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Therapeutic Effect of Yo-Yo Bitters and Evans Healthy Bitters against Acetaminophen-Induced Hepatotoxicity in Mice

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Abstract:- Yo-Yo bitters and Evans healthy bitters are brands of polyherbal preparations/mixtures widely consumed in Nigeria. However, there is scarcity of scientific data on the acclaimed medicinal or therapeutic benefits of these bitters. The effects of these bitters on acetaminophen - induced hepatotoxicity in mice was examined using liver function data obtained from the liver of mice. Thirty Wistar mice divided into 6 groups of 5 mice each were used. Group 1 was injected with 0.15 mL of acetaminophen (AAP) and group 4 (control) was injected with 0.03 mL of normal saline. Group 2 and group 3 were injected with 0.3 mL of Yo-Yo bitters and 0.3 mL of Evans Healthy bitters, respectively. Group 5 and 6 were injected with 0.15 mL of AAP and then treated with 0.3 mL of Evans Healthy bitters and 0.3 mL of Yo-Yo bitters, respectively. The mice were sacrificed four hours after the last treatment and the liver collected for Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) bioassays. Treatment with acetaminophen alone caused significant ($p<0.05$) elevation in the levels of the liver enzymes; ALP by 33.5 %, AST by 21 % and ALT by 22 % when compared to the control while groups treated with Evans Healthy Bitters and Yo-Yo Bitters after injection of AAP resulted in significant ($p<0.05$) depletion in the levels of AST by 13 % and 18 %, ALT by 12 % and 18 %, and ALP by 18.5 % and 20 %, respectively, compared to the AAP injected group. These observations demonstrate that an overdose of acetaminophen is hepatotoxic to healthy liver and that treatment with both Yo-Yo bitters and Evans healthy bitters may attenuate acetaminophen – induced liver damage. Evans healthy bitters appears to be more potent than Yo-Yo bitters. The results also suggest that oral exposure to the selected bitters may not cause liver injury and may even possibly help maintain the integrity and health of the liver. However, further studies are required to elucidate the actual bioactive compounds present in the two bitters and validate the other acclaimed therapeutic potentials.

Keywords:- *Evans healthy bitters, Yo-Yo bitters, Acetaminophen, Hepatoprotective, Liver.*

I. INTRODUCTION

Medications-related hepatotoxicity is a possible challenge of nearly all prescribed drugs, due to the pivotal role the liver plays in the metabolism of all chemical substances. Many of the hepatotoxic chemicals (including acetaminophen, also known as paracetamol) cause injury to the liver cells. Acetaminophen (AAP) is an extensively used pain killer and relatively safe if taken at the therapeutically prescribed dose [1, 2, 3] and it is the preferred analgesics in children. However, liver injury resulting from paracetamol is the major cause of drug-induced liver dysfunction in the developed countries and an overdose can lead to severe liver damage that can transform into liver failure [3, 4].

Traditional herbal treatment of liver diseases has been from time immemorial, and medicinal plants and other derived chemicals of natural product origin continue to gain usage worldwide for one purpose or the other [5]. Experimental assessment of plants more often than none reveals that the bioactive secondary metabolites in these plants are responsible for their efficacy. Many medicinal plants have been evaluated and discovered to possess active principles with therapeutic capabilities against several ailments and disorders. Hepatoprotective plants have many secondary metabolites such as flavonoids, essential oil, phenols, glycosides, monoterpenes, xanthenes, carotenoids, organic acids, lipids, coumarins, alkaloids and lignans, [6]. Recent findings have revealed that drugs of natural product origin are relatively non-poisonous, safe and have little or no side effects. Liver damage is detectable by the level of certain biochemical markers such as ALT, ALP and bilirubin. Cellular elevations of bilirubin and ALT or ALP indicates a liver injury. The factors influencing drug toxicity include age, ethnicity and race, drug dosage and duration, underlying liver disease, gender, alcohol ingestion, genetic factors and nutritional status [5, 7, 8]. Many scientists have reported the hepatoprotective effect of several medicinal plants as well as polyherbal formulation against acetaminophen-induced hepatotoxicity [3, 9, 10, 11, 12, 13, 14].

