Silymarin attenuates Oxidative stress and Nephrotoxicity induced by 5-Fluorouracil in rats

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Abstract. The study aimed to appraise attenuator effect of the Silymarin (SLY) on Oxidative stress and Nephrotoxicity in 5- Fluorouracil treated rats. The study including 60 male rats weighting (180- 200 g) divided into 6 groups including group F (control) receive 0.5 ml normal saline and group A (5-Flu 5 mg /kg), group B (5-Flu 5mg /kg + SLY 3mg /kg), group C (SLY 3mg /kg) for 3o days, group D (SLY 3mg /kg for 15 days after that 5-Flu 5mg /kg for 15 days), group E (5-Flu 5mg /kg for 15 days after that SLY 3mg /kg for 15 days), after animals autopsied, the kidney samples obtained for histological examination and serum collected and stored at – 43 ℃ for biochemical tests. GSH test show no significant difference between group 5-Flu treated rats and control group, while groups treated SLY+5-Flu and SLY only showed a marked increase compared to control at ($P \leq 0.05$). Also the MDA test show groups treated with 5-Flu and 5-Flu +SLY significantly increase compared to control, while group treated with SLY show no significant difference compared to control at ($P \leq 0.05$). The histological examination show the acute tubular necrosis damage percentage elevated compared to control in group treated 5-Flu only, while groups treated with SLY+5-Flu and SLY showed improvement in damage percentage at ($P \leq 0.05$). In conclusion, the Silymarin may provide attenuator role against Oxidative stress and nephrotoxicity induced by 5-Flu drug in the groups that underwent different treatment periods.

1 Introduction

The Reactive oxygen species (ROS) are product from normal cellular metabolism, and they are often associated with the principle of oxidative stress, which suggests that ROS induce pathology by damaging lipids, proteins, and DNA [1]. Oxidative stress (OS) is play a role in the development of many disorders such as Alzheimer's disorder, Parkinson's disorder, diabetes-induced pathologies, rheumatoid arthritis, motor neuron disorders, neuro-degeneration and Oxidative damage in DNA can cause cancer [2]. In particular, the pathological mechanism of cell damage is triggered by the chemotherapy drugs that extreme production of radical oxygen species (ROS) and inflammatory mediators, which alters the cells normal physiological parameters [3]. The 5-Fluorouracil is classified as an anti-metabolic agent and impact the synthesis of DNA and RNA in normal and cancer cells, the majority of 5-Flu is eliminated through liver metabolism and only a Small fraction is removed

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from the body via kidney excretion [4]. The ROS levels are increased after 5-Flu treatment and its induction is also critical to promote both inflammation and drug-mediated apoptotic cell death [5]. Nephrotoxicity caused by chemotherapeutic agents such as 5-Flu drug remainder a remarkable complication limiting their clinical uses and nephrotoxicity induced by 5-Flu was certain by histological changes including glomerular and tubular degeneration and tubular necrosis [6]. Studies have indicated that clinical use of anticancer agents in the fight against malignancies can causes a variety of renal disorders [7]. Meanwhile, other studies have confirmed that 5-Fluorouracil is one of the anticancer drugs that have been related with renal dysfunction [8]. Antioxidants are chemical substances that may protect cells from the damage occurs by unstable molecules known as free radicals, the source and origin of antioxidants which include fruits and vegetables, meats, poultry and fish were treated in many studies, there various types of antioxidants such as ascorbic acid, glutathione, melatonin, tocopherols and tocotrienols [9]. The Silymarin is a polyphenolic composite obtained from the milk thistle (Silybum marianum). The primary constituent of Silymarin is silybin, which is largely responsible for its phytochemical benefits [10]. Numerous studies have highlighted Silymarin abilities, showcasing its antioxidant, anti-inflammatory, anti-neoplastic, anti-fibrotic, and immunomodulatory attributes. Acting as an antioxidant, Silymarin counters ROS, neutralizes free radicals, and bolsters the body's natural antioxidant defenses by enhancing vital antioxidant enzymes like glutathione (GSH) and superoxide dismutase (SOD) [11]. Additionally, it has been observed that Silymarin can restore MDA levels, which suggests its protective role against oxidative damage and lipid peroxidation. Several studies have demonstrated the beneficial effects of silymarin in protecting the liver tissue of rats from various hepatotoxic agents, such as diethylnitrosamine [12], lead acetate, carbon tetrachloride, and acetaminophen [13]. Silymarin can also improve the renal function and prevent the histopathological changes in the kidney tissue. Silymarin can be a potential therapeutic agent for preventing or treating acute kidney injury in humans.

