

Hepatoprotective, Antioxidant and Anti-inflammatory Actions of a Novel 5-Thiocyanauracil Compound and Ascorbic Acid against Paracetamol-Induced Hepatotoxicity in Female Rats

Ibtihal J. Naor¹, Nadheerah F. Neamah², Jawad K. Hasan³

¹Al Madinah Hospital, Basrah Health Directorate, Iraq. E-mail: Ibtihaljumaa3@gmail.com

²Department of Pharmacology and Toxicology, College of Pharmacy, University of Basrah, Iraq. E-mail: Fanma-64@hotmail.com

³Department of Pharmacology, College of Medicine, University of Basrah, Iraq. E-mail: albazoonyjawad@gmail.com

Abstract

The 5-Thiocyanauracil (TCU) is a novel synthetic molecule that has been proposed for its hepatoprotective, antioxidant, and anti-inflammatory action. Overdosing on paracetamol has been linked to hepatic damage, which is a major health danger, especially for women. Our research's objective was to determine the induced hepatic, antioxidant, anti-inflammatory, and coagulopathy effects of 5-Thiocyanauracil against liver damage caused by paracetamol in rats.

Using fifty female rats (10 each group), the following treatments were administered to the groups: Control (D.W or C) group, Paracetamol or PA (500 mg/kg/day) group, TCU (50 mg/kg/day), PA & Ascorbic Acid (AA) (AA 50 mg/kg/day), and PA+TCU group. For 25 days, treatments were given orally. Rats' hearts were directly used to draw blood samples.

Prothrombin Time (PT) was evaluated using an auto-analyzer (Huma Clot Junior), whereas protein carboxylase (PC), Glutathione peroxidase (GPX), and interleukin 6 (IL-6) were detected in serum and liver homogenate using the ELISA method.

TCU treatment dramatically increased GPX activity, decreased IL6 and PC levels as compared to rats given PA. This finding supports its ability to reduce the PT/INR trend to undergo coagulopathy and to inhibit oxidation, inflammatory progressions caused by PA over dosage.

In conclusion, TCU influenced potential hepatoprotective action that could have partially attributed to its anti-inflammatory and antioxidant properties in addition to PT/INR tests against PA-induced acute liver injury in a rat model.

Keywords: Thiouracil, Paracetamol, Antioxidant, Ant Inflammation, Prothrombin Time.

DOI: 10.47750/pnr.2022.13.S03.190

INTRODUCTION

The organic moiety's sulfur atom is bonded to the carbon atom on one side and a nitrile group known as thiocyanates (-SCN) on the other. Due to these substances' remarkable stability, many organic compounds are prepared using them as intermediates. These organic (-SCN) chemicals were interesting since they are found naturally in animal extracellular fluids and are crucial to the defense system. The cyanates in organic chemistry have been the subject of several reviews. Due to these substances' unique qualities in the field of chemotherapy for cancer prevention, they have become extremely important. Agrochemical, polymer science, medicinal chemistry, and industries all employ heterocyclic organic molecules. Additionally, it has chemical structures in which its structural unit may be found in many naturally occurring products, including vitamins,

hormones, and antibiotics, in addition to several manufactured compounds, including veronal and barbituric acid, which are utilized as hypnotic agents (Abdellattif, M. H., 2021).

In the present study, TCU was prepared easily with good yield from the reaction of KSCN with 5-Iodouracil in aqueous medium. The resulting compound was characterized using spectroscopic methods which confirm the proposed structure of the product. One of the most significant heterocyclic organic molecules found in many natural products is uracil (Lu 2019). Because uracil and its derivatives have considerable pharmacological applications as anti-inflammatory, antioxidants, anti-viruses, anti-fungi, anti-bacteria, and anti-cancer... etc., they are of tremendous significance in the field of drug development. (Elwahy, A 2021).

In this investigation, the reaction of KSCN with 5-iodouracil

in aqueous medium produced TCU with acceptable yield and ease of preparation. Spectroscopic procedures have been used to characterize the final chemical, and the results support the product's hypothesized structure. Antioxidants are a group of compounds that aid in capturing and neutralizing free radicals, therefore reducing the harm that free radicals may do to the body. Additionally, antioxidants defend against a number of disorders, such as cancer and inflammation brought on by oxidative stress (OXS) (Mo *et al.*, 2021).

Reactive species are particularly unstable and reactive in the human body. The imbalance between the generation of reactive species and antioxidants leads to OXS. It is recognized as a factor in the development or exacerbation of more than a hundred pathological disorders and is known to damage biomolecules (proteins, DNA, and lipids) (Schaffer, T.K. 2016). Reactive oxygen species (ROS) is start to have a toxic effect on lipids via being complex in lipid oxidation to malondialdehyde (MDA), peroxidation of guanine in to 8-oxo-deoxyguanosine in DNA, and PC (Singh *et al.*, 2019). PC are irritable species formed indirectly or directly by lipid peroxidation intermediates may also change protein (Fragopoulou *et al.*, 2018). However, PC are essential not only as a biomarker for protein peroxidation in disease and aging. Carbonylation may change the sequence of the polypeptide, which leads to total or partial suppression of proteins (Finelli, 2020).

