Tropical Journal of Pharmaceutical Research May 2023; 22 (5): 1023-1029
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i5.13

Original Research Article

Evaluation of the therapeutic potential of vomit seed (*Strychnos nux-vomica*) homeopathic treatment in acute acetaminophen-induced toxicity

Ikram Toumi[1,2]*, Ifriqya Medila[1,2], Seghir Karoui[2]
[1]Biology, Environment and Health Laboratory, [2]Department of Cellular and Molecular Biology, Life and Natural Sciences Faculty, University of El Oued, El Oued, Algeria

For correspondence:* ***Email:* *toumi-ikram@univ-eloued.dz*

Sent for review: 13 January 2023 *Revised accepted: 4 May 2023*

Abstract

Purpose: *To investigate the effect of vomit seed homeopathic drugs (14CH and 30CH) on paracetamol-induced toxicity in rats.*
Methods: *Thirty-two male Wistar rats were divided into 4 groups of 8 animals each (n = 8) and treated for 12 days. During the treatment period, group (G) one (G1) which constitutes the control group received filtered water, whereas groups two (G2), three (G3), and four (G4) were gavaged with a toxic dose of paracetamol 1/4 of LD_{50}. Next, Strychnos nux-vomica (0.5 mg/kg) homeopathic dilutions of 14CH and 30CH were administered to G3 and G4, respectively. Hematological, biochemical, and antioxidant markers were assessed.*
Results: *Homeopathic drug groups, namely G3 and G4, showed significant recovery in some hematological (red blood cells count and hemoglobin concentration; $p < 0.01$) and biochemical parameters (urea, creatinine, glutamic transaminase, and alkaline phosphatase; $p < 0.001$). The level of malondialdehyde significantly decreased while glutathione level significantly increased in treated rats.*
Conclusion: *The antioxidant and anti-hemolytic properties of vomit seeds protect against paracetamol-induced acute toxicity in rats by reducing oxidative stress, hepatotoxicity, and nephrotoxicity in rats.*

Keywords: *Homeopathic, Strychnos nux-vomica, Oxidative stress, Paracetamol-induced toxicity*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Acetaminophen, (paracetamol) is a popular analgesic and antipyretic that is often prescribed and used as a primary treatment. It is widely known that excessive intake of paracetamol may result in acute liver failure due to the generation of reactive oxygen species and formation of toxic metabolite N-acetyl-benzoquinone imines [1]. Administering high doses of paracetamol may result in severe renal failure which is frequently accompanied or preceded by substantial hepatic dysfunction [2].

Strychnos nux-vomica (Loganiaceae) is an evergreen tree native to South-East Asia and India. It is a medium-sized tree that grows in largely open environments [3]. There are at least 84 compounds known to exist in *Nux vomica*, including alkaloids, glycosides, flavonoids,

terpenoids, steroids, and organic acids, according to previous characterization studies [4]. Seeds of *Strychnos nux-vomica L* are used in traditional medicine to treat a variety of health conditions including anemia, lumbago, asthma, pneumonia, constipation, hyperglycemia, malaria, skin problems, paralysis, muscle spasms, and decreased appetite [5]. Before taking it orally, *Nux vomica* is processed by extraction and standardization with other suitable excipients. Its dosage is usually carefully monitored, and it is not taken frequently.

Homeopathy is a well-regarded complementary medicine, particularly in Europe and India. In homeopathy, organic and inorganic natural substances are used to restore "vital power," alleviate illnesses and restore health [6]. Few research has been published on the use of homeopathy against the toxicity of paracetamol. The present study attempts to examine the effectiveness of the microdoses (14CH) and (30CH) of *Strychnos nux-vomica* as a homeopathic drug against paracetamol poisoning.

EXPERIMENTAL

Plant material

Seeds of *Strychnos nux-vomica* were identified and sorted after rigorous quality control. The seeds were washed and air-dried. Then, they were crushed and stored in glass vials until use.

