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ESTER PRODRUG OF PARACETAMOL: SYNTHESIS, CHARACTERIZATION AND EVALUATION

Arun Parashar*

VNS Faculty of Pharmacy, Neelbud, Bhopal-462044.

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***Correspondence for Author**
Dr. Arun Parashar
VNS Faculty of Pharmacy, Neelbud, Bhopal-462044.

ABSTRACT

Background: Besides being the most widely consumed drug, paracetamol is also the leading cause for hepatic failure in many of the western countries. According to the USFDA acute overdoses of paracetamol can cause potentially serious liver damage. **Objective:** This study aimed toward designing and evaluating a safer analog (in terms of hepatotoxicity) of paracetamol in the form of a prodrug. The prodrug was evaluated for the proverbial therapeutic effects of paracetamol i.e. analgesic & antipyretic, together with gastric lesion healing and hepatotoxicity studies. Prodrug was given to animals in a dose molecularly equivalent to that of paracetamol. The dose was calculated using molecular weight of paracetamol and the prodrug. **Methodology:** Paracetamol prodrug was synthesized by esterification between carboxyl group of amino acid and hydroxyl group of paracetamol. Analgesic, antipyretic, ulcer healing and hepatotoxic activities were performed in this study. **Results:** Prodrug showed a 36% inhibition in writhings as compared to 53.3% of paracetamol. Maximum antipyretic effect was observed 3 hours after the drug administration. Fever reduction was greater in paracetamol group. Prodrug showed excellent gastroprotective effects as it reduced the gastric lesions by 20.3%. The prodrug exhibited a hepatoprotective effect by preventing the rise in liver enzymes (SGOT, SGPT and bilirubin). The most notable effect of prodrug was in preventing the fall of hepatic glutathione (GSH) levels as it is one of the major antioxidant of our body. **Conclusion:** Prodrug showed excellent hepatoprotective and gastroprotective effects, although it's therapeutic efficacy was compromised.

KEYWORDS: Paracetamol, Prodrug, Hepatotoxicity, Amino acid, Alanine.

INTRODUCTION

When it comes to pain and fever, paracetamol is the most widely consumed medicine, and because of this it has also been one of the most widely studied in many fields of pharmaceutical sciences, especially in toxicology, pharmacokinetics and drug metabolism.[1] Paracetamol, the drug we consider safe, is the leading cause of acute liver failure in the United States, the United Kingdom, Australia and New Zealand. [2,3,4,5,6] The recommended dose of paracetamol is believed to be safe, although in few cases liver toxicity in pediatric patients has been reported even after a single paracetamol dose of 120- 150 mg/kg of body weight. [7, 8] But of utmost concern, is the overdosage that leads to potential toxic effects varying from upper gastrointestinal complications such as stomach bleeding to liver failure. USFDA states that paracetamol can cause serious liver damage if more than directed is used. [9] According to the American Association of Poison Control Centers, more than 127,000 exposures involving paracetamol were reported in 2003. There were 214 deaths involving overdose of an analgesic agent. In 62 of these cases, Paracetamol was solely responsible agent. [10,11,12] The toxic effects of paracetamol are attributed to formation of a toxic metabolite N-acetyl-p-benzoquinoneimine (NAPQI), which is detoxified by reaction with glutathione leading to glutathione depletion and cell death. [13,14] Using prodrug as combat strategy could prove helpful. In this context, phenol drugs are attractive targets for prodrug designing because the OH group is very convenient to attach a wide range of promoieties. [15] Majority of the work on phenols for prodrug designing has focused on corresponding ester or ether. [16, 17, 18, 19] Since amino acids are normal dietary constituents and are non-toxic in moderate doses as compared to other promoieties, their incorporation as promoiety might serve beneficial in overcoming the toxic effects of paracetamol. Paracetamol was given in the recommended therapeutic doses and so was the prodrug, except for the hepatotoxicity studies where the prodrug was given in molecular equivalent amount of paracetamol that induces hepatotoxicity.