Antioxidants, which are chemicals that can block or delay a molecule's oxidation, can protect against oxidative damage. Antioxidants can be either enzymatic (made internally) or non-enzymatic. Superoxide dismutase (SOD), catalase (CAT), and other enzymatic processes are included (Schaffer, T. K2016), CAT is shared antioxidant enzyme existing nearly in all active tissues that use oxygen. The enzyme catalyzes the break down or decrease of hydrogen peroxides (H_2O_2) to H_2O and O_2 therefore concluding the SOD-copied detoxifying process (Ighodaro & Akinloye, 2018). SOD is identified as a protein containing copper. Superoxide anion (O_2^-) is dismutase by the SOD enzyme to produce H_2O_2 and O_2 (Zulaikhah, n.d.). The essential intracellular enzyme GPX converts lipid peroxides to their equivalent alcohols and degrades H_2O_2 to water, primarily in the mitochondria but also occasionally in the cytosol. Most of the time, selenium, a small nutritional cofactor, is required for its function. GPX is frequently referred to as a seleno cysteine peroxidase for this reason. The enzyme protects cells from OXS by playing a more crucial function in halting the process of lipid oxidation (Ighodaro & Akinloye, 2018). Using glutathione (GSH) as a co-substrate, GPX assisted in the reduction of H_2O_2 . GSSG (oxidized glutathione) is then reduced to GSH by glutathione reductase consuming NADPH. The series between these two conditions aids in free radical and poisonous substance metabolism (Kükürt, A, *et al.*, 2021).

Non enzymatic antioxidants are biological particles that can

act as antioxidants through either directly reducing a free radical or indirectly through helping free radical shifting process. Such antioxidants include GSH, transferrin, vitamin E, AA, beta carotene, and Q10(Nega & Mulata, 2017).

In regard to oxidizable substrates, antioxidants are often characterized as chemicals that considerably suppress or delay oxidative processes while they oxidize themselves (Kükürt, A, *et al.*, 2021). The rising use of analytical tools for the evaluation of these compounds' anti-oxidant effectiveness complements the growing interest in employing them (Lee, D.-Y.2020). Antioxidants inhibit of ROS formation and shifting of free radicals (Elsayed Azab *et al.*, 2019). A water-soluble antioxidant is AA.

That vitamin possesses ability to act as an electron donor, deactivate ROS, and reduce ROS levels, protecting cells from reactive radical damage.

Ascorbate has the ability to directly absorb ROS, whether with or without an enzyme facilitator. Ascorbate be able to bond directly with H_2O , (O_2^-), or tocopherol radicals (Zulaikhah, n.d.). AA can be recognized to its organic roles as a co-factor for a numeral of enzymes, most especially hydroxylases involved in collagen production (Bratovcic, 2020). Ascorbate can indirectly decrease its action via rotating tocopherol to a reduced feature. Ascorbate stimulates antioxidant links of the membrane for example α -tocopherol bind to peroxy (ROO^\bullet) and singlet oxygen (1O_2), AA acts synergistically with vitamin E (Zulaikhah, n.d.).

An early inflammatory reaction often aims to eliminate the harmful stimulation environment. However, low-grade, chronic inflammatory reactions have a considerable impact on the pathological process of many illnesses, including arthritis, autoimmune disorders, type 2 diabetes, and cancer. Nitric oxide (NO), a pro-inflammatory mediator, is necessary for the release of proinflammatory cytokines like IL-6 and TNF. It may be beneficial to inhibit NO generation to prevent a number of illnesses brought on by excessive inflammation. (Pan, G., 2021).

A pro-inflammatory cytokine IL-6 that is secreted through a variety of cells, including malignant cells; as a pyrogen, IL6 causes fever in infectious, non-infectious, and autoimmune disorders. Wherever there is inflammation, whether chronic or acute, the body produces IL-6. This covers conditions as burns, infection, injury, and cancer. The IL-6 test may be helpful as a measure of immune system activation since it aids in the regulation of immunological responses. With infection, certain malignancies, cardiovascular illnesses, autoimmune conditions, and inflammation, IL-6 levels can be increased (Masjedi, A2018). These actions are facilitated by (NF- κ B) stimulation, which controls the transcription of numerous pro-inflammatory genes. The transcriptional action of NF- κ B is excellently dependent on GSH levels. Certainly, GSH originators (e.g., N-acetyl-cysteine (NAC))increased the content of the two NF- κ B forms (Limongi *et al.*, 2019).

A widely used mild analgesic and antipyretic, PA also known as acetaminophen is available in a variety of prescription and OTC preparations. In patients with chronic liver disease (CLD), it is regarded as a first-line analgesic, particularly because of worries of serious side effects of opioid-derived and non-steroidal anti-inflammatory drugs. It is well known that PA has a wide range of hazardous effects, from anorexia and nausea to severe liver damage and death as the sulphation pathway becomes critically saturated. (Hayward, K. L 2016).

PA considered harmless through limited adverse effects, on the other hand when taken for an prolonged period or in an over dosage, it can cause hepatotoxicity, and even liver failure (Al-Doaiss, 2020). Liver cells metabolize PA via microsomal cytochrome P450 (CYP450) into harmless byproducts, N-acetyl-p-benzoquinone imine (NAPQI) (Rotundo & Pysopoulos, 2020). Commonly, NAPQI is quickly detoxified through conjugating with GSH. When processing enzymes are saturated after PA overdose, too much NAPQI deplete GSH, principal to covalent binding of thiol groups in cellular proteins, particularly mitochondrial proteins. This effects in mitochondrial OXS and dysfunction, finally hepatocytes necrosis (Yan et al., 2018).

