**Evaluation of High Mobility Group Box 1 (Hmg-B1) and some Liver Enzymes on Consumers of Polyherbal Medicine (Agbo), Cigarette and Alcohol in Nnewi Metropolis.**

**ABSTRACT**

Background: The consumption of polyherbal medicines, cigarette and alcohol is on the increase especially in Nnewi metropolis with a significant consequence on the health status of the consumers.

Objectives: We evaluated the serum levels of liver enzymes (ALT, AST, ALP) and HMG-B1 in consumers of polyherbal medicine, alcohol and cigarettes and control subjects, correlated the duration, age and frequency of the consumption of polyherbal medicine, alcohol and cigarette with liver enzymes (ALT, AST, ALP) and HMG-B1 in various groups.

Methods: This is a cross sectional study involving 102 participants divided into the following groups: 34 consumers of alcohol, agbo and cigarette; 34 consumers of alcohol and cigarette; and 34 non-consumers of alcohol, agbo and cigarette of the same age range 30-60yrs. The liver enzymes (ALT, ALP AST) were analyzed spectrophotometrically while High mobility group box 1 was analyzed using the ELISA method and SPSS version 26 was used for the statistical analysis.

Results: The result showed that the serum levels of the liver enzymes (ALT, ALP and AST) did not differ significantly among the various groups (P > 0.05), while the level of HMG-B1 differed significantly among the three groups (P < 0.05). Multiple comparison showed that the mean level of HMG-B1 is significantly lower in control group when compared to AAC and AC groups (P < 0.05). Moreso, the mean of HMG-B1 was significantly higher in AAC when compared with AC (P < 0.05). There were no significant correlations between duration of intake, frequency of intake and age of participants with the various biomarkers in various groups (P > 0.05). In conclusion, there was a significant raise in HMG-B1 levels in test groups which is suggestive of early liver damage.

Keyword: high mobility group box 1, Liver enzymes, Polyherbal medicines, Alcohol, Cigarette.

1. **INTRODUCTION**

Polyherbal medicine is a combination of two or more plant extracts used to prevent and treat diseases. The use of polyherbal medicine is widespread in developing countries, including Nigeria, and is often used as a substitute for modern medicine due to its affordability and accessibility (Oyebode & Humphreys, 2011). Agbo is a popular polyherbal medicine used in Nigeria, which consists of a mixture of plant extracts believed to have therapeutic properties. However, the safety and efficacy of Agbo remains largely unknown, and there is limited scientific evidence to support its use (Nwagwu, 2020). Polyherbal medicines has been reported to have hepatoprotective properties and is used to treat liver diseases (Adebayo *et al.,* 2021). However, the use of polyherbal medicine can lead to liver damage and dysfunction (Ekor, 2013), for example, a study conducted in Ghana found that the use of polyherbal medicine was associated with elevated levels of liver enzymes in patients with liver disease (Sekyere, 2024).

Cigarette smoking causes relevant ill effects on many of the organs of our body especially the liver which is seldom understood and given very little importance by the general public due to the lack of knowledge and mere ignorance (Chanda M, 2021) These changes that smoking brings to the body may sound less harmful at first, but they may even be permanently irreversible when they progress to an advanced level. Smoking reduces the generalized immune status of an individual, and smokers are more prone to ailments than those who don't smoke (Varghese and Munto de Gharde, 2023).

Alcohol, specifically known as ethanol, is a psychoactive substance found in alcoholic beverages that can lead to dependence and has a significant impact on both individual and societal health, with global consequences (Leshargie *et al*., 2019). This psychoactive substance is found in alcoholic beverages that can lead to intoxication and has various health effects, both positive and negative thereby affecting the brain and the nervous system. It has a rich history, dating back to the Neolithic period around 7000 BC, with evidence of early winemaking in Georgia and beer production in the Middle East (WHO 2024). It plays a significant role in many cultures, often being part of social gatherings, religious ceremonies, and medicinal practices. However, its consumption also poses health risks, including addiction, liver disease, and increased risk of certain cancers, especially when consumed excessively (Griswold *et al*., 2018).