Animals

Male Wister rats (n = 42) weighing between 165 and 247 g, were acquired from the Animal Laboratory of Natural and Life Science Faculty of El Oued University. Animal study protocol was approved by the Ethics Committee of the University of El Oued, El Oued, Algeria (approval no. 42/EC/DCMB/FLNS/UE2020). All experiments were carried out in accordance with the guidelines for Animal Research: Reporting *in vivo* experiments 2.0 [7].

Preparation of homeopathic drugs

Homeopathic strain preparation

Using the *Strychnos nux-vomica* seeds, a homeopathic strain remedy was prepared. The parent solution is a basic component that serves as a starting point for manufacturing homeopathic medicine. The preparation of the mother tincture was made by maceration in a water/ethanol mixture at 80 % v/v.

Preparations for centesimal Hahnemannian (CH) dilutions

The CH dilution equation is presented in Eq 1.

x CH = 10 - 2x …….. (1)

One volume of the mother tincture was added to 99 volumes of solvent 80 % v/v ethanol to obtain a CH dilution. The dilution was vigorously agitated as a result of dynamization action. This allows for creation of the first level of dilution, or 1 CH = 1 % = 0.01. Repeating the process, one volume of 1 CH solution was made up with 99 volumes of solvent. Subsequently, the solution was electrified to produce 2 CH. This process was continued until appropriate dilution in CH was achieved. There are two steps in the formulation stage of homeopathic remedies: dilution and potency. The solution was shocked repeatedly and strongly agitated after each dilution. This dynamization would discharge therapeutic substances by causing the formation of nanoparticles that keep onto the imprint of original substances and the information it contains. The solvent then grasps this information during subsequent dilutions [8].

Study design

Rats were acclimatized for two weeks, during which they were fed standard food and kept at room temperature, constant humidity, and a 12-hour photoperiod. The animals were equally divided into one control group (G1) and three experimental groups (G2, G3, and G4). The control group received filtered water, whereas the experimental groups were provided with a toxic dose of paracetamol equivalent to 1/4 of the LD_{50} (485 mg/kg) by gavages. In addition to the toxic dose of paracetamol, G3, and G4 were treated with the homeopathic drug (14CH and 30CH) of *Strychnos nux-vomica* (0.5 mg/kg), respectively.

Blood and tissue samples

To conduct biochemical analysis, blood samples in heparinized tubes were drawn from the jugular vein of rats. After dissection, the liver, heart, and kidneys were separated, washed with physiological water, weighed, and fixed in formalin to use in the histopathological study. One gram of tissue (heart, liver, kidney) from each rat was ground and homogenized in TBS and then centrifuged at 3000 rpm for 15 min. The supernatant was filtered and used to determine oxidative stress parameters.

Evaluation of parameters

Antioxidant properties

The stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical scavenging activity was used to measure the antioxidant activity of plant extracts and the standard Butylated hydroxytoluene (BHT). The DPPH radical-scavenging assay was carried out according to the method described by Mubarak *et al* [9] with some modifications. Eq 2 was used to compute the radical scavenging activity.

$RS\ (\%) = ((A_b-A_s)/A_b)100$ …….. (2)

where RS is DPPH radical scavenging, A_b is the absorbance of blank, and A_s is the absorbance of sample.

Hemolysis assay

Hemolysis assay was done as described by Henkelman *et al* [10]. Blood (5 ml) was collected from healthy volunteers in tubes containing 5.4 mg of EDTA to prevent coagulation and centrifuged at 1000 rpm for 10 min at 4 ºC. The erythrocytes were washed three times with PBS. The washed RBCs were diluted to a hemoglobin concentration of 10 g/L with ultra-pure water, which resulted in complete lysis, and aliquots were stored at −80 °C. Fifty microliters of ten dilutions of erythrocytes suspension were mixed with 100 µL of test sample (S) (*Strychnos nux-vomica*), and the reaction mixture was incubated at 37 ºC water bath for 60 min. The absorption of hemoglobin was measured at 450 nm. Percentage hemolysis (H) was calculated using Eq 3.

$H\ (\%) = 100 – (S \div C)100$…… (3)

Where C is the control

Serum biochemical parameters

A type auto-analyzer (BIOLIS 24j) with an appropriate reagent package for each parameter was used to determine blood glucose, urea, creatinine, alanine aminotransferase, and aspartate aminotransferase using the colorimetric method.