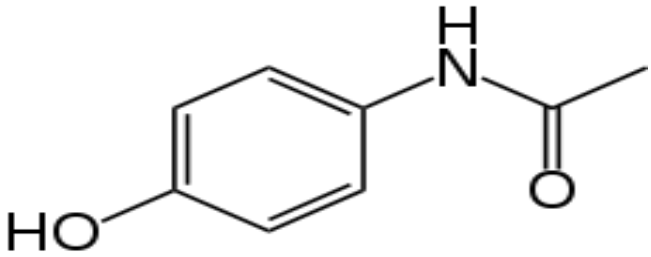

Fig 1:- Chemical structure of Paracetamol

➢ *Mechanism of action of acetaminophen*

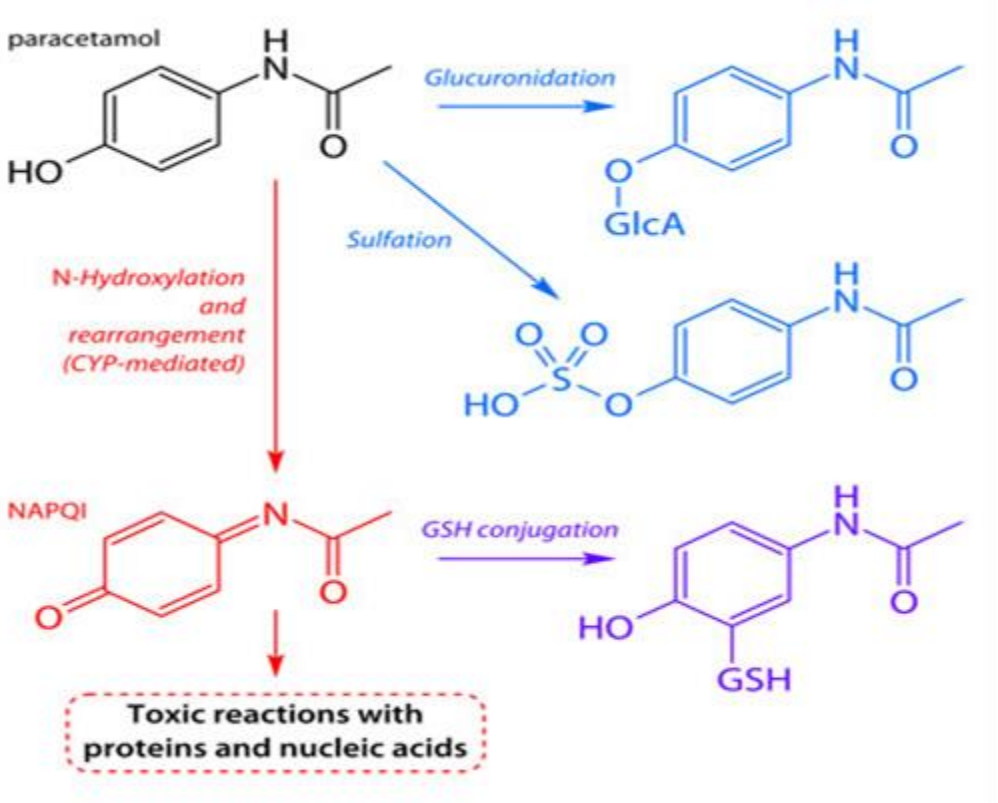

Fig 2:- The main pathways of paracetamol metabolism

Yoyo Bitters is made from these medicinal plants: *Acinos arvensis*, *Aloe*, *Chenopodium murale*, *Cinnamomum aromaticum*, *Citrus aurantifolia*, *Citrus aurantifolia* and *Cinnamomum aromaticum* Evans Healthy Bitters are manufactured mainly from *Alhagi camelorum Cassia Angustifolia Commiphora myrrha , Andrographis peniculata, Picrorhiza kurroa*, *Tinospora cordlifolia*, *Aloe barbadens* and *Crocus sativus*.