The PT is one of the blood tests regularly employed in clinical practice to evaluate a patient's coagulation status. More specifically, PT is utilized to evaluate the intrinsic and common pathways of coagulation, which would reveal low fibrinogen concentrations as well as factors II, V, VII, and X Thromboplastin time (PT), which is a mixture of tissue factor, calcium, and phospholipid added to a patient's plasma sample, is measured in seconds. Even when utilizing the same plasma, the many different thromboplastin reagent preparations that are available can produce varying PT results. The International Normalized Ratio (INR), which was developed by the World Health Organization (WHO) in response to this variability, is now the accepted reporting format for PT findings (Barcellona, D., 2017).

METHODS AND MATERIALS

Drug source: PA powder was providing from (GSK co, EGYPT).

Ascorbic acid (AA) powder achieved by TM Media which it originated from India.

5-thiolcyaauracil (TCU) was synthesized by the author (Nadheerah F. Neamah), at pharmacology department, college of Pharmacy/ University of Basrah.

Animal preparation

For an experiment that will run from 2022 to 2022, fifty female rats were purchased in January from University of Qadsia/ Veterinary Medicine College. The rats were measured, weighed, and kept in polypropylene cages lined with sawdust (5 rats per cage). Rats had access to clean

water and were fed rat pellets. The rats had to get used to the lab's natural illumination and $21\pm 4^{\circ}\text{C}$ temperature at first. The cages were clustered together and had labels on them. The rats classified to five groups each consist of ten. Distilled water was given to Group I, which served as the negative control. As the positive control, a single oral dose of 500 mg of PA/kg/day was given to Group II. TCU was administered to Group III as a single oral dose at a dosage of 50 mg/kg/day. Two hours after consuming an oral dose of 500 mg/kg/day of PA, Group IV was administered a single dose of an oral solution containing 50 mg of (TCU)/kg/day.

Group V was given a single oral dosage of a solution containing 50 mg of (AA) /kg/day within two hours of the delivery of the PA solution (500 mg/kg). The therapy regimen lasted about 25 days. After chloroform anesthesia, all rats were sacrificed. Blood was collected from the heart, centrifuged at 3000 g for 10 min at 4°C . Serum was collected, then separated in vials, and stored at -20°C to assist biochemical research. In order to extract plasma for PT and INR testing, two ml of blood samples from rats stored in plastic tubes containing sodium citrate were centrifuged at 3000 rpm for 15 minutes.

Tissue Homogenate Training

Parts of liver were cut and homogenized with lysis buffer then centrifuged for 15 minutes at 10000r on 4°C . The supernatant was collected, kept at serum collected tubes at -20°C for promote examination.

GPX activity, PC and IL-6 determination

All those enzymes concentrations were estimated by means of the Sandwich enzyme-linked immunosorbent assay (ELISA kit for rat: ELK Biotechnology Co. Ltd. China). The measurement results of GPX, PC and IL-6 were achieved within 3 hours.

PT determination

The separated plasma was analyzed to do PT and INR. PT was estimated using Huma-Clot Junior (BIO LABO, BIO-TP kit).

Analytical Statistics

All data were studied analyzed by the SPSS 16.0 program (IBM, Armonk, NY, USA).

One-way (ANOVA) was used to conclude statistical significance, followed by the Tukey post-hoc test. Data were identified as mean \pm standard deviation (SD). Significant was determined to be $P < 0.05$.

RESULT

GPX activity in Serum and Liver Tissue: As showed in the Figure 1, and table (1). Hepatotoxic group (PA) compared to the negative control group(C) there was no

important alteration at both serum and tissue levels of GPX. Administration of TCU increased significantly GPX activity in serum at TCU group compared to PA; however, TCU group exerts high concentration of GPX the control group(C) and PA at hepatic tissue.

While GPX levels were significantly increased in serum and tissue following PA treatment in the PA&TCU, and PA&AA groups than in PA group. Administration of TCU after PA treatment avoided decrease of GPX in serum and liver tissues. The AA after PA treatment increase GPX

activity in serum and tissues in comparison with C, PA, and PA &TCU groups.

Protein Peroxidation showed insignificant changes at serum PC concentration of all test groups when compared with PA group. However, at tissue level, PA group revealed significantly increased in PC concentration than C group. Conversely, treatment by TCU or AA after PA treatment significantly inhibited of PC concentrations, in comparison with PA group, as illustrated at fig.2 and table 1.