Oxidative stress parameters

The thiobarbituric acid (TBA) method was used to determine Malondialdehyde (MDA) level. The MDA reacts with TBA to give pink-absorbing chromophores at 532 nm. Glutathione was determined by a spectrophotometer using the colorimetric method [11].

Statistical analysis

One-way analysis of variance was used to statistically assess the impact of homeopathic medicine. A Duncan's multiple comparison post hoc test was used to detect whether any statistically significant difference exists between the obtained means. Differences were considered significant at $p < 0.05$.

RESULTS

Biological activities of homeopathic strain

Antioxidant property

The researchers first assessed the antioxidant capacity of the homeopathic strain (hydroalcoholic extract of Vomit seeds). Figure 1 demonstrates the DPPH radical-scavenging activity of plant extract and Butylated hydroxytoluene (BHT) at various doses. The *S. nux-vomica* seed extract showed the highest inhibitory action, attaining a peak of 97.35 % at a dose of 0.5 mg/mL, while BHT showed a maximum inhibitory activity of 77.63 % at the same concentration. The extract of seeds has an IC_{50} of 6.86 g/mL. With respect to the common BHT, IC_{50} equals 257 g/mL.

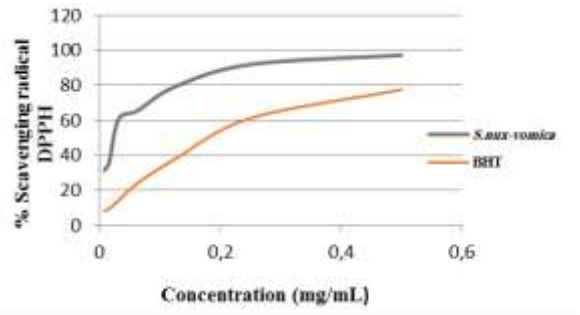

Figure 1: DPPH free radical inhibition of seed extract of *S. nux-vomica* and standard BHT

Hemolytic activity

Figure 2 displays the anti-hemolytic action of *S. nux-vomica* seeds extract at various concentrations (20 – 80 g/mL) on human blood erythrocytes. The obtained findings in this respect were negative. The antihemolytic activity demonstrated a percentage of hemolysis inhibition that rises with an increase in the concentrations ($R^2 = 0.895$) of plant seed extract. At 80 g/mL, inhibition of hemolysis was 95.8 %. These results demonstrate the protective effect of *S. nux-vomica* seed extracts.

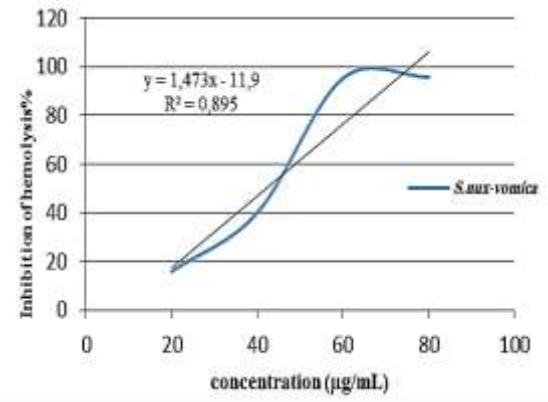

Figure 2: Hemolytic inhibition

Hematological parameters

The effects of homeopathic formulations 14CH and 30CH on hematological parameters in control and acetaminophen-intoxicated rats are presented in Table 1. The red blood cell (RBC) count and hemoglobin (Hg) concentration significantly decreased after the paracetamol intake in experimental groups compared to control group.

Biochemical parameters

Table 2 shows the effect of *Strychnos-nux-vomica* homeopathic drugs on serum biochemical markers in control and paracetamol-intoxicated rats. The latter showed a significant increase in the serum levels of urea, creatinine, glutamic transaminase (GTO and GTP), and alkaline phosphatase compared to their counterparts in control group. The kidney and liver injuries were reduced significantly after the concurrent intake of 14CH and 30CH *S. nux Vomica* extracts.

