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Protective Potential of Ashwagandha, Against Pyrifluquinazon Induced Hepato-And Nephrotoxicity in Albino Mice

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ABSTRACT

Globally, due to rise in dependence on the agricultural sector for food supply, the use of insecticides i.e., "Chemicals used to kill insects", has been increasing day by day. One of these is Pyrifluquinazon. The emerging concern is that after field spray, this insecticide has the tendency to be incorporated into surface water and become a part of the food chain and so becomes toxic for both aquatic and terrestrial organisms. **Objective:** To investigate the protective potential of Ashwagandha against damaging effect of Pyrifluquinazon (PQZ) on liver and kidney of mature and healthy male albino mice. **Methods:** Albino mice were divided into four groups, each group contained five mice. First group was control, supplied with water while, three were treated groups (Dose, Dose + Antidote and Antidote group respectively) to check protective potential of Ashwagandha against PQZ induced hepato and nephrotoxicity in mice. The dose group was supplied with PQZ while Dose + Antidote group with PQZ and Ashwagandha as an antidote while the Antidote group was supplied with Ashwagandha. This was accomplished for 20 days trial. Blood sampling and dissection was done at 20th day. Blood sample were taken using intra cardiac sampling method for biochemistry while liver and kidney were separated from each group for histopathological studies. **Results:** The liver function test and kidney function were normal and indicated restoration of their normal function. Histopathological findings also revealed regeneration in damaged areas of liver and kidney tissues. **Conclusions:** The findings of this study clearly revealed the protective potential of Ashwagandha against PQZ induced hepato and nephrotoxicity.

INTRODUCTION

Insects are probably one of the largest group of organisms on this earth. Social insects such as wasps, bees and termites are the main components of many ecological ecosystems [1]. So, being a part of earth's environment, insects also interact with many other species like animals, plants and human directly or indirectly for resources such as food. Unfortunately, the role of insects is more harmful than beneficial economically. They act as either pests, parasites or pathogens, so produce a multitude of disorders in animals and plants [2]. There are insect pests that are beneficial in other realms i.e. wasps, bees and lacewings which participate in ecosystems, and provide services in the form of pollination, producing food such as honey and so on. About 35 percent of world food production depends on pollinators [3] and these useful insect pests aid in biological control and reduce pests of crops, for example, aphids [4]. In order to control these insects, insecticides have been used voraciously. Many of these insecticides have a diverse range of chemical compositions such as organophosphates, carbamates and phytochemicals among others. Some of these are easy to get rid of but others tend to stay in the tissues of those organisms who consume them through their diet, thus resulting in bioaccumulation of hazardous substances which can either immediately or in the long run harm the plant consumer due to the insecticides that are applied on

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those plants [5]. Pyrifluquinazon is a very novel and unique insect-behaviour regulator (IBR), broad spectrum, 'Chordotonal organ TRPV channel modulator' (according to the Insecticide Resistance Action Committee-IRAC) type of insecticide which depends upon modern and advanced chemistry. It is efficient against sucking and piercing type of insects such as scales, aphids, especially white flies present on ornamental crops and vegetables. It works by effecting the chordotonal organs of insects thereby disabling them from using their feeding organs and causing death through starvation. It was registered in Japan firstly under the brand named as 'Colt' in 2010 for use on horticultural crops and in 2014 as under the brand name 'RYCAR' in the United States by SePRo [6]. It is very toxic and has been shown to affect the kidneys and livers of animals. The liver and kidneys play a vital role in detoxification and removal of harmful chemicals which are absorbed and are considered among the most important organs of the body to whom little injury can cause life threatening consequences [7]. The kidney is as an advanced and effective mechanism for filtration, secretion, and reabsorption [8]. The liver plays a key role in detoxifying harmful substances and absorbing nutrients in the body. Many diseases have secondary complications that can cause oxidative stress that puts pressure on the liver to protect us against these affects. So, there have been efforts to find ways to combat oxidative stress due to external factors that give the least amount of side effects. As such people have started to turn to traditional means of cure to such harmful chemicals in the form of CAM (complementary and alternative medicine). It is composed of Avurveda, homeopathy and traditional Chinese medicinal practices among others. Since these kids of practices have the least amount of side effects, they have started to gain popularity. Some of these natural remedies tend to have a general healing effect and are not specific in their action, while others are specific in their mode of action [9-11]. In an experiment to assess toxic effects of insecticides, it wasseen that there was an increased concentration of calcium in serum beside its low level in muscle, liver, kidney and brain of *C. batrachus*, having 0.24 ppm and 2.03 ppm of carbaryl and carbofuran, for the period of 15 days. It was hypothesized that increased level of calcium was the result of discharge of Ca^{+} ions in to the blood from important organs because of poisonous effects of insecticides [12-14]. Thus, the effects of insecticides need to be countered and effectively mitigated to secure renal and hepatic health.