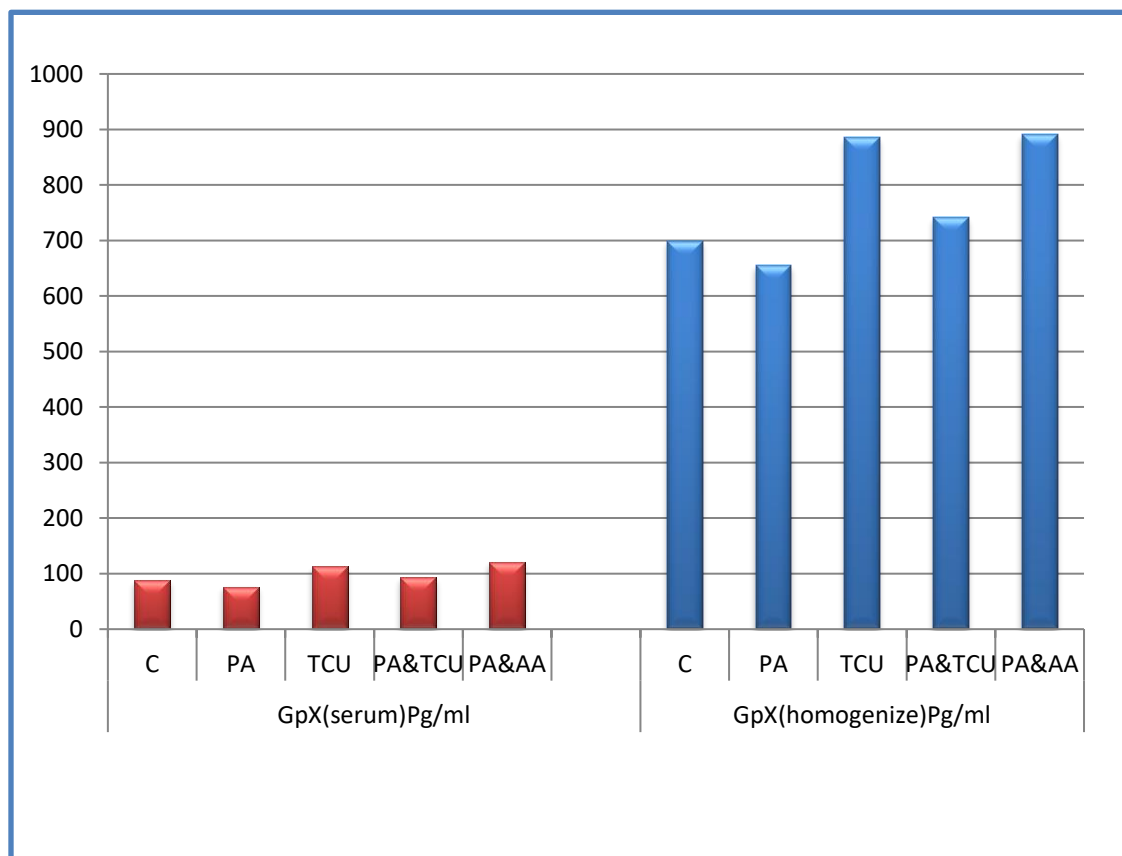


Fig 1: showed the levels of GPX at both serum and liver homogenize

Table 1: represent both Serum and hepatic GPX and PC concentrations

Serum		
groups	PC(nmol/ml)	GPX(Pg/ml)
C	1.195417429±0.455	87.8±25.862
PA	1.552083 ±0.8867	76±16.255
TCU	1.028073 ± 0.397	112.5±33.96b
PA&TCU	1.131107± 0.3657	92.6±40.885b
PA&AA	1.208556 ±0.2009	120.6±27.55abc
LSD	NS	28.0

Homogenize		
groups	PC(nmol/ml)	GPX(Pg/ml)
C	17.7517± 3.43	699.14±110.87
PA	21.808± 3.21a	655.71±103.57
TCU	16.034±4.445b	885±117.5ab
PA&TCU	15.7± 4.29b	741.8±187.35b
PA&AA	15.025± 2.33b	891.5±141.3abc
LSD	4.05673	149.67

a =significant differences with C group, b= significant differences with PA group, c= significant differences with PA& TCU group. P<0.05.

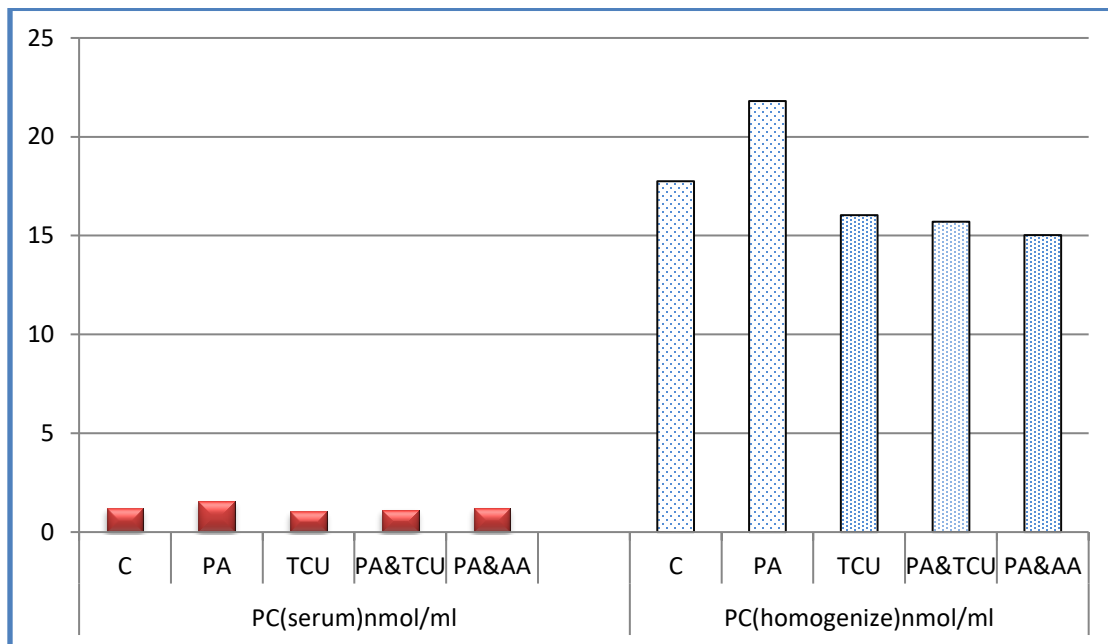


Fig. (2): Serum and liver tissue PC concentrations

IL6 levels at Serum and Liver Tissue

The results of the current study that only TCU group exhibited significant ($p < 0.05$) decrease in IL6 concentrations than PA group IL6 levels at both serum and tissue. There were no significant differences among test groups and C group. Table 2 and fig 3 reported IL6 concentrations results.