II. MATERIALS AND METHODS

A. Animals

The mice were obtained from the Animal House University of Lagos, Idi-Araba, Lagos State, Nigeria. They were kept in the animal house of the Department of Chemical and Food Sciences, Bells University of Technology, Ota, Ogun State, Nigeria. The Animal House was maintained on a 12 – hour light/dark cycle. The animals were fed with mice feeds containing at least 20% protein, 3.5% fat, 9.0% fibre, 1.2% calcium, 0.7% phosphorus, vitamin, mineral per mix, carbohydrates etc. from Graceline Feeds, Ota, Ogun State, Nigeria. Drinking water were made available *ad libitum* throughout the experimental period. A two-week adaptation period was allowed to acclimatize the mice to the food and housing.

B. Yoyo Bitters and Evans Healthy Bitters

Yoyo Bitters and Evans Healthy Bitters were purchased from a licensed pharmacy store in Ota, Ogun State, Nigeria.

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C. Animal Treatment

- Group 1: Subcutaneous injection of 0.15 mL of Paracetamol (Negative Control).
- Group 2: Subcutaneous injection of 0.03 mL of Yo-Yo bitters only.
- Group 3: Subcutaneous injection of 0.03 mL of Evans healthy bitters only.
- Group 4: Subcutaneous injection of 0.3 mL of Normal Saline (Positive Control).
- Group 5: Subcutaneous injection of 0.03 mL of Evans healthy bitters, 30 minutes after being injected with 0.15 mL of paracetamol.
- Group 6: Subcutaneous injection of 0.03 mL of Yo-Yo bitters thirty minutes after being injected with 0.15ml of paracetamol.

D. Preparation of Post Mitochondria Liver Fraction

The liver samples were washed in 5 mL ice-cold KCl and blotted with filter paper and weighed. They were then minced and homogenized in 4 volumes of phosphate buffer (PH 7.4) using Elvehjem apparatus with fitted Teflon pestle. The resulting homogenate was centrifuged at 17,000 g for 20 minutes at -4 ^{0}C in a high-speed cold centrifuge to obtain the post mitochondria liver fraction. The supernatant was decanted and stored in the freezer at -4 ^{0}C until the liver enzymes activities were determined.

AST, ALT and ALP activity was determined according to the method described by [15]. Randox diagnostics kits (Randox Laboratories Ltd, UK) were used for the assays.

E. Statistical Analysis

Statistical analysis was done using one-way ANOVA and data are expressed as mean ± standard error of mean

III. RESULTS

There was a significant increase ($p<0.05$) in the AST activity of the acetaminophen-treated group (group 1) when compared to the control group (group 4). Meanwhile, the AST activity was significantly ($p<0.05$) reduced in both group 5 and 6 that were treated with Evans bitters and Yo-Yo bitters after AAP administration. Table 1 illustrates the result of AST.

Groups	AST (UI/L)
1	22.00 + 3.83
2	16.00 + 2.45
3	14.50 + 3.87
4	13.75 + 2.87
5	15.25 + 2.87
6	16.00 + 2.45

Table 1:- Result of AST activity in acetaminophen-induced liver injury in mice

AAP administration significantly ($p<0.05$) increased ALT activity. Treatment with Yo-Yo bitters and Evans bitters alone showed a significant ($p<0.05$) reduction in ALT activity when compared to the control. Similarly, administration of Evans healthy bitters and Yo-Yo bitters after treatment with AAP caused a significant ($p<0.05$) depletion in ALT activity compare to the control group. The result of ALT activity obtained for all the groups are depicted in table 2.

Groups	ALT (UI/L)
1	24.00 + 3.83
2	18.00 + 2.00
3	19.00 + 2.31
4	14.50 + 2.89
5	14.50 + 2.89
6	20.00 +2.00

Table 2:- Result of ALT activity in acetaminophen-induced liver injury in mice

The ALP activity of the group given only paracetamol had about 3.87 folds increase when compared to the control group. No significant ($p<0.05$) change was observed in the groups treated with Yo-Yo bitters and Evans healthy bitters alone compared to the control group. The administration of Yo-Yo bitters and Evans bitters following AAP treatment resulted in significant ($p<0.05$) reduction in the ALP activity by 13 % and 18 %, respectively compared to the group treated with AAP alone (group 1). Table 3 shows the result of ALP activity for all the groups.