2 Materials and Methods

2.1 Experimental animals

The current study conducted in the Animals House, College of Science, University of Kufa during the period from 14/ 3/ 2023 to 12 / 4 / 2023 under typical condition were followed, including a temperature range of 25–28 °C and a 12-hour light–dark cycle with access to chow and water ad libitum. The rats were adapted for one week before the beginning of the experiment.

2.2 Experimental design

The study including 60 male rats weighting (180- 200 g). The rats divided into 6 groups, each group was consisting of 10 rats and the rats administrated 5-Flu 5mg/kg (Onko llac San, Turkey) (intraperitoneally) and SLY 3mg/kg (NATROL, USA) were dissolved in water and administered orally by gavage needle in variable periods of time.

2.3 Blood and Organs collection

The Blood were collected intracardially directly after animals autopsied. Heart aperture was done by a disposable syringe, 2-5 ml blood was drawn very softly and quietly and placed in a gel tube. The Blood sample centrifuged at 5000 x for 15 min, and serum collected and stored at – 43 ℃ for biochemical tests. The abdomen was unlocked throughout a midline

incision and kidney were readily removed and washed after that taken segment from kidney and set in formalin 10 % for tissue processing.

2.4 Antioxidants Analysis (GSH & MDA tests)

Reduced glutathione in the tissue was determined according to the method of Moron *et al.* (1979) [15]. Whereas Malondialdehyhe was estimated by Thiobarbituric acid (TBA) assay method of Buege & Aust (1978) [16] on spectrophotometer.

2.5 Histological study

The process of preparing histological sections of the kidney, then studying the changes that appear in the sections. When the rats are sacrificed, then the organs come out immediately. The samples are received to make tissue slides according to the method Bancroft and Stevens (1999) [17]; Alturkistani *et al*, (2015) [18].

2.6 Statistical Analysis

Data was analyzed using SPSS (version 26, SPSS Inc. Chicago, Illinois, USA) Statistical analysis was carried out by One-way ANOVA at $p \leq 0.05$ using Duncan's Multiple Range test. The value of $p \leq 0.05$, 0.01 was considered to be statistically significant.

3 Results

3.1 Role Silymarin in reduce ROS production induced by 5-Flu

The results of GSH levels indicates to no significant difference between group A (5 Flu for 30 days) (65 µg/l) and group F (control) (68.24 µg/l), While the group A (5 Flu for 30 days) (65 µg/l) showed a marked decrease compared to groups C (SLY only for 30 days) (95.80 µg/l), E (5-Flu for 15 days and after that SLY for 15 days) (93.92 µg/l), D (SLY for 15 days and after that 5-Flu for 15 days) (91.34 µg/l) and B (5-Flu and SLY for 30 days) (84.66 µg/l) respectively at ($P \leq 0.05$). The group C (SLY only for 30 days) (95.80 µg/l), group E (5-Flu for 15 days after that SLY for 15 days) (93.92 µg/l), groups D (SLY for 15 days after that 5-Flu for 15 days) (91.34 µg/l) and group B (5-Flu and SLY for 30 days) (84.66 µg/l) respectively showed a marked increase compared to group F (control) (68.24 µg/l) at ($P \leq 0.05$). The results of MDA levels of group A (5 Flu for 30 days) (40.99 mmol/l), D (SLY for 15 days after that 5 Flu for 15 days) (40.87 mmol/l), E (5-Flu for 15 days after that SLY for 15 days) (40.73 mmol/l) and B (5-Flu + SLY for 30 days) (39.63 mmol/l) respectively showed a marked increase significantly compared to the group F (control) (27.86 mmol/l) at ($P \leq 0.05$). While no statistically significant differences were found between group C (SLY only for 30 days) (27.41 mmol/l) and group F (control) (27.86 mmol/l) in this regard ($P \leq 0.05$) table (1).