METHODOLOGY

Synthesis of Prodrug (Fig 1): Synthesis comprised of a three step process involving amino acid protection, reaction with paracetamol and finally the deprotection. All the chemicals used were purchased from Loba Chemie except for trifluoroacetic acid (TFA) and dichloromethane (DCM) which were purchased from Fisher scientific. In order to synthesize the ester prodrug, first of all the amino group of amino acid was protected so that it could not react with the paracetamol molecule. This was achieved by use of phthalic anhydride by a

process known as phthaloylation. In this process alanine was fused with phthalic anhydride. The second step included the reaction of protected amino acid with paracetamol. This reaction required use of special catalysts like N, N-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP). Both of these catalysts are used in peptide synthesis mostly. The phthalic group was removed in the third step using TFA and DCM.

Physiochemical characterization

Characterization and identification of prodrug was done using various methods like TLC, UV spectroscopy and IR spectroscopy.

In Vivo analysis (20)

Wistar rats and Balb/c mice of either sex were used in this research. Study consisted of a control group that received saline (0.9% NaCl) or propylene glycol, a Standard group that received paracetamol and a test group that received alanine prodrug in a dose molecularly equivalent to paracetamol (e.g.: ten molecules of prodrug for ten molecules of paracetamol). All the experiments were conducted in the animal house of VNS Faculty of Pharmacy, approved by the *Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)* (Regd no. 778/PO/a/03/CPCSEA).

Analgesic activity (Writhing test): Acetic acid (0.1 ml of 0.6% v/v) intraperitoneally (i.p) was used to induce pain in mice. Control group received 0.9% w/v NaCl. Standard group received 100 mg/kg paracetamol and test group received 186 mg/kg of prodrug which is molecularly equivalent to that of paracetamol given to standard group.

Antipyretic activity (Brewer's yeast induced hyperpyrexia method): Pyresis was induced using a 20% suspension of Brewer's yeast (Loba Chemie) in 0.9% saline at a dose of 10 mg/kg) by subcutaneous administration. [21] Body temperature was monitored before injecting brewer's yeast suspension rectally. Post 18hrs brewer's yeast injection, the vehicle, standard drug and test drugs were administered to different groups. Propylene glycol at dose of 5 ml/kg was administered orally to the control groups of animals and Paracetamol at dose of 150mg/kg was administered orally to standard group of animals. Test group received 222 mg/kg of Alanine prodrug. Rectal temperature was recorded by clinical thermometer at 0, 1, 2 and 3hrs after drug administration. Anti-Ulcer activity (Aspirin induced gastric lesions method): Amino acids have a healing effect on gastric lesions. [22] Each group received 300 mg/kg of aspirin orally to produce gastric lesions. The animals fasted 24 h before the

experiment, but had free access to water. The test-substances were administered 30 min before Aspirin (300 mg /kg) Paracetamol was given at its therapeutic dose for fever i.e. 150 mg/kg orally. Test group received 222 mg/kg of Alanine prodrug.

Hepatotoxicity: Paracetamol is a potent hepatotoxic agent. Standard group received paracetamol at a dose of 2000 mg/kg or 2 gm/kg, which is reported to produce hepatic tissue injury. Test group received 2.94 gm/kg of alanine prodrug, which is equivalent dose to that of paracetamol. After 3 hours of fasting they were treated with drugs orally. Hepatic parameters like SGOT, SGPT, bilirubin and glutathione (GSH) levels were analysed using biochemical autoanalyser.

Statistical Analysis

Statistical Analysis Values were represented as mean ± SEM. Data was analyzed using one-way analysis of variance (ANOVA) and group means were compared using the Dunnett's multiple comparison test using GraphPad prism v5.03 software. $P < 0.05$ was considered significant.