Groups	ALP (UI/L)
1	296.70 +7.17
2	77.05 + 9.78
3	80.39 + 6.13
4	76.59 + 8.90
5	152.14 + 21.23
6	158.99 +30.56

Table 3:- Result of the ALP activity in acetaminophen-induced liver injury in mice

IV. DISCUSSION

Paracetamol-induced hepatic injury is a model usually employed for hepatoprotective drug evaluation [16] and the degree of hepatic damage is obtained by considering the concentrations of cytoplasmic liver enzymes (ALT, AST and ALP) in the system [17]. Yo-Yo bitters and Evans healthy bitters when administered intraperitoneally exhibited protection against paracetamol-induced hepatotoxicity in mice suggesting hepatoprotective actions. Yo-Yo bitters and Evans healthy bitters administration appear to reverse and improve hepatotoxicity caused by paracetamol as observed by the slowing of the elevation of the liver enzymes (ALP, AST and ALT) levels following exposure to drug overdose [18] and this might be the contributing factor towards the observed hepatoprotection. However, Adeyemi *et al*., [19] reported that Yoyo Bitters appears to promote free radical generation and initiating lipid peroxidation that caused increased superoxide dismutase and eventual dose-related reduction of reduced glutathione levels. Bharali *et al*., [20] observed that the aqueous methanolic bark extract of *Oroxylum indicum* (L.)Vent. showed hepatoprotective property in paracetamol treated rats. There had been other health and therapeutic benefits reported in literature by Biochemists and other scientists. Kayode *et al*., [21] reported that some alcoholic bitters produced and regularly consumed in Nigeria, were found to boost the usual physiological performance of the testes, endogenous free radical scavenging enzymes and secretions of sex hormones. Certain alcoholic bitters studied by Kayode and co-workers were described to be useful in reducing hypercholesterolemia and enhancing antioxidant status in male rats orally exposed to those alcoholic bitters [22].

V. CONCLUSION

The result obtained from the activities of ALT, AST and ALP analyses carried out on the liver of mice suggest that Evans healthy bitters and Yo-Yo bitters offers some degree of hepatoprotection against acetaminophen-induced hepatotoxicity in mice. Hence, their consumption may go a long way in preventing the severity of drug-induced liver damage in humans. Collectively, our result indicates that both Yo-Yo bitters and Evans healthy bitters exhibited protection against paracetamol-induced liver injuries but it seems Evans healthy bitters has a more potent hepatoprotective activity and might be more effective than Yo-Yo bitters. However, further studies are required to identify the bioactive compounds present in these two bitters and their other acclaimed therapeutic potentials.