3.2 Role of Silymarin in Reduce Acute Tubular Necrosis damage induced by 5-Flu

The group A (5-Flu for 30 days) showed significant increase in acute tubular necrosis damage percentage (68 %) compare with a group F (control) at ($P \leq 0.05$), also the group A (5-Flu for 30 days) showed significant increase in acute tubular necrosis damage percentage (68 %) compare with other groups B (5-Flu and SLY for 30 days), D (SLY for 15 days and after that

5-Flu for 15 days), E (5-Flu for 15 days and after that SLY for 15 days) and C (SLY only for 30 days) (45%, 33%, 33%, 32%) respectively (P≤ 0.05). The group B (5-Flu and SLY for 30 days) showed significant increase in acute tubular necrosis damage percentage (45%) compare with group F (control) at (P≤ 0.05), the group B (5-Flu and SLY for 30 days) also showed significant increase in acute tubular necrosis damage percentage (45%) compare with group D (SLY for 15 days and after that 5-Flu for 15 days), E (5-Flu for 15 days after that SLY for 15 days) and C (SLY only for 30 days) (33%, 33%, 32%) respectively (P≤ 0.05), While decrease significantly compare with group A (5 Flu for 30 days) (68%) at (P≤ 0.05). The group C (SLY only for 30 days) showed significant increase in acute tubular necrosis damage percentage (32%) compare with group F (control) at (P≤ 0.05), while show significantly decreased compared with group A (5-Flu for 30 days) (68%) at (P≤ 0.05). The groups D (SLY for 15 days after that 5-Flu for 15 days) and E (5-Flu for 15 days after that SLY for 15 days) showed significant increase in acute tubular necrosis damage percentage (33%,33%) compare with group F (control) at (P≤ 0.05), on other hands no significant difference between groups D (SLY for 15 days and after that 5-Flu for 15 days), E (5-Flu for 15 days after that SLY for 15 days) (33%,33%) and group C (SLY only for 30 days) (32%) at (P≤ 0.05). whereas both groups show significantly decrease compared with group A (5 Flu for 30 days) (68%) at (P≤ 0.05) as the following (table 2) & (figure 1).

Table 1. Mean differences between GSH&MDA

Parameters / Groups	GSH (µg/l)	MDA (mmol/l)
	Mean±S.D	
Group A	65.10±4.9 **a**	40.99±3.7 **c**
Group B	84.66±1.4 **b**	39.63±2.1 **b**
Group C	95.80±3.8 **d**	27.41±2.9 **a**
Group D	91.34±8.9 **c**	40.87±4.7 **c**
Group E	93.92±9.5 **cd**	40.73±4.2 **c**
Group F	68.24±6.2 **a**	27.86±3.1 **a**
LSD(0.05)	**3.894**	**9.041**

Table (1): Show Mean differences between GSH&MDA levels for all groups, group A: 5-Flu (5mg/kg) group B: 5-Flu (5mg/kg) + SLY(3mg/kg) for 30 days, group C: SLY(3mg/kg) only for 30 days, group D: SLY(3mg/kg) for15 days and after that 5-Flu(5mg/kg) for 15 days, group E: 5-Flu(5mg/kg) for 15 days and after that SLY(3mg/kg) for 15 days, group F: Control, normal saline 0.5ml, Different letters refer to significant difference at p≤0.05, S.D = Standard Deviation, % = percentage 100%.