METHODS

A total of 20 male albino mice weighing 26 ± 2 g were acquired from the Veterinary Research Institute (VRI) in Lahore for the purpose of animal rearing. These mice were then placed in a typical habitat with a 12-hour cycle of light and dark at an appropriate room temperature of 27°C along with 40-60% moisture to adapt to the environment. Pyrifluquinazon was dissolved in distilled water in proper amount after determining its LD50 in such a way that 1 ml solution contained required amount of pyrifluquinazon. 0.1 ml Dose was administrated to mice for a time period of 20 days constantly. Fresh *Withania Somnifera* (Ashwagandha) extract having 75 % ethanol by volume was purchased from a popular company. Each group had 5 male albino mice; Control group (C), Pyrifluquinazon (0.1ml of 300 mg/kg) administrated dose group (D), *Withania Somnifera* provided antidote group (AD) and PQZ dose plus antidote (*Withania Somnifera* and Pyrifluquinazon) treated group (AD+D). In control group, five male albino mice were used and fed untreated food and water, in dose group (D) they were fed with PQZ 0.1ml of 300mg/kg/day in parallel with untreated food, in Dose + Antidote group with 0.1ml of PQZ and *Withania somnifera*, while in Antidote group treated with 0.1ml of *Withania somnifera*. After the experiment completion, all the mice were dissected, the kidneys and liver were obtained, preserved and labeled for histopathological study and also biochemical analysis was conducted on blood drawn from mice after dissection. The samples of blood were obtained in special EDTA vials with the aid of 3 ml syringes by puncturing the heart of mice. To isolate the serum, blood samples were spun and serum was then placed at -80° C. After this, serum was analyzed to test various hormones level including LH, FSH and testosterone. After dissection Kidneys and liver were washed with 0.9% saline solution and shifted from formalin fixative to 70 percent ethyl alcohol for the analysis of their morphological features under microscope. The measurement of liver and kidneys was done for the morphometric analysis. Digital weight balance was used for the measurement of weight of such organs. A software SPSS version 24.0 was used to capture and explain the all data. The difference in standard errors and arithmetic means of control was that all the treated groups were studied by utilizing one way ANOVA $p<0.05$ as a significance level. All the graphs were shown as mean S.E values. Tissues were washed with 0.9 percent saline solution and then fixed in 10% formalin solution, later on dehydrated with graded ethanol, for clearing treated with xylene and for block preparation embedded in paraffin wax. Microtome was used to make sections of liver and kidney having thickness five micrometer and stained with eosin and hematoxylin following established protocol [14]. After drying completely, the microphotography was performed and prepared slides were studied under a microscope at 10X and 40X for the purpose of further histopathological

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analysis. The average weight of mice was enhanced when placed in control group 26mg/dl ± 2.3 to 27.1 mg/dl ± 2.2, while in dose group it decreased to 24.1mg/dl ± 0.57 to 23mg/dl ± 0.78, while in Dose + Antidote group increased to 24.1mg/dl ± 0.57 to 23mg/dl ± 0.78 and in Antidote group mice increased noticeably to 29.1mg/dl±0.57 to 30.5mg/dl±0.8, as given table 1.