Table 2: Serum and liver tissue IL6 concentrations

	Serum	Homogenize
groups	IL6 (pg/ml)	IL6(Pg/ml)
C	68.33±18.67	385.33±59.62
PA	89.17±17.84	461.66±84.675
TCU	58.6667*±6.62	337.66*±69.68
PA&TCU	70.16667±9.786	373.2±43.95
LSD	30.5	124

* $p < 0.05$ between TCU and PA groups.

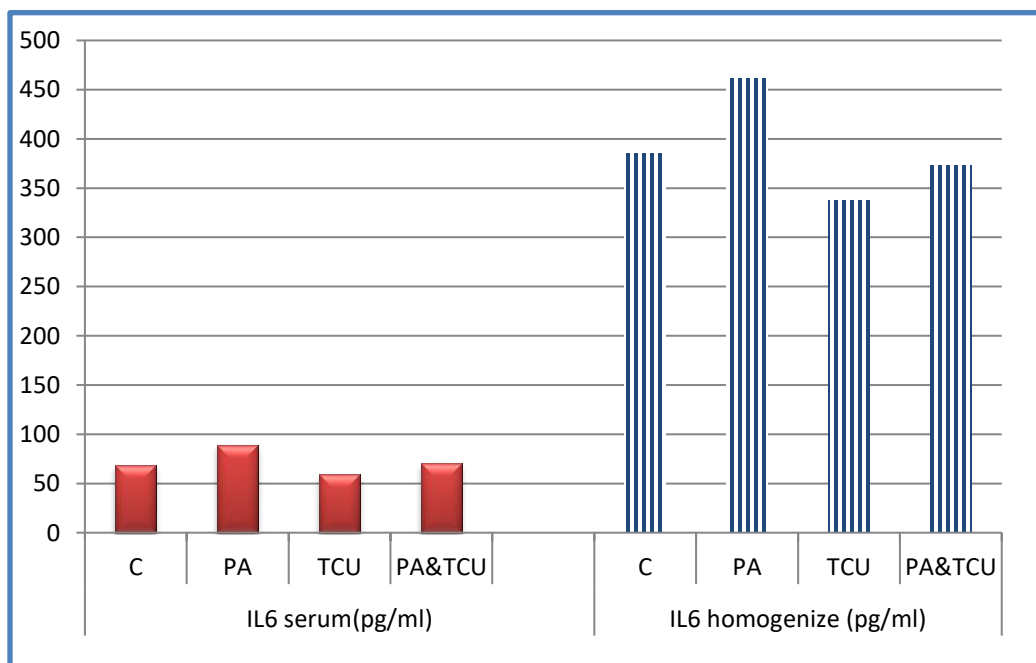


Fig. 3: IL6 serum and liver tissue concentration, * significant differences $p < 0.05$.

Effects on PT and INR

The present study illustrated (as in table 3 and fig. 4) that PT of PA group significantly increased than C group, while TCU group showed statistically significant ($p < 0.05$) decreased than at C and PA groups, while PA&TCU showed only significant alteration ($p < 0.05$) than PA group.

Table (3): Prothrombin time of test groups.

groups	PT (second)	INR
C	19.8±0.725	0.7525±0.037
PA	28.225±5.977a	1.20475±1.2a
TCU	11.9±7.22ab	0.401±0.323ab
PA&TCU	19±0.816b	0.71±0.04b
LSD	7.9	0.49

a significant difference with C group, b significant differences with PA group $p < 0.05$.