Table 2. Mean difference of acute tubular necrosis damage

Parameters / Groups	%
	Mean±S.D
Group A	68±5.9 **d**
Group B	45±5.0 **c**
Group C	32±3.6 **b**
Group D	33±6.7 **b**
Group E	33±4.4 **b**
Group F	0.0±0.0 **a**
LSD(0.05)	**7.872**

Table (2): Show Mean difference of acute tubular necrosis damage percentage in Kidney for all groups, group A: 5-Flu (5mg/kg) group B: 5-Flu (5mg/kg) + SLY(3mg/kg) for 30 days, group C: SLY(3mg/kg) only for 30 days, group D: SLY(3mg/kg) for15 days and after that 5-Flu(5mg/kg) for 15 days, group E: 5-Flu(5mg/kg) for 15 days and after that SLY(3mg/kg) for 15 days, group F: Control, normal saline 0.5ml, Different letters refer to significant difference at $p \leq 0.05$, n =10, S.D = Standard Deviation, % = percentage 100%.

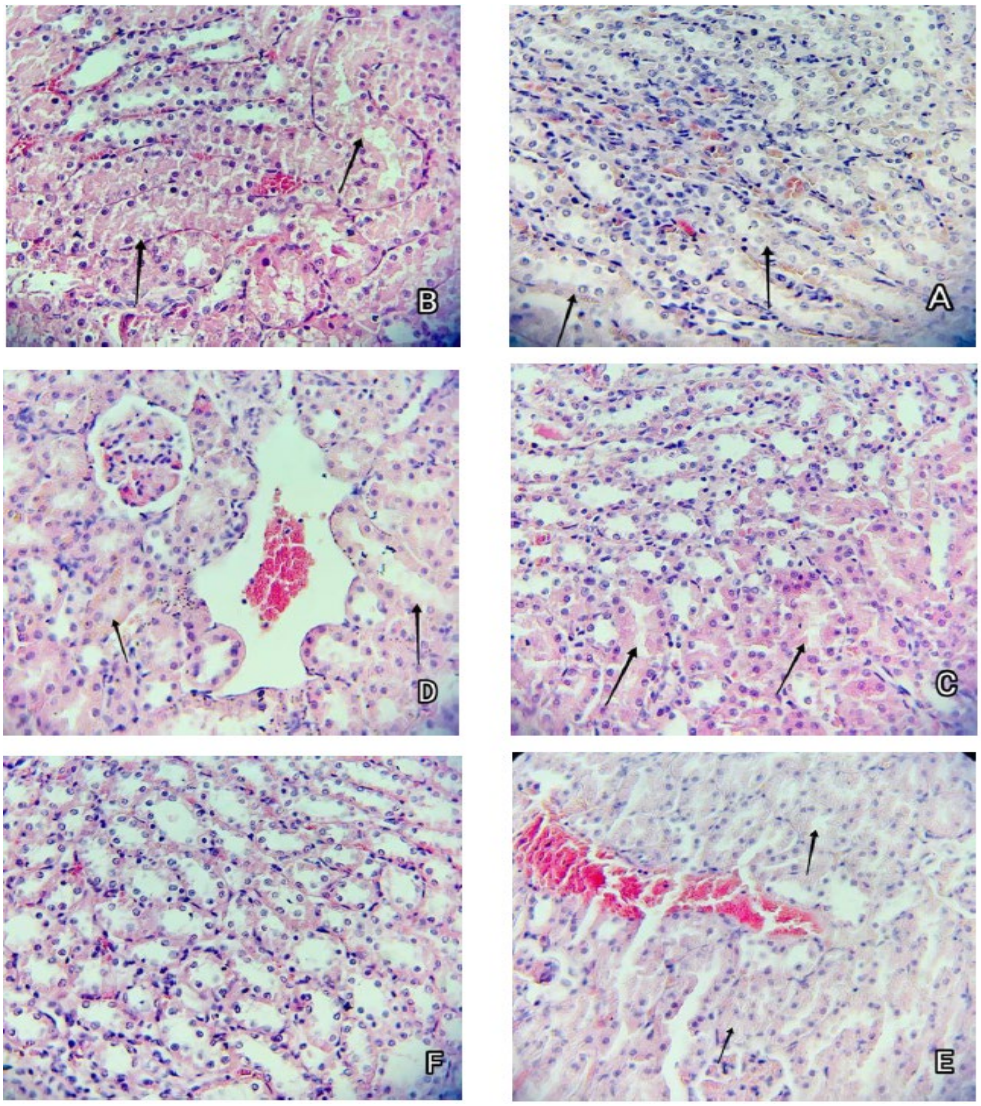

Fig.1. Show kidney acute tubular necrosis damage percentage for all groups.