Table 1: Comparison of Average body weight of mice from every group after experimentation showed significant difference ($p<0.05$)

Groups	Initial Weight (g)	Final Weight (g)
Control	$26^{a} \pm 2.3$	$27.1^{a} \pm 2.2$
Dose	$24.1^{a} \pm 0.57$	$23^{a} \pm 0.78$
Dose + Control	$28.1^{a} \pm 0.85$	$29.1^{a} \pm 1.01$
Antidote	$29.1^{a} \pm 0.57$	$30.5^{a} \pm 0.8$

Histopathological sections of liver of control group at 400X express normal structures of hepatocytes such as proper central nucleus, hepatic lobule, normal hepatic triads, Kupfer's cell while in Dose Group, central vein does not form proper boundary, hepatic lobule disappears, Hepatic Triads are not visible and Kupfer's cell disappear, In Dose + Antidote group hepatocytes structures such as Hepatic lobule, hepatic nucleus are normal, hepatic triad of artery, portal vein and common bile duct, all these structures show mild effect, due to ashwagandha antidote. While in Antidote group, hepatocyte central vein, hepatic lobule, hepatic nucleus, Kupfer cells, lobular space and binucleate hepatocytes are normal, indicating ashwagandha has beneficial effects on liver as liver regenerated its damaged areas, as given in figure 1.

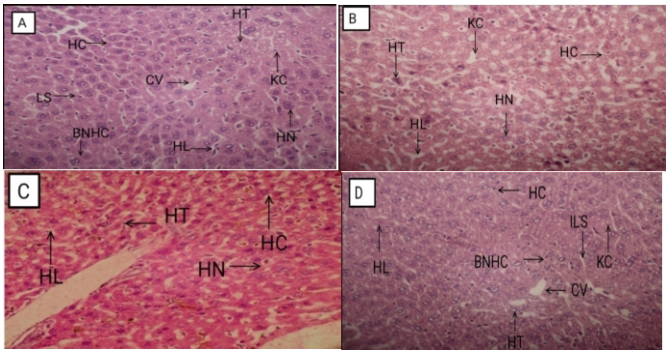

Figure 1: Histological Sections of liver at 400X of A (control), B (Dose), C (Dose + Antidote) and D (Antidote) Groups of *Musmucscullus*

(HL), Hepatic nucleus (HN), HT and KC (Hepatic triads and Kupfer cells) while Section B express damaged structures Central vein (CV)does not form, HN nucleus became dark, KC cells disappear, in section Crenal tubule with juxtamedullary nephron is intact, proximal (PT), Binucleate Hepatocytes (BNHC) and distal tubules (DT) are prominent. In section D, proximal tubule and distal tubules are more prominent. In control Group, nephrocytes shows normal structures as renal tubule with juxtamedullary nephron is intact, proximal and distal convoluted tubule, collecting duct, Loop of Henle are prominent. In Dose Group, renal tubule with juxtamedullary nephron is not intact proximal and distal convoluted tubule is not prominent, capillaries in glomerulus are damaged and becomes dark. In Dose and Antidote group, renal tubule with juxtamedullary nephron is intact and proximal and distal convoluted tubules are prominent. In Antidote group, renal tubule with juxtamedullary nephron is intact, distal and proximal convoluted tubule are prominent and collecting duct appear more prominent than other structures showing restoring effect of liver using ashwagandha (Antidote), as given in figure 2.

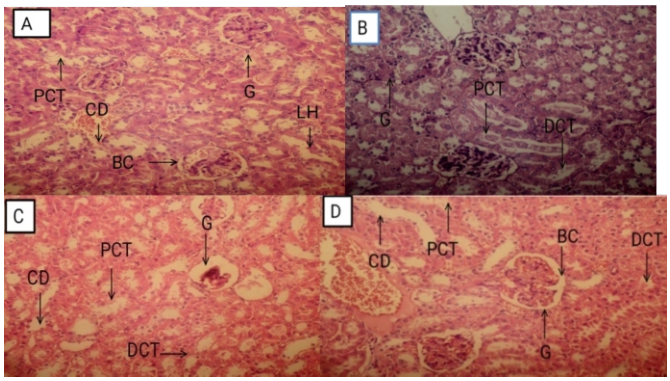

Figure 2: Histological sections A-Dog *Mus musculus* kidneys at 400X respectively showing all nephrocytes., Proximal convoluted tubule (PCT) and Distal convoluted tubule (DCT), Collecting duct (CD). Glomerulus(G), Bowan's capsule(BC), Loop of Henle(LH)