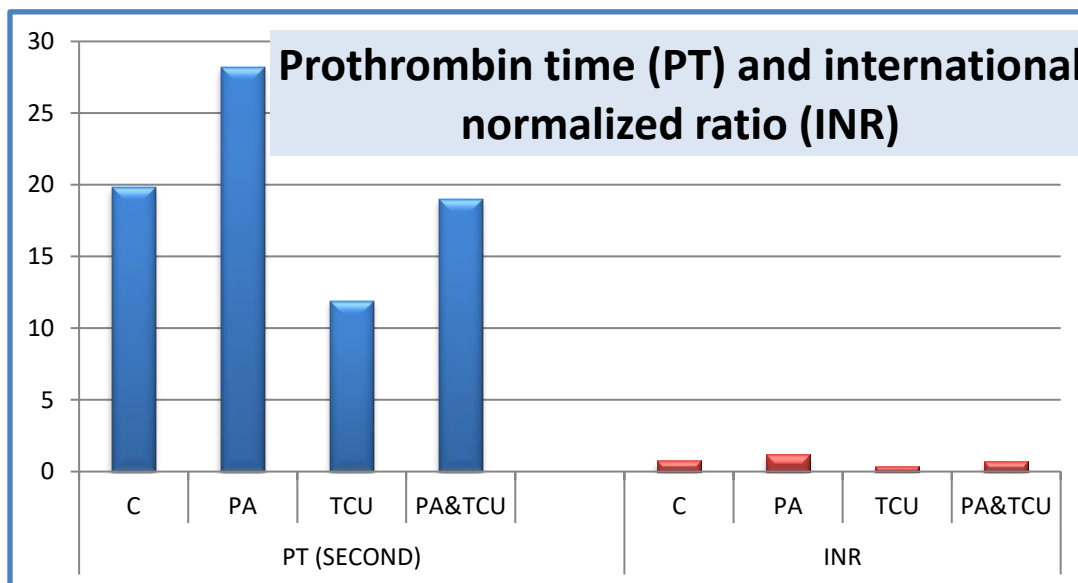


Fig. 4: Prothrombin time and INR of test groups

DISCUSSION

It was documented by a study that synthetic organosulfur compounds have significant antioxidant activity (Sauer, A. 2017). OXS can therefore seriously harm the cell. Thiol is a vital antioxidant that guards the cell from oxidative stress and prevents damage caused by it. Taurine and GSH are two crucial thiols. Thiol status is known to alter in a number of disorders, and thiol/disulphide equilibrium plays an important role in the pathogenesis of digestive, pulmonary, reproductive, urinary, and metabolic systems disorders, as well as cancer. This further demonstrates the critical role that thiol status plays in the development of OXS-mediated illnesses. Therefore, it is believed that measures to enhance thiol status may aid in the prevention or management of disorders linked to OXS (Kükürt, A., 2021).

Mechanisms liver damage initiated by PA are particularly complex, and numerous intracellularly and extracellularly events, such as metabolism of PA, OXS produce in mitochondria, autophagy, microcirculatory dysfunction, sterile inflammation, and liver regeneration, are involved in this pathophysiological process. These events control acute liver injury in a number of ways, including beginning the

damage, directly causing hepatocyte death, restricting cellular stress response, and promoting liver regeneration and repair. Therefore, many other mechanisms in addition to mitochondrial OXS (which is well established) may be viable therapeutic targets to treat acute liver injury. However, it should be emphasized that certain cellular processes could have paradoxical effects throughout various stages of acute liver injury, acting both favorably and unfavorably in the control of PA hepatotoxicity. As a result, therapeutic approaches focused on these events might not be ultimately effective, a worry that has been mirrored in several preclinical investigations. Generally, further research is required just before pinpoint the precise function of these time-dependent processes in Acetaminophen induced liver injury (AILI) and enable the clinical application of late phase therapy (Yan, M., 2018).

The development of derivatives of dietary-interesting polyphenols with improved antioxidant properties that can be used, for example, as additives for food preservation during storage, seems like an obvious choice when using GSH, which is crucial for the detoxification processes and confers water solubility. Antioxidant dietary polyphenols can scavenge reactive nitrogen species (RNS), which are

produced at the acidic PH of the gastric compartments after consumption of food containing nitrite ions up to micromolar levels, such as cured meats, vegetables, etc., to control tumor-initiating events in the gastrointestinal tract. RNS may cause significant biomolecules to be nitrosated, nitrated, or oxidized, which could have toxic, mutagenic, or carcinogenic effects (Alfieri, M.L., 2022).

AA and E, enzymes like SOD, CAT, and GPX, and thiols or sulfhydryl -containing substances like GSH and thioredoxin are some of the cellular antioxidant defense mechanisms. The GSH has received the most in-depth research. It protects cells by acting as an antioxidant defense mechanism as well as through a variety of other processes, such as protein thiolation, drug detoxification, and control of signal transduction that is influenced by oxidation-reduction reactions. The free sulfhydryl group in GSH, which serves as a source of reducing equivalents to scavenge damaging ROS, is responsible for the antioxidant activity. By offering reducing equivalents, GSH also significantly contributes to GPX's antioxidant activity, a defense mechanism against peroxides. NAC replenishes the hepatic GSH pool lost during the drug detoxification process, acting as an antidote to PA toxicity.

NAPQI, an electrophilic and dangerous metabolite of PA, interacts with GSH and neutralizes it. GSH also scavenges ROS and RNS. At therapeutic dosages, PA is regarded as a safe medication. More than 90% of PA is removed under these circumstances by phase II conjugation processes, and less than 10% is converted by cytochrome P450 2E1 to the reactive metabolite NAPQI (Raghu, G., 2021).

A study reported that the main results were the difference in mean PT and INR between the control group and the group receiving 8 months of long-term PA medication. The mean observed PT and INR were considerably increased in participants taking 1 g of PA daily. The PA patients' mean PT was significantly different from the control participants' (12.083 0.077) from the second month of treatment onward (P 0.01). Dakheel, S. (2015), which agree with the results in the current article.

Rats were used to standardize the determination of PT and APTT while accounting for all the intrinsic and extrinsic clotting variables. It is generally recognized that distinct thromboplastins and cephaloplastins have different sensitivity responses depending on the tissues, species, and collecting techniques, which will be reflected in shorter or longer clotting durations (García-Manzano, A., 2001). In the diabetic brain, Cazzola (2021), who confirms our observations, found that NAC reversed the platelet activation, proteins glycation, and pro-coagulation reactions and protected against thrombosis. Additionally, it led to a restoration of intraplatelet GSH in type 2 diabetes patients who had intracellular GSH deficiency, along with a reduction in platelet-monocyte conjugation.

Erdosteine reduced plasma concentration of factors II, VII, VII, IX, and X, prolonged prothrombin time (PT), activated

partial thromboplastin time (aPTT), and antithrombin levels in rats (Cazzola et al., 2021).

Following the administration of NAC, Pakravan and colleagues discovered a substantial decrease in the activity of plasma Vitamin-K dependent clotting factors (II, VII, IX, and X) (Wong & Graudins, 2016) Chemotherapy has been demonstrated to exacerbate the hypercoagulability state, as according numerous studies.

When chemotherapy is used in advanced cancer patients who have a high risk for thrombosis or in patients with additional risk factors, it is important to take into account the tendency toward hypercoagulability that is induced by cyclophosphamide, methotrexate, and fluorouracil.