A (5-Flu5mg/kg for 30 days) with damage involving 68% of the examined kidney (black arrows), B (5-Flu 5mg/kg+SLY 3mg/kg for 30 days) with damage involving 45% of the examined kidney (black arrows), C (SLY 3mg/kg for 30 days) with damage involving 32% of the examined kidney (black arrows), D (SLY for 15 days after that 5-Flu for 15 days) with damage involving 33% of the examined kidney, E (5-Flu for 15 days after that SLY for 15 days) with damage involving 33% of the examined kidney, F (control) normal tissuse. E&H.400X

4 Discussion

The 5-Flu has been reported to have the ability to generate free radicals (ROS). The production of ROS induces oxidative stress on cells, destroys protein, DNA and other cellular components, and ultimately leads to cytotoxicity [19]. The results of GSH levels indicates to

no significant difference between group A (5 Flu for 30 days) and control group, while showed a marked decrease compared to groups C (SLY only for 30 days), E (5-Flu for 15 days and after that SLY for 15 days), D (SLY for 15 days and after that 5-Flu for 15 days) and B (5-Flu and SLY for 30 days) respectively. This may be the result of the cells being exposed to a high level of oxidative stress caused by the drug, so the cells try to neutralize this stress by producing some glutathione, this agreed with Matés *et al*. (2020) [20], who reported that GSH plays an important role in protecting against oxidative stress by interacting with free radicals and chemicals and converting them into harmless substances. They also pointed out the possibility that GSH generates itself by the enzyme glutathione reductase, which uses NADPH as an electron donor. NADPH is a compound that transfers electrons in many biological processes, including glutathione synthesis. NADPH is produced through the pentose phosphate pathway (PPP) or by breaking down glutamine, an amino acid. but due to the amount of stress caused by 5-Flu leads to GSH deficiency and destruction of the cellular antioxidant defense mechanism [21]. The group C (SLY only for 30 days), group E (5-Flu for 15 days after that SLY for 15 days), groups D (SLY for 15 days after that 5-Flu for 15 days) and group B (5-Flu and SLY for 30 days) respectively showed a marked increase compared to group F (control), this may be related to the effect of Silymarin, which reduces the amount of oxidative stress in cells by eliminating free radicals, also the Silymarin reduces 5-Fluorouracil-induced oxidative stress through some mechanisms, such as increasing the activity of antioxidant enzymes such as glutathione reductase in cells and thus increasing the level of GSH [22, 23, 24]. The MDA is a free radical produced by reactive lipid peroxidation metabolites and is commonly used as a biomarker of lipid peroxidation to determine oxidative stress [25]. The results of MDA levels of group A (5 Flu for 30 days), D (SLY for 15 days after that 5 Flu for 15 days), E (5-Flu for 15 days after that SLY for 15 days) and B (5-Flu + SLY for 30 days) respectively showed a marked increase significantly compared to the group F (control).