Results (Fig 2)

Paracetamol was more effective in reducing the number of writhings and hence showed better analgesic activity than its prodrug. Maximum antipyretic of all drugs was achieved 3hours after treatment. Antipyretic effect of paracetamol was greatest, while the prodrug showed a lesser effect. As expected, the prodrug showed a good ulcer healing activity. Surprisingly, paracetamol, instead of being a NSAID didn't increase the severity of gastric lesions. In terms of hepatotoxicity, prodrug significantly protected the rise in hepatic enzymes. But most importantly, the prodrug prevented the depletion of glutathione levels in liver as compared to paracetamol.

DISCUSSION

Paracetamol showed better pharmacological activities than its alanine prodrug. The prodrug scored in the field of ulcer healing and hepatoprotective effects. Most notable effect was with the levels of glutathione (GSH). Alanine-paracetamol prodrug significantly protected the depletion of GSH levels as compared to its parent drug. To summarize, alanine-paracetamol prodrug was of low therapeutic benefit. Further studies are required in this direction using multiple amino acid sequence in different combinations to yield a much better and safer prodrug of paracetamol.

Figures

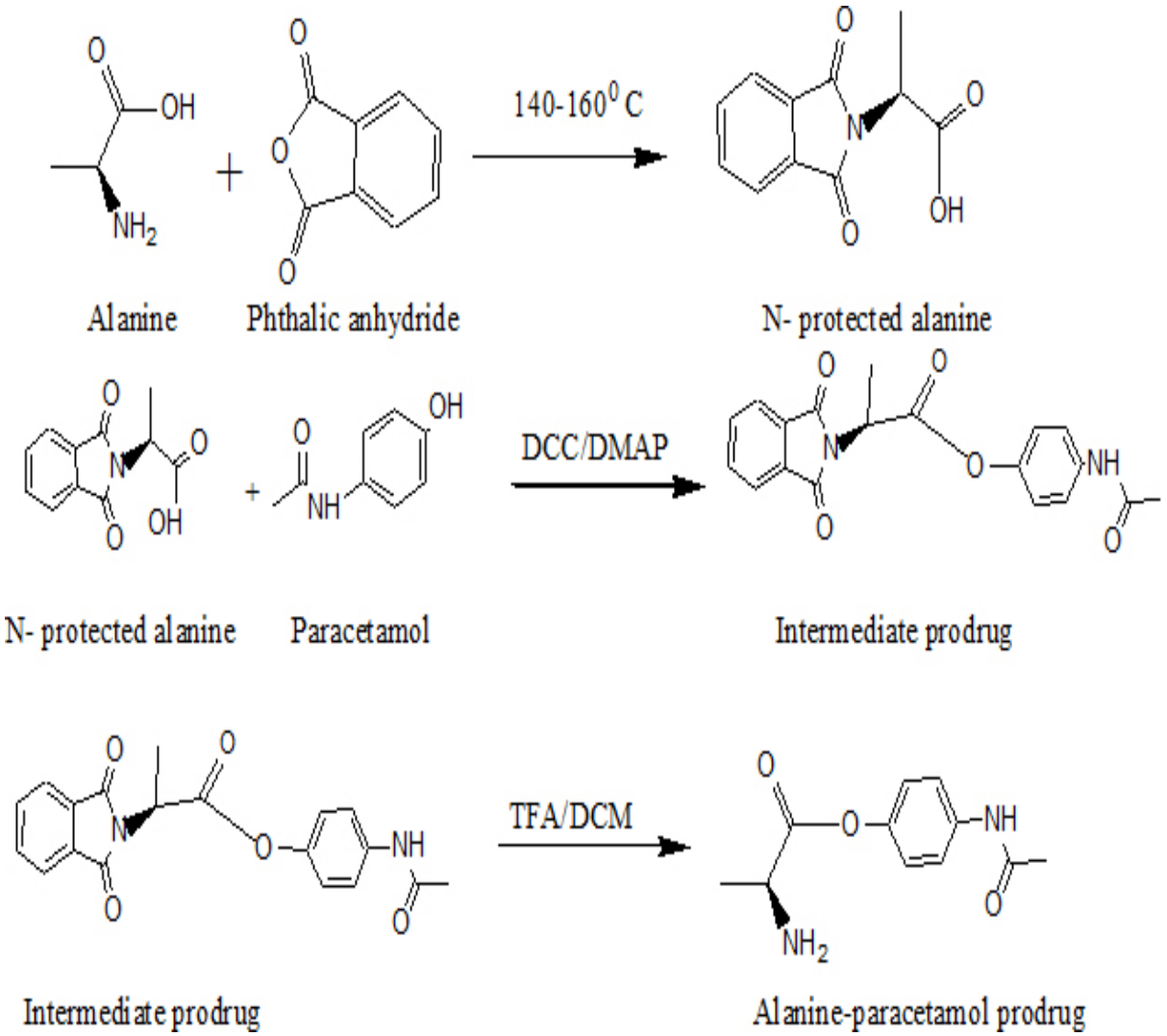

Figure 1: Synthesis of prodrug. It involved three steps viz a) amino group protection of amino acid using phthaloylation, b) reaction of paracetamol with protected amino acid and c) removal of the amino group protection (deprotection).