RESULTS

Liver function test (LFT) of in four groups of mice are displayed as Mean S.E. ANOVA is used for variance analysis, and the Tukey test. The increased level of various factors indicates to damage of liver. Significance difference in all groups at the significance value($p<0.05$) represented by the addition of various alphabets as superscripts. The average mean value of bilirubin total in control group was 0.586mg/dl ± 0.11which is normal range, while of dose group was 1.45mg/dl ± 0.042, higher than normal value indicates that Pyrifluquinazon has damaged the liver, for Dose +Antidote group is 0.638mg/dl ± 0.125 which is lower as compared to the Dose group indicated restoration of liver damaged, for Antidote group is 0.576mg/dl ± 0.126 was in normal range again showing restorative action on liver as compared to Dose + Antidote group as given in table 2. In this group the level of SGPT was in normal range such as its value vary between 7 to 56. The mean value of ALT in table;4.5 is 28.4U/L ± 3.51, showing normal liver functions for dose group is 63^{b} ± 1.64 higher than control group showing liver damage while for Dose +Antidote group is

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31.4[a] ± 3.72i.e., lower than Dose group showing restoring liver damage and respectively for Antidote group was27.1[a] ± 2.46 showed further decrease in ALT as compared to the rest of the groups showing ashwagandha has positive effect on the function of liver as given in table 2. In control normal mean value is 35.4[a] ± 1.80, while of dose group is 35.4[a] ± 1.80 higher than control group showing liver damage while for Dose +Antidote group is 39.2[a] ± 1.68 i.e., lower than Dose group showing restoring liver damage and respectively for Antidote group was 35.4[a] ± 1.804showed further decrease in ALT as compared to the rest of the groups showing ashwagandha has positive effect on the function of liver. In control normal mean value is 0.586[a] ± 0.11. While of dose group is 9.815[b] ± 0.30 higher than control group showing liver damage while for Dose + Antidote group is 7.44[a] ± 0.298 i.e., lower than Dose group showing restoring liver damage and respectively for Antidote group was 7.14[a] ± 0.36 showed further decrease in total proteins as compared to the rest of the groups indicated that liver function normally. In control normal mean value is 4.4[b] ± 0.29. While of dose group is 7.56[c] ± 0.39 higher than control group showing liver damage while for Dose +Antidote group is 4.46[b] ± 0.20 i.e., lower than Dose group showing restoring liver damage and respectively for Antidote group was 3.8[a] ± 0.26showed further decrease in albumin as compared to the rest of the groups indicated that liver function normally as given in table 2.

Table 2: Biochemical analysis in different treatment groups

Groups	ALT (U/L)	AST (U/L)	Alkaline Phosphatase (ALP)	Total Bilirubin (mg/dl)	Albumin (g/dl)	Total Protein (g/dl)
Control	28.4[a] ± 3.51	35.4[a] ± 1.80	0	0.586[a] ± 0.11	4.4[b] ± 0.29	7.4[a] ± 0.314
Dose	63[b] ± 1.64	458.6[b] ± 0.74	0	1.45[b] ± 0.04	7.56[c] ± 0.39	9.815[b] ± 0.30
Dose + Control	31.4[a] ± 3.72	39.2[a] ± 1.68	130.75 ± 8.49	316.2 ± 44.38	4.46[b] ± 0.20	7.44[a] ± 0.298
Antidote	27.1[a] ± 2.46	35.4[a] ± 1.804	157.25 ± 9.40	194.4 ± 17.71	3.8[a] ± 0.26	7.14[a] ± 0.36

Figure 3 shows that level of A/G Ratio increased after exposure with Pyrifluquinazon for the period of 20 days. The increased level of A/G Ratio indicates the damage to liver. Significance difference in all groups at the significance value (p< 0.05) is represented by the addition of various alphabets as superscripts.

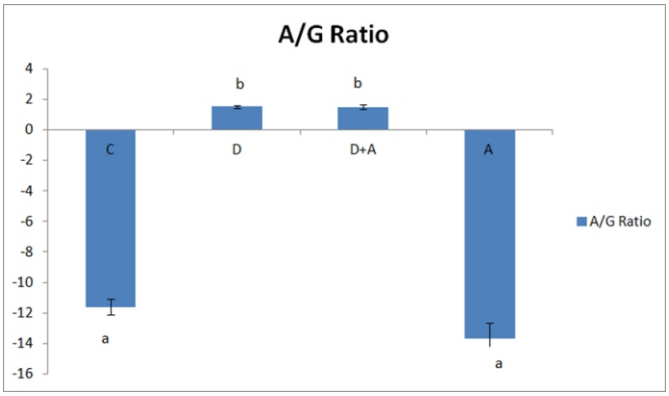

Figure 3: Plotted graph showing the level of A/G Ratio after exposure with Pyrifluquinazon for the period of 20 days (w.r.t. Percentage ± S.E.M)