This can be explained as a result of the effect of the drug, which is its ability to generate a number of free radicals, which leads to an increase in the level of MDA, and this is agrees with Abdulgani *et al*. (2020) [26], found that 5-Flu increased MDA levels in rats, indicating increased oxidative stress and lipid peroxidation. While no statistically significant differences were found between group C (SLY only for 30 days) and group F (control), this agrees with Kim *et al*. (2021) [27], who found that Silymarin can scavenge free radicals, modulate the expression of antioxidant enzymes, and protect the cells from toxins and drugs, suggesting that Silymarin can reduce oxidative stress and lipid peroxidation. There are several possible reasons why high acute tubular necrosis damage in group of rats treated with 5-Flu, one of this reasons is that 5-Flu induces oxidative stress and lipid peroxidation in the kidneys, which can impair the antioxidant defenses and damage the cellular membranes, another reason is that 5-Flu is metabolized in the liver and produces toxic by-products such as ammonia, urea, and carbo dioxide, which can cause nephrotoxicity [28, 29]. The group B (5-Flu and SLY for 30 days) showed significant increase in acute tubular necrosis damage percentage (45%) compare with group F (control) at ($P \leq 0.05$), While decrease significantly compare with group A (5 Flu for 30 days). Although the Silymarin has antioxidant, anti-inflammatory, and immunomodulatory properties, but these may not be sufficient to counteract the complex effects long- term use of 5-Flu on the kidneys and may not be effective enough to protect the kidneys from damage caused by 5-Flu [30]. The group C (SLY only for 30 days) showed significant increase in acute tubular necrosis damage percentage (32%) compare with group F (control), while show significantly decreased compared with group A (5-Flu for 30 days). The effects of Silymarin on the kidney are less clear, and some studies have suggested that Silymarin may have nephron-protective effects against certain renal injuries, such as ischemia-reperfusion and diabetic nephropathy [31]. The Silymarin may exert these effects by scavenging free radicals, reducing inflammation and modulating renal hemodynamic, but

some studies have reported that Silymarin may have adverse effects on the kidney, such as altering the renal blood flow, glomerular filtration rate, or tubular function [32].

The groups D (SLY for 15 days after that 5-Flu for 15 days) and E (5-Flu for 15 days after that SLY for 15 days) showed significant increase in acute tubular necrosis damage percentage compare with group F (control), whereas both groups show significantly decrease compared with group A (5 Flu for 30 days).

This agrees with Noorbakhsh *et al*. (2022) [33], found Silymarin may have some interactions with 5-Fluorouracil that reduce its efficacy or increase its toxicity. For example, Silymarin may affect the metabolism or transport of 5-Fluorouracil in the liver or kidney, leading to altered pharmacokinetics or pharmacodynamics.

5 Conclusions

Silymarin may provide attenuator role in oxidative stress and nephrotoxicity caused by 5-Flu drug.

6 Ethical Approval

All experiments and procedures of this study were reviewed and approved by the Central Committee of Bioethics of University of Kufa.

References

1. J. D. Lambeth, Free Radical Biology and Medicine, **43(3),** 332-347 (2007)
2. A. Wood-Kaczmar, S. Gandhi, N.W. Wood. Trends in molecular medicine, **12(11),** 521-528 (2006)
3. F. Yoshino, A. Yoshida, A. Nakajima, S. Wada-Takahashi, S. Takahashi & M. C. Lee, PLoS ONE, **8**, e82834 (2013)
4. D. B. Longley, D. P. Harkin & P. G. Johnston, Nature Reviews Cancer, **3(5),** 330–338 (2003)
5. U. Ozer, K. W. Barbour, S. A. Clinton & F. G. Berger, Molecular Pharmacology, **88**, 970–981 (2015)
6. B. D. Humphreys, R. J. Soiffer & C. C. Magee, Journal of the American Society of Nephrology, **16(1),** 151-161 (2005)
7. M. A. Perazella & H. Izzedine, Kidney International, **87**, 909–917 (2015)
8. A. Al-Asmari, A. Al-Zahrani, A. Khan, N. Al-Masri, H. Al-Shahrani & M. A. AlAmri, Human & Experimental Toxicology, **35(1),** 10–20 (2016)
9. A. A. Hamid, O. O. Aiyelaagbe, L. A. Usman, O. M. Ameen & A. Lawal, African Journal of Pure and Applied Chemistry", **4(8),** 142-1511 (2010)
10. M. Azizoğlu, S. Arslan, E. Gokalp Özkorkmaz, F. Aşir, E. Basuguy, M. H. Okur, B. Aydoğdu, M. Alagöz Karabel & İ. Kaplan, European Review for Medical and Pharmacological Sciences, **27(1),** 10446-10453 (2023)
11. S. Belhan, S. Yıldırım, C. Kayıkcı, A. Kömüroğlu, U. Özdek & Y. Kuşcu, Turk J Zool, **45(3),** 267-276 (2021)
12. R. A. Sallam, M. Hooda, J. K. Das, A. Arshad, Z. S. Lassi, P. Middleton & Z. A. Bhutta, Journal of Adolescent Health, **59(2),** S29-S39 (2016)