The liver is an essential organ responsible for detoxifying harmful substances in the body, regulating metabolism, and producing bile to aid digestion. Liver enzymes are proteins that speed up chemical reactions in the liver (MedlinePlus, 2019). They include Alanine transaminase (ALT), which is an enzyme found mostly in liver cells and also found in heart and muscle cells, helps to convert alanine into pyruvate, for cellular energy production  (Sharma *et al*, 2013), Aspartate aminotransferase (AST) which catalyzes the reversible transfer of an α-amino group between aspartate and glutamate (Chanda M et al, 2024) and, as such, is an important enzyme in amino acid metabolism which is found in the liver, heart, skeletal muscle, kidneys, brain, red blood cells and gall bladder (Ighodaro *et al*., 2020) and Alkaline phosphatase (ALP) which are biomarkers used to assess liver function and are a group of isoenzymes located on the outer layer of the cell membrane that catalyzes the hydrolysis of organic phosphate esters found in the extracellular space (Lowe *et al*., 2023). Elevated levels of liver enzymes indicate liver damage or disease (Murhekar *et al*., 2021).

High mobility group box-1 (HMG-B1) is a ubiquitous protein that was initially thought to be simply an architectural protein due to its DNA binding ability, it is a key protein participating in the pathogenesis of acute liver injury and chronic liver disease (Kim *et al*., 2017). HMG-B1 was originally discovered as a nuclear protein however, when it is passively released or actively secreted after injury or cell stimulation, it meets all the criteria of a damage associated molecular pattern (DAMP) and also work as a necrosis signal for the immune system through cell surface receptor (Kim *et al*., 2017). It is also known as a nuclear protein released into the bloodstream during liver cell death which gains attention as a potential marker for early liver injury including that from hepatotoxic herbs (Yokoi *et al*., 2018). Despite the widespread use of polyherbal medicine in Nigeria, there is limited scientific evidence on its effects on liver function (Yokoi *et al*., 2018). It is essential to investigate the effects of polyherbal medicine on liver enzymes and HMG-B1 levels to guide healthcare providers and consumers on the potential risks associated with its use.

**MATERIALS AND METHOD**

**Study design and population**

This is a cross-sectional study designed to evaluate the serum activity of Alanine transaminase, Aspartate transaminase, Alkaline phosphatase, and High Mobility Group Box1 in consumers of polyherbal medicine (Agbo) in Nnewi metropolis. A total of hundred and two (102) participants, 34 consumers of alcohol, agbo; cigarette named as AAC, 34 consumers of alcohol and cigarette named as AC; and 34 non-consumers of alcohol, agbo and cigarette named as control were recruited for this study from different motor parks in Nnewi metropolis. Simple random sampling was used to select 102 consecutive consenting adults into these 3 groups of 34 participants each.

The inclusion criteria include individuals who reside in Nnewi; individuals who consume agbo, alcohol and cigarette, and individuals between the age of 30-60yrs. Individuals with chronic renal, liver and cardiac diseases and those that declined consent were excluded from this work. Also, individuals under the age of 25 and above the age of 60 were excluded from this study. This study design was approved by the Ethics Committee of Faculty of Medical Laboratory Sciences, College of Health Sciences and Technology, Nnamdi Azikiwe University Nnewi Campus Anambra State.

**Sample Size**

G-power software version 3.1.9.4, was used to determine the sample size and power of the study. The predicted sample size of 102 participants has an error probability of 0.05 and a 95% power to detect variations in replies as small as 0.4 (effect size).

**Sample Collection and Biochemical Analysis**

Five millilitres (5ml) of venous blood were aseptically drawn from each subject's ante-cubital vein using a plastic syringe and dispensed into plain tubes. Following centrifugation at 4000rpm for 5 minutes, the serum was separated from the plain tubes using a micro pipette and then stored in separate plain tubes. The samples were kept frozen at a temperature of -20C until the biochemical analysis of the activities of alanine transaminase, aspartate transaminase, alkaline phosphatase, and ELISA analysis of High mobility group box1 was carried out within one month of storage.

Alanine Transaminase (ALT) levels was determined using the enzymatic method as described by Reitman and Frankel (1957) with the use of Randox test kit. Aspartate Transaminase (AST) levels was determined using the enzymatic method as described by Reitman and Frankel (1957) with the use Randox test kit. Alkaline Phosphatase (ALP) levels was determined using the enzymatic method as described by Schlebusch *et al.*, (1974) with the use of Randox test kit. High Mobility Group Box1 (HMG-B1) levels was determined using the ELISA method as described by (Chen *et al*., 2021) with the use of ELISA test kit (MornMed, China).

**Statistical Analysis**

The Statistical Package for the Social Sciences (SPSS) version 26 was used for the analysis of the results. The data obtained was presented as mean ± standard deviation (SD) and was analyzed statistically using one-way analysis of variance (ANOVA), Post-hoc test, and Pearson correlation. The level of significance was set at p˂0.05.

**RESULT**

Table 1 revealed that there was a significant raise in the HMG-B1 level in control when compared to group AAC and group AC (p<0.05). However, no significant difference was observed in the activities of ALT, ALP and AST in the control group when compared to Group AAC and group AC (p>0.05).