The current study added to the clinical evidence in the area, (WANG et al., 2015), which the current article accepts with its findings.

CONCLUSION

In conclusion, TCU has been shown to have protective properties against PA induced acute hepatic injury in a rat model by the decreased level of PC and increase level of GPX in serum and homogenized tissue. TCU and other drugs had clear influence on IL-6 that suggesting TCU capability to reduce the inflammatory processes.

FINANCIAL SUPPORT AND SPONSORSHIP

Nil.

CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES

- Abdellattif, M. H., Shahbaaz, M., Arief, M. M. H., & Hussien, M. A. (2021). Oxazinethione Derivatives as a Precursor to Pyrazolone and Pyrimidine Derivatives: Synthesis, Biological Activities, Molecular Modeling, ADME, and Molecular Dynamics Studies. *Molecules*, 26(18). doi: 10.3390/molecules26185482
- Al-Doaiss, A. A. (2020). Hepatotoxicity-Induced by the Therapeutic Dose of Acetaminophen and the Ameliorative Effect of Oral Co-administration of Selenium/ Tribulus terrestris Extract in Rats Hepatotoxicidad Inducida por la Dosis Terapéutica de Acetaminofén y el Efecto de Mejora de la Administración Conjunta de Extracto de Selenio Tribulus terrestris en Ratas. In *Int. J. Morphol* (Vol. 38, Issue 5). www.theplantlist.org
- Alfieri, M. L., Panzella, L., Amorati, R., Cariola, A., Valgimigli, L., & Napolitano, A. (2022). Role of Sulphur and Heavier Chalcogens on the Antioxidant Power and Bioactivity of Natural Phenolic Compounds. *Biomolecules*, 12(1), 90.
- Barcellona, D., Fenu, L., & Marongiu, F. (2017). Point-of-care testing INR: an overview. *Clin Chem Lab Med*, 55(6), 800-805. doi: 10.1515/cclm-2016-0381
- Bratovcic, A. (2020). Antioxidant Enzymes and their Role in Preventing Cell Damage. *Acta Scientifci Nutritional Health*, 4(3), 01–07. https://doi.org/10.31080/asn.2020.04.0659
- Cazzola, M., Rogliani, P., Salvi, S. S., Ora, J., & Matera, M. G. (2021). Use of Thiols in the Treatment of COVID-19: Current Evidence. In