13. A. A. Elberry, F. M. Harraz, S. A. Ghareib, S. A. Gabr, A. A. Nagy & E. Abdel-Sattar, International Journal of Diabetes Mellitus, **3(1),** 37-442 (2015)
14. C. Jia, Z. Zhang, J. Wang & Z. Nie, Human and Experimental Toxicology, **41(1),** 1-8 (2021)
15. M.S. Moron, J.W. Depierre, and B. Mannervik, Biochim Biophys Acta., **582**, 67-78 (1979)
16. J. A. Buege, and S. D. Aust, Methods in Enzymology, **52**, 302-310 (1978)
17. J. Bancroft and A. stevens, *Theory and Practice of Histological Techniques*, 4th. (New York: Churchill Livingstone, 1999) 113-118.
18. H. A. Alturkistani, F. M. Tashkandi & Z. M. Mohammedsaleh, Global Journal of Health Science, **8(3),** 72–79 (2015)
19. J. Ali, A. A. Khan, A. Ahmad, M. Akhtar & M. Ahmad, Life Sciences, **239**, 116888 (2019)
20. J. M. Matés, J. A. Campos-Sandoval, J. de Los Santos-Jiménez & J. Márquez, Archives of Toxicology, **94(8),** 2603-2623 (2020)
21. Q. K. Alabi, R. O. Akomolafe, J. G. Omole, A. C. Akinmoladun, M. T. Olaleye & B. V. Owoyele, Biomedicine & Pharmacotherapy, **103**, 812-822 (2018)
22. A. Gillessen & H. H.-J. Schmidt, Advances in Therapy, **37(4),** 1279-1301 (2020)
23. S. Belhan, S. Yıldırım, C. Kayıkcı, A. Kömüroğlu, U. Özdek & Y. Kuşcu, Turkish Journal of Zoology, **45(3),** 267–276 (2021)
24. D. Doğan, İ. Meydan & A. U. Kömüroğlu, International Journal of Clinical Practice, **76(1),** e6541026 (2022)
25. V. Shofia, Aulanni'am, & C. Mahdi, Journal of Chemistry, University of Brawijaya, **1(1),** 119-125 (2013).
26. N. Abdulgani, D. Hidayati, R. Adinovitasary, V. Oliviatie & A. D. Sekartaji, Nusantara Bioscience, **12(1),** 50–54 (2020).
27. S. H. Kim, G. S. Choo, E. S. Yoo, J. S. Woo, J. H. Lee, S. H. Han, S. H. Jung, H. J. Kim & J. Y. Jung, Oncology Letters, **22(1),** 127 (2021)
28. W. H. Abdel-Ghaffar & E. A. Abdelghffar, Beni-Suef University Journal of Basic and Applied Sciences, **11(1),** 1-152 (2022)
29. M. C. Da Silva, A. C. de Oliveira, A. P. de Oliveira, C. M. de Souza, M. R. de Souza, R. F. de Souza, & J. de Oliveira, Antioxidants, **12(5),** 1005 (2023)
30. S. Ghosh, S. Banerjee, P. C. Sil, & D. Roy, Frontiers in Pharmacology, **7**, 481 (2016)
31. M. Mirjalili, E. Mirzaei & A. Vazin, European Journal of Medical Research, **27(64)** (2022)
32. E. Sengul, V. Gelen, S. Yildirim, E. Senturk, Y. Dag, G. Eser & M. Gok, Investigation of Effects of Silymarin in 5-Fluorouracil Hepatotoxicity and Nephrotoxicity in Mice. Research Square (2021)
33. M. F. Noorbakhsh, N. Ahmadi, S. Nazifi, A. Nikravesh & E. Hosseinifard, Iranian Veterinary Journal, **18(3),** 103–113 (2022)