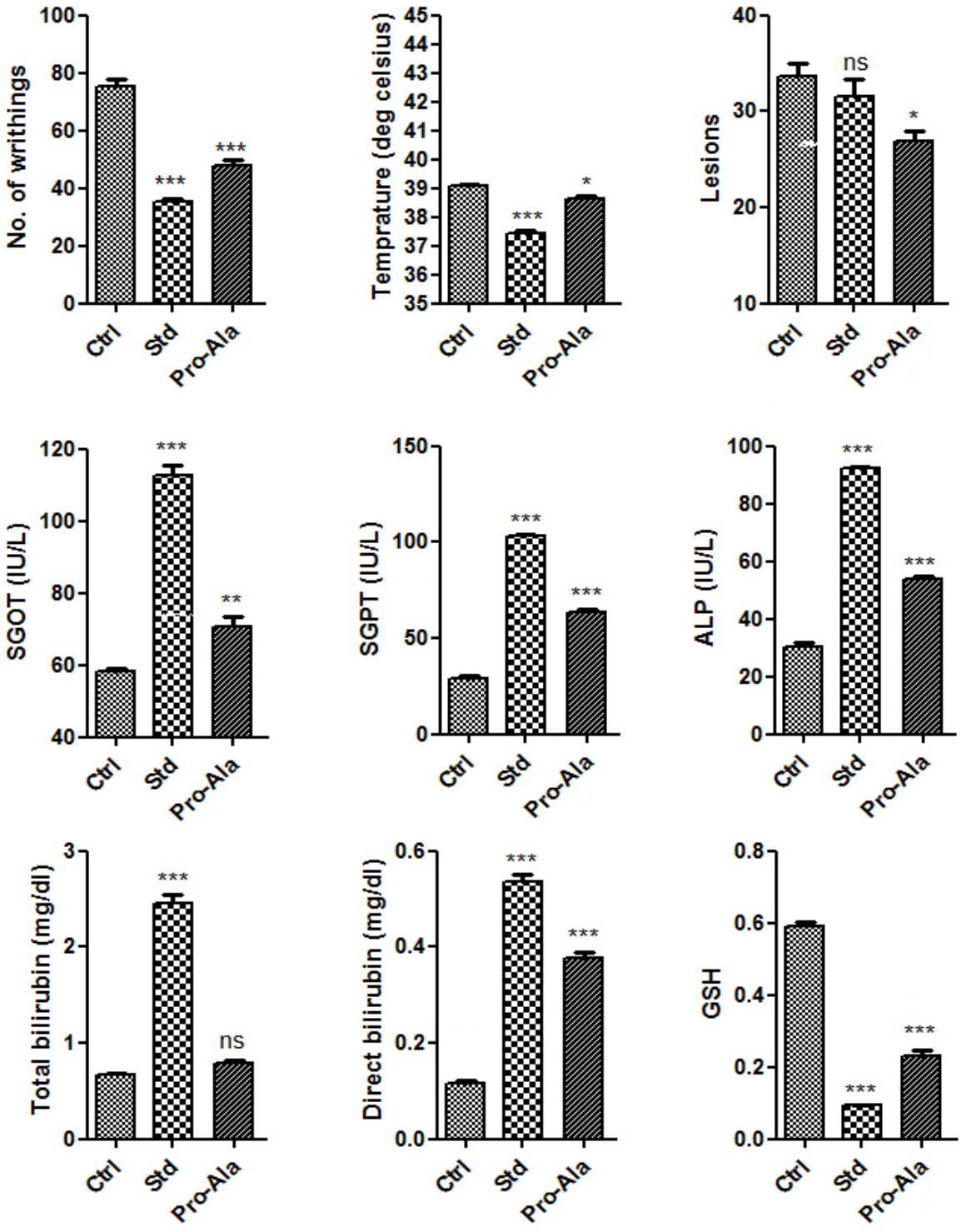

Fig 2: In-vivo activities performed for the screening of alanine-paracetamol prodrug. First row (left to right) analgesic activity, antipyretic activity (post 3 hr), ulcer healing activity, SGOT and SGPT. Second Row (left to right) ALP, total bilirubin, direct bilirubin and Glutathione (GSH). N = 6 wistar rats per group, tabular value represents mean ± SEM, *: significantly different with p < 0.05, **: significantly different with p < 0.01, *: significantly different with p < 0.001, ns: insignificant different with p > 0.05.**

CONCLUSION

Prodrug showed excellent hepatoprotective and gastroprotective effects, although it's therapeutic efficacy was compromised. This research opens new doors in designing safer analogs of paracetamol. Continuous efforts will someday lead to emergence of a non toxic and therapeutically active form of paracetamol.

Authors' contributions

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REFERENCES

1. Chamberlain J. Paracetamol (Acetaminophen). A Critical Bibliographic Review Laurie F. Prescott Published 1996 Taylor & Francis, London x + 708 pages ISBN 0 7438 0136 9 £90.00. Journal of Pharmacy and Pharmacology, 1996; 48(8): 882.
2. Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, et al. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatology, 2005; 42(6): 1364-72. Epub 2005/12/01.
3. Ryder SD, Beckingham IJ. ABC of diseases of liver, pancreas, and biliary system. Other causes of parenchymal liver disease. BMJ, 2001; 322(7281): 290-2. Epub 2001/02/07.
4. Daly FF, Fountain JS, Murray L, Graudins A, Buckley NA. Guidelines for the management of paracetamol poisoning in Australia and New Zealand--explanation and elaboration. A consensus statement from clinical toxicologists consulting to the Australasian poisons information centres. The Medical journal of Australia, 2008; 188(5): 296-301. Epub 2008/03/04.
5. Hawkins LC, Edwards JN, Dargan PI. Impact of restricting paracetamol pack sizes on paracetamol poisoning in the United Kingdom: a review of the literature. Drug safety: an international journal of medical toxicology and drug experience, 2007; 30(6): 465-79. Epub 2007/06/01.
6. Khashab M, Tector AJ, Kwo PY. Epidemiology of acute liver failure. Current gastroenterology reports, 2007; 9(1): 66-73. Epub 2007/03/06.
7. Henretig FM, Selbst SM, Forrest C, Kearney TK, Orel H, Werner S, et al. Repeated acetaminophen overdosing. Causing hepatotoxicity in children. Clinical reports and literature review. Clinical pediatrics, 1989; 28(11): 525-8. Epub 1989/11/01.