Oxidative stress markers

The obtained results are presented in Table 3. Paracetamol induced significant increases in liver and kidney malondialdehyde (MDA) levels against a significant decrease in glutathione (GSH) levels of the experimental groups compared to control group. Unlike paracetamol-intoxicated rats, the 14CH and 30CH homeopathic treated groups significantly alleviated disharmony and restored normal secretion of oxidative stress markers.

Table 1: Hematological parameters in control and Acetaminophen intoxicated rats

Group	RBC 10^6 (cells/µL)	LYM (%)	WBC 10^3 (cells/µL)	Hb (g/dL)	Platelets 10^3 (cells/µL)
Control	7.1±0.06	72±5.2	6.1±0.25	12.4±0.2	480±0.2
Paracetamol	5.4±0.23**	90±3.7**	12.1±0.34***	7.4±0.42***	686.5±0.6**
14CH	7.3±0.19^b	68±8.4^b	5.79±0.39^c	13.1±0.36^c	479.4±0.8^b
30CH	6.98±0.25^b	70±1.3^b	5.88±0.12^c	12.9±0.16^c	501.2±0.4^b

*P < 0.05, **p < 0.01, *** < 0.001 compared to control group; $^a p$ < 0.01, $^b p$ < 0.01, $^c p$ < 0.001 compared to paracetamol group

Table 2: Biochemical data for control and acetaminophen-intoxicated rats

Group	Urea (g/lL)	Creatinine (mg/mL)	GTO (µg/L)	GTP (µg/L)	PAL (µ/L)
Control	0.37±0.09	8.89±1.19	52.13±4.5	249.2±7.2	185±4.9
Paracetamol	0.83±0.02***	13.19±0.06***	103.09±8.2***	324.1±4.31***	271±5.2**
14CH	0.36±0.14^c	7.97±2.6^c	50.96±9.1^c	215.4±9.06^c	178±3.6^b
30CH	0.35±0.03^c	8.48±1.08^c	64.01±8.7*^{c}	272±6.2*^{c}	187±6^b

*P < 0.05, **p < 0.01, ***p < 0.001 compared to control group; $^a p$ < 0.01, $^b p$ < 0.01, $^c p$ < 0.001, compared to paracetamol group

Table 3: Oxidative stress markers in control and acetaminophen intoxicated rats

Group	MDA (nmol/g tissue)		GSH (nmol/g tissue)	
	Liver	*Kidney*	*Liver*	*kidney*
Control	10.34±0.6	12.67±0.19	33.9±0.4	11.88±0.2
Paracetamol	32.46±0.4***	28.1±0.03**	10.49±0.3***	7.64±0.1***
14CH	12.86±0.3^c	15.7±0.6^b	21.9±0.8*^{c}	13.4±0.1^c
30CH	13.5±0.4^c	16.4±0.8^b	18.8±0.6*^{c}	13.6±0.4^c

*P < 0.05, **p < 0.01, ***p < 0.001 compared to control group; $^a p$ < 0.01, $^b p$ < 0.01, $^c p$ < 0.001, compared to paracetamol group

DISCUSSION

The concept of "let like be healed by like" underlies one of the most significant applications of alternative and integrative medicine (homeopathy). Homeopathy treats a variety of illnesses by using highly diluted medicines [12]. The goal of current investigation was to assess the biological characteristics and protective effect of homeopathic remedies prepared from Vomit seeds against paracetamol-induced toxicity in rats. The damaging effects of oxidative stress are inhibited or avoided thanks to natural antioxidants found in plants. It is widely demonstrated in literature that free radical scavengers such as polyphenols, flavonoids, glycosides, steroids, tannins, alkaloids, and phenolic substances are present in *S. nux-vomica* extract [5]. Hemolysis of human RBCs is a highly useful model to examine the effects of free radicals on membrane oxidative damage and assess the antioxidant activity of novel drugs. The erythrocyte membrane is prone to damage, and it may lose its integrity as a result of lipid peroxidation, releasing hemoglobin (hemolysis) and intracellular K^+ ions [13].