Table 2 showed no significant correlation between duration of intake and other biochemical parameters in group AAC and AC (p>0.05).

Table 3 showed no significant correlation between age and other biochemical parameters in the three groups (p>0.05).

Table 4 showed no significant correlation between frequency of consumption and other biochemical parameters in group AAC and AC (p>0.05)

**Table 1. The serum levels of ALT, ALP, AST and HMG-B1 of study participants with control group.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Groups** | **ALT****(U/I)** | **ALP****(U/I)** | **AST****(U/I)** | **HMG-B1****(mg/dl)** |
| Control (A) | 6.88 ± 2.59 | 76.82 ± 33.99 | 12.09 ± 4.57 | 32.19 ± 6.31 |
| AAC (B) | 7.53 ± 5.67 | 78.44 ± 33.06 | 15.71 ± 18.93 | 44.84 ± 12.32 |
| AC (C) | 7.41 ± 4.27 | 61.79 ± 38.90 | 12.12 ± 10.88 | 39.58 ± 8.71 |
| F-value | 0.212 | 2.285 | 0.887 | 15.37 |
| P-value | 0.809 | 0.107 | 0.415 | <0.001 |
| B vs A | 1.000 | 1.000 | 0.749 | <0.001 |
| C vs A | 1.000 | 0.250 | 1.000 | 0.005 |
| C vs B | 1.000 | 1.000 | 0.749 | <0.001 |

**Table.2. correlation between the duration of consumption with other parameters among the participants in various groups.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Group** | **Parameters** | **r** | **p-value** |
| **AAC** | Dur vs ALT | -0.268 | 0.125 |
|  | Dur vs ALP | 0.023 | 0.896 |
|  | Dur vs AST | -0.203 | 0.250 |
|  | Dur vs HMGB1 | 0.261 | 0.136 |
|  |  |  |  |
| **AC** | Dur vs ALT | -0.039 | 0.827 |
|  | Dur vs ALP | 0.026 | 0.884 |
|  | Dur vs AST | -0.207 | 0.241 |
|  | Dur vs HMGB1 | 0.118 | 0.507 |

**Table 3. correlation between the age with other parameters among the participants in various groups.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Group** | **Parameters** | **r** | **p-value** |
| **CONTROL** | Age vs ALT | -0.138 | 0.437 |
|  | Age vs ALP | 0.089 | 0.615 |
|  | Age vs AST | -0.104 | 0.558 |
|  | Agee vs HMGB1 | -0.088 | 0.619 |
|  |  |  |  |
| **AAC** | Age vs ALT | -0.179 | 0.310 |
|  | Age vs ALP | -0.211 | 0.231 |
|  | Age vs AST | -0.162 | 0.360 |
|  | Age vs HMGB1 | 0.173 | 0.327 |
|  |  |  |  |
| **AC** | Age vs ALT | -0.010 | 0.957 |
|  | Age vs ALP | 0.022 | 0.901 |
|  | Age vs AST | -0.099 | 0.577 |
|  | Age vs HMGB1 | 0.016 | 0.927 |

**Table 4. correlation between the frequency of consumption with parameters among the participants in various groups.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Group** | **Parameter** | **r** | **p-value** |
| **AAC** | Freq vs ALT | 0.335 | 0.053 |
|  | Freq vs ALP | -0.082 | 0.647 |
|  | Freq vs AST | 0.369 | 0.322 |
|  | Freq vs HMGB1 | -0.181 | 0.305 |
| **AC** | Freq vs ALT | 0.182 | 0.303 |
|  | Freq vs ALP | 0.080 | 0.651 |
|  | Freq vs AST | 0.102 | 0.568 |
|  | Freq vs HMGB1 | -0.100 | 0.573 |

**DISCUSSION**

The evaluation of traditional polyherbal medicines, such as Agbo, and their potential impact on human health is of paramount importance in developing countries such as Nigeria. This study aimed to assess the effects of polyherbal medicine, cigarette and alcohol consumption on liver enzymes (ALT, AST, ALP) and high mobility group box 1 (HMG-B1) levels, contributing to the understanding of its possible hepatoprotective properties or potential adverse effects on liver function.