In renal function test only level of urea was assessed. In control group, mean value of urea is 40.4mg/dl± S.E (standard error of the mean).Its value is between normal rage in blood, it indicated that kidney is functioning normally while in Dose Group value of urea level rises to 70.8mg/dl ±S.E 1.85 higher than control indicating toxic effects of pyrifluquinaon, in Dose + Antidote group value of urea was 46.8mg/dl ± S.E 2.03expressing restoring function of kidneys and in Antidote group the mean value of urea was 46.8mg/dl± S.E 2.03 indicating that ashwagandha as an antidote has positive effect on kidney function and urea level remains in normal range as given in table 3.

Table 3: Ranges of urea in control and treatment groups

Groups	Urea (Mean ± SE)
Control	40.4[ab] ± 3.26
Dose	70.8[c] ± 1.85
Dose + Control	46.8[b] ± 2.03
Antidote	34.44[a] ± 4.23

DISCUSSION

Pesticide contamination is a world-wide public health and environmental issue having impact on all types of living organism because of highly toxic and carcinogenic affects. The research discussed here is a basic step in the assessment of the feasibility of prescription of Ashwagandha as a possible antidote, due to protective effect of *Withania Somnifera* against Pyrifluquinazon induced hepato-and nephrotoxicity in albino mice. Creatinine levels, urea and uric acid are the major significant medical parameter for evaluating anomaly in kidney function. In fact, creatinine is a more consistent sign of nephrotoxicity because its level is enhanced in first stage of renal disease [15, 16]. In related research, the treatment of rats with *Withania Somnifera* at the dose of 500 mg/kg significantly reduced the high level of biomarkers i.e. alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP),

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bilirubin total when applied with hepato-toxic dose of paracetamol [17]. In our study as well, the histopathology of the liver and kidney, the slides of control group showed normal growth in both of these organs. While the histopathology of the kidney and the liver slides of dose group showed bad effects of the pyrifluquinazon dose on both of these organs. In the dose-group slides of kidneys, abnormal structure of the kidneys section was observed such as bowman capsule, glomerulus was damaged more badly and glomerulus became destroyed and its capillaries become dark. So, the main function of kidney such as removal of the urea and creatinine was upset and the serum level of urea and creatinine increased. In another research, the toxic effects of bromobenzene were judged by using similar criteria of blood urea, uric acid and creatinine levels, the levels of which were seen to be high in the renal tissues. This was caused by the decrease in antioxidants in the blood serum, which decreased the kidney's ability to get rid of harmful chemicals in the serum. After administering Ashwagandha, the antioxidant levels also increased. Pre-administration of bromo-benzene given rats with *Withania Somnifera* was also able to compensate the levels of glutathione and antioxidants in such a way that it counter-acted most of the negative effect of bromobenzene toxicity. The proposed reason for this counter healing could be due to alkaloids, sitoinosides and steroidal lactones [18]. Thus, strengthening the hypothesis that Ashwagandha has curative properties that makes it a viable candidate as a non-allopathic, natural medicine [19, 20].

CONCLUSIONS

The present research showed the healing aspect to Ashwagandha, which has antioxidant and anticarcinogenic ability against pyrifluqinazon induced hepato and nephrotoxicity in mice.

Authors Contribution

Conceptualization: AUR, SA
Methodology: AA, AUR, SA, HF, FA, RB
Formal analysis: AA
Writing-review and editing: AUR, AA, HF, FA, RB

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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REFERENCES

[1] Ramos DL, Cunha WL, Evangelista J, Lira LA, Rocha MV, Gomes PA *et al*. Ecosystem services provided by insects in Brazil: What do we really know?. Neotropical Entomology. 2020 Dec; 49: 783-94. doi: 10.1007/s13744-020-00781-y.

[2] Resh VH and Cardé RT, editors. Encyclopedia of insects. Academic Press; 2009.

