- 280 rat.," *IOP Conf. Ser. Mater. Sci. Eng.*, vol. 578, 2019.
- 281 [44] A. Salazar-Gómez, J. C. Ontiveros-Rodríguez, S. S. Pablo-Pérez, E. Vargas-Díaz, and
- 282 L. Garduño-Siciliano, "The potential role of sesquiterpene lactones isolated from
- 283 medicinal plants in the treatment of the metabolic syndrome-A review," *South African*
- 284 *J. Bot.*, vol. 135, pp. 240-251, 2020.
- 285 [45] Sancho-Martinez, Sandra M, J. M. Lopez-Novoa, and Lopez-Hernandez,
- 286 "Pathophysiological role of different tubular epithelial cell death modes in acute kidney
- 287 injury," *Clin. Kidney J.*, vol. 8, no. 5, pp. 548-559, 2015.
- 288 [46] Melfi Francesco *et al.*, "Natural products as a source of new anticancer chemotypes,"
- 289 *Expert Opin. Ther. Pat.*, vol. 33, no. 11, pp. 721-744, 2023.
- 290 [47] jemilat A. ibrahim, O. H. Egharevba, and K. S. Gamaniel, "Chemical and biological
- 291 screening approaches to phytopharmaceuticals," *J. Int. Sci.*, vol. 6, no. 10, pp. 22-31,
- 292 2017.
- 293 [48] U. Mariko, "Isoflavone metabolism and bone-sparing effects of daidzein-metabolites,"
- 294 *J. Clin. Biochem. Nutr.*, vol. 52, no. 3, pp. 193-201, 2013.
- 295 [49] S. Lecomte, F. Demay, F. Ferriere, and Pakdel Farzad, "Phytochemicals targeting
- 296 estrogen receptors: beneficial rather than adverse effects?," *Int. J. Mol. Sci.* , vol. 18,
- 297 no. 7, p. 1381, 2017.
- 298 [50] Q. Wang, B. Spenkelink, R. Boonpawa, and I. M. Rietjens, "Use of physiologically
- 299 based pharmacokinetic modeling to predict human gut microbial conversion of daidzein
- 300 to S-Equol. ," *J. Agric. Food Chem.*, vol. 70, no. 1, pp. 343-352, 2021.
- 301 [51] J. Ebbage, "Avocado Nutrition Desktop Research and Best Minds Panel," *Hortic. Innov.*
- 302 *Aust. Sydney.*, 2015.

- 303 [52] P. Mani and A. Rohatgi, "Niacin therapy, HDL cholesterol, and cardiovascular disease:
304 is the HDL hypothesis defunct?," *Curr. Atheroscler. Rep.*, vol. 17, pp. 1-9, 2015.
- 305 [53] S. Rahman, "Effect of Avocades to LDL Cholesterol as a preventive risk of
306 atherosclerosis," *Atherosclerosis*, vol. 4, p. 6, 2019.
- 307 [54] B. Olas, J. Bialecki, K. Urbanska, and M. Brys, "The effects of natural and synthetic
308 blue dyes on human health: A review of current knowledge and therapeutic
309 perspectives," *Adv. Nutr.*, vol. 12, no. 6, pp. 2301-2311, 2021.
- 310 [55] S. A. Hussain, N. R. Panjagari, and G. R. Patil, "Potential herbs and herbal
311 nutraceuticals: food applications and their interactions with food components," *Crit.
312 Rev. Food Sci. Nutr.*, vol. 55, no. 1, pp. 94-112, 2015.
- 313 [56] J. Qishu, R. Wang, Y. Jiang, and B. Liu, "Study on the interaction between active
314 components from traditional Chinese medicine and plasma proteins," *Chem. Cent. J.*,
315 vol. 12, pp. 1-20, 2018.
- 316