The results revealed higher level of HMG-BI amongst consumers of Agbo, alcohol and cigarette which indicates possible early liver damage. It has been shown that HMG-B1 is a potential marker for early liver injury including that from hepatotoxic herbs (Yokoi *et al*., 2018; Chanda M, et al., 2022). However, the consumption of these substances (Agbo, alcohol and cigarette) did not have any impact on the liver enzymes. These findings are consistent with studies conducted by Chumpolphant *et al*., (2022) who observed similar outcomes in their study on the effects of herbal medicine on liver enzymes. They postulated that the complex interactions between the diverse bioactive compounds present in herbal formulations might contribute to the absence of significant alterations in liver enzymes. Ezeugwunne *et al*. (2018) found significant decreases in ALP and AST with no effect on ALT activities following the administration of *Phyllantus amarus* in albino rats which partly agrees with the current reports. On the other hand, Analike *et al*. (2018) reported significant decreases in ALT, AST and ALP activities after the administration of *Sida corymbosa* in albino rats which is invariance with the current findings.

However, the absence of a significant correlation in duration of consumption and frequency of intake with some liver enzyme and HMG-BI in polyherbal consumers, smokers and alcoholic in this work suggest that the impact is not dose or frequency dependent. The findings from this work highlight the possibility that the mechanism of action of these polyherbal remedies is independent of individual characteristics such as age or long-term usage or frequency. A study done by Mussarat *et al*., (2021) underscored that presence of potentially harmful compounds, such as pyrrolizidine alkaloids or heavy metals, in poorly regulated herbal products has been linked to liver damage, including liver fibrosis, cirrhosis, and even liver failure.

**Conclusion**

There was a significant raise in HMG-B1 levels in individuals who consume Agbo, alcohol and cigarette which is suggestive of early liver damage.

**RECOMMENDATION**

Regular examination of early markers of liver damage in individuals who frequently consume these substances is imperative to enable early detection and intervention.

**LIMITATION**

A longitudinal follow-up study should be used to verify this study.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

**REFRENCES**

Adebayo, C., Adepoju, D. and Oseghale, A. (2021). Analysis of Food Security and Coping Strategies among Maize Based Farmers in Niger State, Nigeria. *International Journal Of Agricultural Economics*, [online] 11(1), p.9. Available at: <http://repository.futminna.edu.ng:8080/jspui/bitstream/123456789/18974/1/Adebayo-et-al-FS%20among%20maize%20farmers.pdf>

Analike, R.A., Ezeugwunne, I.P., Ogbuebuna, U.E., Ogbodo, E.C., Oguaka, V.N., Amah, A.K., Ahaneku, J.E., & Ahaneku, G.I. (2018). Effect of *Sida corymbosa* leaf extract on the activity of serum liver enzymes of alloxan-induced diabetic albino wistar rats in College of Health Science and Technology, Nnamdi Azikiwe, Nnewi Campus, Anambra State, Nigeria. *Acta Medica Scientia,* 5(2), 5-7.

Chen, M., Zhu, L., Xue, M., Zhu, R., Jing, L., Wang, H. and Qin, Y. (2021). HMGB1, anti-HMGB1 antibodies, and ratio of HMGB1/anti-HMGB1 antibodies as diagnosis indicator in fever of unknown origin. *Scientific Reports*, [online] 11(1).

Chanda M, Biswas T, Roy MN, Sampa SR, Saha P, Sharna RJ, Islam S, Mahbub A, Rahman MA. Association of Liver Enzymes and Lipid Profile in Adults at Tertiary Level Hospitalin Bangladesh. J Natl Inst Lab Med Ref Bangladesh. 2021 Jun;1(1):17-24.

Chanda M, Biswas T, Tannim N, MahbubaA, Yasmin R, Islam S, Rahman MA.Association of Serum Uric Acid and LipidProfile in Adults at a Tertiary Level Hospitalin Bangladesh. Ad-din Sakina Women’sMedical College Journal. 2024; 5(1): 28-37

Chumpolphant, S., Suwatronnakorn, M., Issaravanich, S., Tencomnao, T. and Prasansuklab, A. (2022). Polyherbal formulation exerts wound healing, anti-inflammatory, angiogenic and antimicrobial properties: Potential role in the treatment of diabetic foot ulcers. *Saudi Journal of Biological Sciences*, 29(7), p.103330.

Chanda M, Biswas T, Amiruzzaman M, Begum H, Tabassum F, Munmun ST, Rahman A, Akram A. Association of Serum Uric Acid and Liver Enzymes in Adults at Tertiary Level Hospital in Bangladesh. Bangladesh Medical Journal. 2022;51(3):18-27.

Ekor, M. (2013). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, [online] 4(177).