The results showed that plants, particularly phenolic compounds which are responsible for the protection of red blood cells against lipid peroxidation, are rich in antioxidants [5]. High-dose paracetamol destroyed RBCs and also produced thrombocytopenia and hemolytic anemia [14]. Paracetamol may stop the kidneys from releasing erythropoietin. The number of RBC and Hb, values significantly decreased after paracetamol overdose, but the number of WBC significantly rose. Paracetamol toxicity is substantially correlated with the development of inflammatory reactions and immune cell dysfunction [15]. The co-administration of homeopathic treatments 14CH and 30CH reduced considerably the toxic effects of paracetamol and reversed these alterations. According to Patel *et al* [3], *Strychnos-nux-vomica* has bioactive compounds such as alkaloids, saponins, tannins, flavonoids, and glycosides. This explains their therapeutic actions. The results of this study revealed that kidney damage that was caused by paracetamol overdose was characterized by a significant increase of creatinine and blood urea in the paracetamol-intoxicated rats' group compared to control group. The obtained findings also showed that an overdose of paracetamol caused severe hepatotoxicity in rats as explained by an increase in hepatic enzymes GOT, GPT, and PAL when compared to control group.

Numerous harmful substances are known to build up in the liver, where they are detoxified. It was reported that a high intake of paracetamol damages the kidneys and liver by producing reactive oxygen species, increasing lipid peroxidation, depleting antioxidant enzymes, and activating cytokines that cause cell death and tissue damage [16]. The harmful effects of paracetamol on the kidneys and liver were significantly reduced after administering the homeopathic S. *nux vomica* extract.

According to the findings, treatment with paracetamol induced a considerable increase in MDA levels while concurrently inhibiting the level of GSH in the liver and kidney of rats [17]. As a result of toxicity, there is a fairly significant association between the rise in lipid peroxidation (LPO) and the fall in reduced glutathione (GSH). Malonaldehyde (MDA), a byproduct of the degradation of lipids in LPO, damages the structure of membranes as a result of a series of peroxidative processes [18]. Cytochrome P450 transforms paracetamol into a toxic metabolite (N-acetyl-p-benzoquinone imine). The glutathione detoxifies this metabolite, forming an acetaminophen-glutathione conjugate (APAP-SG). Increased APAP-SG synthesis reduces GSH levels, which results in cell death [19]. In the event of hepatotoxicity, free radicals are produced. Glutathione has antioxidant properties manifested through scavenging of free radicals. Thus, the *S. nux-vomica* homeopathic drug has antioxidant properties that prevent lipid peroxidation through decreasing MDA.

CONCLUSION

The study reveals the presence of antioxidant and anti-hemolytic principles in vomit seeds. Homeopathic treatment demonstrates superior recovery in renal parameters and liver enzyme levels. The results suggest that acetaminophen toxicity is effectively reversed with *Strychnos nux-vomica* 14CH and 30CH homeopathic treatment. Strategies to examine the findings of this study in humans should be explored.

DECLARATIONS

Acknowledgements

We thank Biology laboratory staff of University of El Oued, El Oued, Algeria for their assistance during the analysis.

Funding

None provided.

Ethical approval

Approval for this work was obtained the Ethics Committee of the University of El Oued, El Oued, Algeria (approval no. 42/EC/DCMB/FLNS/ UE2020).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Ikram Toumi designed the study, Seghir karoui collected and analyzed the data, Ikram Toumi and Ifriqya Medila supervised the data collection, analyzed the data, and reviewed the draft of the manuscript. All authors read and approved the manuscript for publication.