[3] McElwee P, Fernández-Llamazares Á, Aumeeruddy-Thomas Y, Babai D, Bates P, Galvin K *et al*. Working with Indigenous and local knowledge (ILK) in large-scale ecological assessments: Reviewing the experience of the IPBES Global Assessment. Journal of Applied Ecology. 2020 Sep; 57(9): 1666-76. doi: 10.1111/1365-2664.13705.

[4] Stern VM. Economic thresholds. Annual Review of Entomology. 1973 Jan; 18(1): 259-80. doi: 10.1146/annurev.en.18.010173.001355.

[5] Singh NN, Das VK, Srivastava AK. Insecticides and ionic regulation in teleosts: a review. Zoologica Poloniae. 2002; 47(3/4): 49-64.

[6] Maienfisch P. Selective feeding blockers: pymetrozine, flonicamid, and pyrifluquinazon. Modern Crop Protection Compounds. 2019 Mar; 3: 1501-26. doi: 10.1002/9783527699261.ch34.

[7] Ding L, Han M, Wang X, Guo Y. Ochratoxin A: Overview of Prevention, Removal, and Detoxification Methods. Toxins. 2023 Sep; 15(9): 565. doi: 10.3390/toxins15090565.

[8] Brooks J, Cervantes-Godoy D, Jonasson E. Strategies for smallholders in developing countries: Commercialisation, Diversification and Exit. 2009.

[9] Okokon JE, Okokon PJ, Dar Farooq A, Choudhary MI. Anti-inflammatory and antinociceptive activities of Homalium letestui. Pharmaceutical Biology. 2013 Nov; 51(11): 1459-66. doi: 10.3109/13880209.2013.799707.

[10] Charlton D, Rutledge Z, Taylor JE. Evolving agricultural labor markets. Handbook of Agricultural Economics. Elsevier. 2021 Jan; 5: 4075-133. doi: 10.1016/bs.hesagr.2021.10.003.

[11] Memon RA and Lohana K. Covid-19 outbreak: challenges and opportunities towards agriculture sector. International Journal of Agricultural Extension. 2021 Sep; 9(3): 409-16. doi: 10.33687/ijae.009.03.3622.

[12] Ram R and Schultz TW. Life span, health, savings, and productivity. Economic Development and Cultural Change. 1979 Apr; 27(3): 399-421. doi: 10.1086/451107.

[13] Wielenberg EJ. The Absurdity of life in a Christian Universe as a reason to prefer that god not exist. InDoes God Matter? Routledge; 2017 Dec: 147-163. doi: 10.4324/9781315210995-7.

[14] Suvarna SK, Layton C, Bancroft JD. The hematoxylins

DOI: https://doi.org/10.54393/mjz.v4i02.82

and eosin. Bancroft's Theory and Practice of Histological Techniques. E-Book 8th Edition. Nottingham, UK: Elsevier; 2018. 173-186.

[15] Cervantes-Gody D and Dewbre J. Economic importance of agriculture for poverty reduction. OECDiLibrary. 2010.

[16] Mehreen R, Niaz F, Rehman AU, Shah SJ, Fayaz M, Khan F. To Evaluate the Level of Serum Cholesterol, Uric Acid, Urea, Creatinine and Urine Albumin in Diabetic Patients from Peshawar. Pakistan Journal of Medical & Health Sciences. 2022 Nov; 16(09): 630. doi: 10.53350/pjmhs22169630.

[17] Li J, Stein TD, Johnson JA. Genetic dissection of systemic autoimmune disease in Nrf2-deficient mice. Physiological Genomics. 2004 Aug; 18(3): 261-72. doi: 10.1152/physiolgenomics.00209.2003.

[18] 18.Vedi M, Rasool M, Sabina EP. Protective effect of administration of *Withania somifera* against bromobenzene induced nephrotoxicity and mitochondrial oxidative stress in rats. Renal failure. 2014 Aug; 36(7): 1095-103. doi: 10.3109/0886022X.2014.918812.

[19] Tavafi M and Ahmadvand H. Effect of rosmarinic acid on inhibition of gentamicin induced nephrotoxicity in rats. Tissue and Cell. 2011 Dec; 43(6): 392-7. doi: 10.1016/j.tice.2011.09.001.

[20] Amarasiri SS, Attanayake AP, Jayatilaka KA, Mudduwa LK. South Asian medicinal plants and chronic kidney disease. Traditional Medicine Research. 2020; 5(5): 389. doi: 10.53388/TMR20200603189.