Ezeugwunne, I.P., Umeaka, B.K., Ogbodo, E.C., Okpogba, A.N., Analike, R.A., Okwara, J.E., Onyegbule, O.A., Okwara, E.C. (2018). Effect of *Phyllantus amarus* leaf extract on the serum liver enzymes of alloxan-induced diabetic albino wistar rats in College of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria. *European Journal of Biomedical and Pharmaceutical sciences*, 5(2), 61-66

Griswold, M.G., Fullman, N., Hawley, C., Arian, N., Zimsen, S.R.M., Tymeson, H.D., Venkateswaran, V., Tapp, A.D., Forouzanfar, M.H., Salama, J.S., Abate, K.H., Abate, D., Abay, S.M., Abbafati, C., Abdulkader, R.S., Abebe, Z., Aboyans, V., Abrar, M.M., Acharya, P. and Adetokunboh, O.O. (2018). Alcohol use and burden for 195 countries and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet*, [online] 392(10152), pp.1015–1035.

Kim, M.S., Lee, S., Jung, N., Lee, K., Choi, J., Kim, S.-H., Jun, J., Lee, W.-M., Chang, Y. and Kim, D. (2017). The vitamin D analogue paricalcitol attenuates hepatic ischemia/reperfusion injury through down-regulation of Toll-like receptor 4 signaling in rats. *Archives of Medical Science*, 2(11), pp.459–469.

Leshargie, C.T., Alebel, A., Kibret, G.D., Birhanu, M.Y., Mulugeta, H., Malloy, P., Wagnew, F., Ewunetie, A.A., Ketema, D.B., Aderaw, A., Assemie, M.A., Kassa, G.M., Petrucka, P. and Arora, A. (2019). The Impact of Peer Pressure on Cigarette Smoking among High School and University Students in Ethiopia: a Systemic Review and meta-analysis. *PLOS ONE*, [online] 14(10), p.e0222572.

MedlinePlus (2019). *Liver Function Tests: MedlinePlus Lab Test Information*. [online] Medlineplus.gov. Available at: <https://medlineplus.gov/lab-tests/liver-function-tests/>.

Murhekar, M.V., Bhatnagar, T., Vivian, W., Saravanakumar, Muthusamy Santhosh Kumar, Sriram Selvaraju, Rade, K., C. Ganesh Kumar, R. Sabarinathan, Ashok Kumar Turuk, Asthana, S., Rakesh Balachandar, Sampada Dipak Bangar, Avi Kumar Bansal, Chopra, V., Das, D., Alok Kanti Deb, Kangjam Rekha Devi, Vikas Dhikav and Dwivedi, G. (2021). SARS-CoV-2 seroprevalence among the general population and healthcare workers in India, December 2020–January 2021. *International Journal of Infectious Diseases*, 108(11), pp.145–155.

Mussarat, S., Adnan, M., Begum, S., Ur Rehman, S., Hashem, A. and Abd\_Allah, E.F. (2021). Antimicrobial screening of polyherbal formulations traditionally used against gastrointestinal diseases. *Saudi Journal of Biological Sciences*, [online] 28(12), pp.6829–6843.

Nwagwu, E.J. (2020). Nwagwu, E. J., Udegbunam, K. C., & Uwaechia, O. G. (2020). Federal Road Safety Corps and Administration of Traffic Laws in South-East Nigeria An Appraisal. International Journal of Injury Control and Safety Promotion, 27, 510-519.

Oyebode, F. and Humphreys, M. (2011). The future of psychiatry. *British Journal of Psychiatry*, 199(6), pp.439–440.

Reitman, S. and Frankel, S. (1957). A Colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases. *American Journal of Clinical Pathology*, 28(1), pp.56–63.

Schlebusch H, Rick W, Lang H, Knedal M. (1974). Standards in the activities of clinically important enzymes. *Deutsche Medizinische Wochenschrift.* 99: 765-766.

Sekyere, J.O. (2024). *Google Scholar*. [online] Google.com. Available at: https://scholar.google.com/scholar?q=Sekyere+et+al. [Accessed 26 Sep. 2024].

Sharma, U., Pal, D. and Prasad, R. (2013). Alkaline Phosphatase: An Overview. *Indian Journal of Clinical Biochemistry*, 29(3), pp.269–278.

Varghese, J. and MuntodeGharde, P. (2023). A Comprehensive Review on the Impacts of Smoking on the Health of an Individual. *Cureus*, [online] 15(10).

WHO (2024). *Alcohol*. World Health Organization. Available at: <https://www.who.int/news-room/fact-sheets/detail/alcohol>.

Yokoi, T., Yokoyama, Y., Kokuryo, T., Yamaguchi, J. and Nagino, M. (2018). Inhibition of Toll-like receptor 4 ameliorates experimental postischemic injury in the cholestatic liver through inhibition of high-mobility group box protein b1 (HMGB1) signaling. *Surgery*, 163(2), pp.270–276.