8. Alander SW, Dowd MD, and Bratton SL, Kearns GL. Pediatric acetaminophen overdose: risk factors associated with hepatocellular injury. Archives of pediatrics & adolescent medicine, 2000; 154(4): 346-50. Epub 2000/04/18.
9. USFDA. Acetaminophen Information. USFDA; 2014 [updated 05/28/2014; cited 2014]; Available
From: http://www.fda.gov/Drugs/DrugSafety/InformationbyDrugClass/ucm165107.htm.
10. Watson WA, Litovitz TL, Klein-Schwartz W, Rodgers GC, Jr., Youniss J, Reid N, et al. 2003 annual report of the American Association of Poison Control Centers Toxic Exposure Surveillance System. The American journal of emergency medicine, 2004; 22(5): 335-404. Epub 2004/10/19.
11. Ostapowicz G, Fontana RJ, Schiodt FV, Larson A, Davern TJ, Han SH, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Annals of internal medicine, 2002; 137(12): 947-54. Epub 2002/12/18.
12. Rowden AK, Norvell J, Eldridge DL, Kirk MA. Updates on acetaminophen toxicity. The Medical clinics of North America, 2005; 89(6): 1145-59. Epub 2005/10/18.
13. Vermeulen NP, Bessems JG, Van de Straat R. Molecular aspects of paracetamol-induced hepatotoxicity and its mechanism-based prevention. Drug metabolism reviews, 1992; 24(3): 367-407. Epub 1992/01/01.
14. Dahlin DC, Nelson SD. Synthesis, decomposition kinetics, and preliminary toxicological studies of pure N-acetyl-p-benzoquinone imine, a proposed toxic metabolite of acetaminophen. Journal of medicinal chemistry, 1982; 25(8): 885-6. Epub 1982/08/01.
15. Friis GJB, H, Povl Krogsgaard-Larsen, Hans Bundgaard. A Textbook of Drug Design and Development. Harwood Academic. 1996.
16. Wasdo SC, Sloan KB. Topical delivery of a model phenolic drug: alkyloxycarbonyl prodrugs of acetaminophen. Pharmaceutical research, 2004; 21(6): 940-6. Epub 2004/06/24.
17. Majumdar S, Sloan KB. Synthesis, hydrolyses and dermal delivery of N-alkyl-N-alkyloxycarbonylaminomethyl (NANAOCAM) derivatives of phenol, imide and thiol containing drugs. Bioorganic & medicinal chemistry letters, 2006; 16(13): 3590-4. Epub 2006/04/18.
18. Thomas JD, Sloan KB. In vitro evaluation of alkylcarbonyloxymethyl (ACOM) ethers as novel prodrugs of phenols for topical delivery: ACOM prodrugs of acetaminophen. International journal of pharmaceutics, 2008; 346(1-2): 80-8. Epub 2007/07/17.

19. Majumdar S, Sloan KB. Synthesis and topical delivery of N-alkyl-N-alkyloxycarbonylaminomethyl prodrugs of a model phenolic drug: acetaminophen. International journal of pharmaceutics, 2007; 337(1-2): 48-55. Epub 2007/01/30.
20. H. Gerhard Vogel HGV, Jochen Maas, Alexander Gebauer. Drug Discovery and Evaluation: Methods in Clinical Pharmacology. Springer, 2011; 12. Springer Berlin Heidelberg.
21. Adams SS, Hebborn P, Nicholson JS. Some aspects of the pharmacology of ibufenac, a non-steroidal anti-inflammatory agent. The Journal of pharmacy and pharmacology, 1968; 20(4): 305-12. Epub 1968/04/01.
22. Okabe S, Takeuchi K, Honda K, Takagi K. Effects of various amino acids on gastric lesions induced by acetylsalicylic acid (ASA) and gastric secretion in pylorus-ligated rats. Arzneimittel-Forschung, 1976; 26(4): 534-7. Epub 1976/04/01.