REFERENCES

[1]. S.P. Danque, T.G. Mant, J.H. Tempowski, G.N. Volans, and J.C. Talbot, "Adverse Reactions of Drugs and Effects of Overdose". Journal of Clinical Research, 2000, 289 (6439): 217–9.
[2]. E. Bjomsson, and R. Olsson, “Suspected Drug-induced Liver Fatalities Reported to the WHO Database”. Digest. Liver Dis., 2006, 38:33-38.
[3]. K.R. Mahaboob, P.S. Evan, L. Kumar, and N. Pichandi, “Therapeutic Effect of India Ayurvedic Herbal Formulation Triphala on Acetaminophen-Induced Hepatotoxicity in Mice”. Journal of Pharmacology and Toxicology, 2007, 2 (8) 725-7.
[4]. W.M. Lee, R.H. Squires Jr, S.L. Nyberg, E. Doo, and J.H. Hoofnagle, “Acute Liver Failure: Summary of a Workshop”. Hepatology, 2008, 47: 1401- 1415.
[5]. J. Luming, and S. Crabb, "A Development of a Database Analysis that Provides Putative Metabolic Enzyme Reactions for Ligand-based Drug Design". Computational Biology and Chemistry, 2002, 33 (2): 149–159.
[6]. B. Sharma, J.G. O'Grady, G.J. Alexander, K.M. Hayllar, and R. Williams, "Early Indicators of Prognosis in Fulminant Hepatic Failure". Gastroenterology, 2009, 97 (2): 439–45.
[7]. S.K. Kurose, “Plants Having Hepatoprotective Activity”. Phytochemistry and Pharmacology; 2001, 2, 253-70.
[8]. M. Nilesh, “An Up-and-Down Procedure for Acute Toxicity Testing”. Fundamental and Applied Toxicology 1985, 5: 151–157.
[9]. C. Girish, B.C. Koner, S. Jayanthi, K.R. Rao, B. Rajesh and S.C. Pradhan, “Hepatoprotective Activity of Six Polyherbal Formulations in Paracetamol-Induced Liver Toxicity in Mice”. Indian J Med Res 129, May 2009, pp 569-578.
[10]. K. Prabu, N. Kanchana, and A. Mohamed Sadiq, “Hepatoprotective Effect of *Eclipta alba* on Paracetamol Induced Liver Toxicity in Rats”. J. Microbiol. Biotech. Res., 2011, 1 (3): 75-79.
[11]. K. Zahra, M.A. Malik, M.S. Mughal, M. Arshad and M.I. Sohail, “Hepatoprotective Role of Extracts of *Momordica charantia* in Acetaminophen-Induced Toxicity in Rabbits”. The Journal of Animal & Plant Sciences, 22(2): 2012, Page: 273-277.
[12]. D. Balne, P. Pallerla, S.R. Vanapatla, R.K. Bobbala, “Hepatoprotective Effect of Whole Plant Extract Fractions of *Marsilea minuta* Linn. Asian J Pharm Clin Res, Vol 6, Issue 3, 2013, 100-107
[13]. R. Gupta, A. Gupta, R.L. Singh, “Hepatoprotective Activities of Triphala and Its Constituents”. International Journal of Pharma Research & Review, Jan 2015; 4(1):34-55
[14]. G.S. Sreejith, J.A. Ittoop, M.G. Anitha, B. Ravishankar, “Hepatoprotective Activity of Dushivishari Agada in

Paracetamol Induced Hepatotoxicity of Wistar Rats". Int. J. Res. Ayurveda Pharm. 6 (5). Sept. –Oct. 2015.
[15]. S. Reitman, and S. Frankel, "A Colorimetric Method for Determination of Serum Glutamate Oxaloacetate and Glutamic Pyruvate Transaminase". America Journal of Clinical Pathology, 1957, 28: 56-58.
[16]. P.Z. Plaa, and H.L. Hewit, "Evaluation of Hepatoprotective Activity of *Cleome viscosa* Linn Extract". Indian journal of pharmacology; 2002, 41, 36-40.
[17]. D. Sallie, E.A. Khairallah, "Selective Protein Arylation and Acetaminophen Induced Hepatotoxicity. Drug Metabolism, 2000, 29: 59- 77.
[18]. J. Aeschbach, A.V. Appelboam, P.I. Dargan, J. Knighton, "Fatal anaphylactoid reaction to N-acetylcysteine: caution in patients with asthma". Emergency medicine journal: 1994, 19 (6): 594–5.
[19]. O. S. Adeyemi, M. Fambegbe, O.R. Daniyan, and I. Nwajei, "Yoyo Bitters, a Polyherbal Formulation Influenced Some Biochemical Parameters in Wistar rats". J Basic Clin Physiol Pharmacol, 2012, 23(4):135–138.
[20]. M. K. Bharali, H. Konya, L.B Chetry, "Protective Effect of *Oroxylum indicum* on Acetaminophen-Induced Liver Injury in Rat. International Current Pharmaceutical Journal, January 2014, 3(2): 223-227.
[21]. O.T. Kayode, A.A.A. Kayode and C.O. Nwonuma, "Alcoholic Bitters Modulates Sex Hormones and Some Biochemical Parameters of Testicular Function in Male Wistar Rats". F1000Research, 2018b: http://www.doi.org/10.5256/f1000research.16648.d225117 (https://doi.org/).
[22]. O.T. Kayode, A.A.A. Kayode, M.E. Iyobhebhe, R. Ojji, and R.F. Maimako, "Metabolic Alterations in Male Rats after Oral Exposure to Alcoholic Bitters". NISEB Journal Vol. 18, No. 3. September, 2018.