- Lung (Vol. 199, Issue 4, pp. 335–343). Springer. <https://doi.org/10.1007/s00408-021-00465-3>
- Dakheel, S. (2015). Low Doses of Acetaminophen Prolonged Prothrombin Time and Increased International Normalized Ratio in Patients Receiving Long-Term Therapy. *Clinical & Experimental Pharmacology*, 05. doi: 10.4172/2161-1459.1000187
- Elsayed Azab, A., A Adwas, Almokhtar, Ibrahim Elsayed, A. S., A Adwas, A., Ibrahim Elsayed, Ata Sedik, & Quwaydir, F. A. (2019). Oxidative stress and antioxidant mechanisms in human body. *Journal of Applied Biotechnology & Bioengineering*, 6(1), 43–47. <https://doi.org/10.15406/jabb.2019.06.00173>
- Elwahy, A., Diab, H., Salem, M., & Abdelhamid, I. (2021). Aminouracil and aminothiouracil as versatile precursors for a variety of heterocyclic systems. *ARKIVOC*, 2021. doi: 10.24820/ark.5550190.p011.474
- Finelli, M. J. (2020). Redox Post-translational Modifications of Protein Thiols in Brain Aging and Neurodegenerative Conditions—Focus on S-Nitrosation. In *Frontiers in Aging Neuroscience* (Vol. 12). Frontiers Media S.A. <https://doi.org/10.3389/fnagi.2020.00254>
- Fragopoulou, E., Gavriil, L., Argyrou, C., Malagaris, I., Choleva, M., Antonopoulou, S., Afxentiou, G., & Nikolaou, E. (2018). Suppression of DNA/RNA and protein oxidation by dietary supplement which contains plant extracts and vitamins: A randomized, double-blind, placebo-controlled trial. *Lipids in Health and Disease*, 17(1). <https://doi.org/10.1186/s12944-018-0836-z>
- García-Manzano, A., González-Llaven, J., Lemini, C., & Rubio-Póo, C. (2001). Standardization of rat blood clotting tests with reagents used for humans. *Proceedings of the Western Pharmacology Society*, 44, 153-155.
- Hayward, K. L., Powell, E. E., Irvine, K. M., & Martin, J. H. (2016). Can paracetamol (acetaminophen) be administered to patients with liver impairment? *Br J Clin Pharmacol*, 81(2), 210-222. doi: 10.1111/bcp.12802
- Ighodaro, O. M., & Akinloye, O. A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*, 54(4), 287–293. <https://doi.org/10.1016/j.ajme.2017.09.001>
- Kükürt, A., Gelen, V., Başer, Ö. F., Deveci, H. A., & Karapehlivan, M. (2021). Thiols: Role in Oxidative Stress-Related Disorders. In (Ed.), *Accenting Lipid Peroxidation*. IntechOpen. <https://doi.org/10.5772/intechopen.96682>
- Limongi, D., Baldelli, S., Checconi, P., Marcocci, M., De Chiara, G., Fraternali, A., Magnani, M., Ciriolo, M. R., & Palamara, A. T. (2019). GSH-C4 acts as anti-inflammatory drug in different models of canonical and cell autonomous inflammation through NFκB inhibition. *Frontiers in Immunology*, 10.
- Lee, D.-Y., Park, Y.-J., Song, M.-G., Kim, D. R., Zada, S., & Kim, D.-H. (2020). Cytoprotective Effects of Delphinidin for Human Chondrocytes against Oxidative Stress through Activation of Autophagy. *Antioxidants*, 9(1), 83. <https://doi.org/10.3389/antiox.2019.00155>
- Lu, G. Q., Li, X. Y., Mohamed, O. K., Wang, D., & Meng, F. H. (2019). Design, synthesis and biological evaluation of novel uracil derivatives bearing 1, 2, 3-triazole moiety as thymidylate synthase (TS) inhibitors and as potential antitumor drugs. *Eur J Med Chem*, 171, 282-296. doi: 10.1016/j.ejmech.2019.03.047
- Masjedi, A., Hashemi, V., Hojjat-Farsangi, M., Ghalamfarsa, G., Azizi, G., Yousefi, M., & Jadidi-Niaragh, F. (2018). The significant role of interleukin-6 and its signaling pathway in the immunopathogenesis and treatment of breast cancer. *Biomedicine & Pharmacotherapy*, 108, 1415-1424. doi: <https://doi.org/10.1016/j.biopha.2018.09.177>
- Mo, Y. N., Cheng, F., Yang, Z., Shang, X. F., Liang, J. P., Shang, R. F., Hao, B. C., Wang, X. H., Zhang, H. J., Wali, A., Lu, C. F., & Liu, Y. (2021). Antioxidant Activity and the Potential Mechanism of the Fruit From *Ailanthus altissima* Swingle. *Frontiers in Veterinary Science*, 8. <https://doi.org/10.3389/fvets.2021.784898>
- Nega, M. H., & Mulata, H. N. (2017). Role of Endogenous and Exogenous Glutathione in the Detoxification of Free Radicals Genetic determinants of response to chemicals View project Role of Endogenous and Exogenous Glutathione in the Detoxification of Free Radicals. 7(1), 1–14. www.stmjournals.com
- Pan, G., Li, Y., Che, X., Tian, D., Han, W., Wang, Z.,.... Kong, F. (2021). New Thio-Compounds and Monoterpenes With Anti-inflammatory Activities From the Fungus *Aspergillus* sp. CYH26. *Front Microbiol*, 12, 668938. doi: 10.3389/fmicb.2021.668938
- Raghu, G., Berk, M., Campochiaro, P. A., Jaeschke, H., Marenzi, G., Richeldi, L.,.... Calverley, P. M. A. (2021). The Multifaceted Therapeutic Role of N-Acetylcysteine (NAC) in Disorders Characterized by Oxidative Stress. *Curr Neuropharmacol*, 19(8), 1202-1224. doi: 10.2174/1570159x19666201230144109
- Rotundo, L., & Pysropoulos, N. (2020). Liver injury induced by paracetamol and challenges associated with intentional and unintentional use. *World Journal of Hepatology*, 12(4), 125–136. <https://doi.org/10.4254/wjh.v12.i4.125>
- Singh, A., Kukreti, R., Saso, L., & Kukreti, S. (2019). Oxidative stress: A key modulator in neurodegenerative diseases. In *Molecules* (Vol. 24, Issue 8). MDPI AG. <https://doi.org/10.3390/molecules24081583>
- Schaffer, T. K., Wohlenberg, M. F., Medeiros, N., Martins, J. B., Agostini, F., Funchal, C., & Dani, C. (2016). Evaluation of antioxidant activity of grapevine leaves extracts (*Vitis labrusca*) in liver of Wistar rats. *An Acad Bras Cienc*, 88(1), 187-196. doi: 10.1590/0001-3765201620140658
- Sauer, A. C., Leal, J. G., Stefanello, S. T., Leite, M. T. B., Souza, M. B., Soares, F. A. A.,.... Dornelles, L. (2017). Synthesis and antioxidant properties of organosulfur and organoselenium compounds derived from 5-substituted-1,3,4-oxadiazole/thiadiazole-2-thiols. *Tetrahedron Letters*, 58(1), 87-91. doi: <https://doi.org/10.1016/j.tetlet.2016.11.106>
- WANG, Z., DANG, C., ZHU, K., ZHANG, Y., CHANG, D., XIA, P., SONG, Y., & LI, K. (2015). Cyclophosphamide, epirubicin and fluorouracil chemotherapy-induced alteration of haemostasis markers in breast cancer patients. *Molecular and Clinical Oncology*, 3(5), 1088–1092. <https://doi.org/10.3892/mco.2015.584>
- Wong, A., & Gaudins, A. (2016). N-acetylcysteine regimens for paracetamol overdose: Time for a change? *EMA - Emergency Medicine Australasia*, 28(6), 749–751. <https://doi.org/10.1111/1742-6723.12610>
- Yan, M., Huo, Y., Yin, S., & Hu, H. (2018). Mechanisms of acetaminophen-induced liver injury and its implications for therapeutic interventions. In *Redox Biology* (Vol. 17, pp. 274–283). Elsevier B.V. <https://doi.org/10.1016/j.redox.2018.04.019>
- Zulaikhah, S. T. (n.d.). The Role of Antioxidant to Prevent Free Radicals in The Body. In *Sains Medika* (Vol. 8, Issue 1). <http://jurnal.unissula.ac.id/index.php/sainsmedika>