Open Access

REFERENCES

1. *Rajasekaran A, Periyasamy M. Hepatoprotective effect of ethanolic extract of Trichosanthes lobata on paracetamol-induced liver toxicity in rats. Chin Med 2012; 7(1): 1-6.*
2. *Canayakin D, Bayir Y, Kilic BN, Sezen KE, Atmaca HT, Kocak OFB, Keles MS, Halici Z. Paracetamol-induced nephrotoxicity and oxidative stress in rats: The protective role of Nigella sativa. Pharm Biol 2016; 54(10): 2082-2091.*
3. *Patel DK, Patel K, Duraiswamy B, Dhanabal SP. Phytochemical analysis and standardization of Strychnos nux-vomica extract through HPTLC techniques. Asian Pac J Trop Dis 2012; 2: S56-S60.*
4. *Guo R, Wang T, Zhou G, Xu M, Yu X, Zhang X, Sui F, Li C, Tang L, Wang Z. Botany, phytochemistry, pharmacology and toxicity of Strychnos nux-vomica L: A review. Am J Chinese Med 2018; 46(01): 1-23.*
5. *Bhati R, Singh A, Saharan VA, Ram V, Bhandari A. Strychnos nux-vomica seeds: Pharmacognostical standardization, extraction, and antidiabetic activity. J Ayurveda Integr Med 2012; 3: 80-84.*
6. *Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker, M, Browne WJ, Clark A, Cuthill IC, Dirnagl U et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. J Cereb Blood Flow Metab 2020; 40(9): 1769-1777.*
7. *Frenkel M. Is there a role for homeopathy in cancer care? Questions and challenges. Curr Oncol Rep 2015; 17: 1-6.*
8. *Chemouny B. Dictionnaire des médicaments et des traitements homéopathiques. ed 1. Odile Jacob, Paris, 2006.*
9. *Mubarak A, Croft KD, Bondonno CP, Din NS. Comparison of liberica and arabica coffee: Chlorogenic acid, caffeine, total phenolic and DPPH radical scavenging activity Part of the Dietetics and Clinical Nutrition Commons. Asian J Agric Biol 2019; 7(1): 130-136*
10. *Henkelman S, Rakhorst G, Blanton J, van Oeveren W. Standardization of incubation conditions for hemolysis testing of biomaterials. Mater Sci Eng C 2009; 29(5): 1650-1654.*
11. *Toumi I, Medila I, Lifa S. Hypolipidemic and cardioprotective effects of Taraxacum officinal aqueous extract in obese rats. Vet Stanica 2021; 52(4): 405-412.*
12. *Şenel E. Evolution of homeopathy: A scientometric analysis of global homeopathy literature between 1975 and 2017. Complement Ther Clin Pract 2019; 34: 165-173.*
13. *Kunwar A, Mishra B, Barik A, Kumbhare LB, Pandey R, Jain VK, Priyadarsini KI. 3, 3'-Diselenodipropionic acid, an efficient peroxyl radical scavenger and a GPx mimic, protects erythrocytes (RBCs) from AAPH-induced hemolysis. Chem Res Toxicol 2007; 20(10): 1482-1487.*
14. *Banerjee A, Kunwar A, Mishra B, Priyadarsini KI. Concentration-dependent antioxidant/pro-oxidant activity of curcumin: Studies from AAPH induced hemolysis of RBCs. Chem Biol Interact 2008; 174(2): 134-139.*
15. *Thornton JR, Losowsky MS. Severe thrombocytopenia after paracetamol overdose. Gut 1990; 31(10): 1159-1160.*
16. *Craig DG, Kitto L, Zafar S, Reid TW, Martin KG, Davidson JS, Hayes PC, Simpson, KJ. An elevated neutrophil–lymphocyte ratio is associated with adverse outcomes following single time-point paracetamol (acetaminophen) overdose: a time-course analysis. Eur J Gastroenterol Hepatol 2014; 26(9): 1022-1029.*
17. *Yousef MI, Omar SA, El-Guendi MI, Abdelmegid LA. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions, and*

haematotoxicity in rats. Food Chem Toxicol 2010; 48(11): 3246-3261.

18. *Ahmad B, Yousafzai AM, Khan AA, Attaullah M, Ali W, Ahmad S, Zamani GY, Naz F, Raziq S, Ali A. Therapeutic effects of Typha elephantina leave's extract against paracetamol-induced renal injury in rabbits. Saudi J Biol Sci 2021; 28(8): 4324-4328.*
19. *Athersuch TJ, Antoine DJ, Boobis AR, Coen M, Daly AK, Possamai L, Nicholson JK, Wilson ID. Paracetamol metabolism, hepatotoxicity, biomarkers and therapeutic interventions: a perspective. Toxicol Res 2018; 7(3): 347-357.*