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Erythropoietic and hepatocurative profile of Yoyo Bitters® - A pilot study

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ABSTRACT

The present study evaluated the effects of a branded Nigerian polyherbal formulation, Yoyo® bitters on the haematological and biochemical status of hydrogen peroxide (H₂O₂)-intoxicated rats. Thirty (30) healthy male Wistar rats used in this study were divided into 6 groups of 5 rats each. Group 1 served as normal control and received 3 ml/kg body weight (b.w.) of distilled water only while group 2 served as experimental control and was intoxicated with H₂O₂ without treatment. Rats in groups 3-5 were H₂O₂-intoxicated and treated with 1, 2 and 3 ml/kg b.w. of Yoyo® bitters respectively while rats in group 6 were H₂O₂-intoxicated and treated 100 mg/kg. b.w. of silymarin. H₂O₂ was administered intraperitoneal on day 0 while drugs were orally administered from days 0 to 14. The rats were sacrificed on day 15 and blood samples collected were subjected to haematological and biochemical analyses. Intoxication with H₂O₂ significantly ($p < 0.05$) induced haematotoxicity, oxidative stress and hepatotoxicity when compared with control. Treatment of intoxicated rats with the formulation restored the haematological and biochemical status to normal, suggesting that Yoyo® bitters has erythropoietic and hepatocurative effects. However, future studies are required to confirm these effects in different in vivo models.

Keywords: Yoyo® bitters, hepatocurative, haematology, antioxidants, silymarin, acute toxicity

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1. Introduction

Majority of the world population is using mono and polyherbal formulations for effective treatment and management of different diseases because they believe the formulations have fewer side effects compared to synthetic drugs (Ahmed *et al.*, 2016). Herbal bitters are aqueous or alcoholic polyherbal formulations commonly used as hepatoprotective, immunity enhancer and detoxifying agents with antimicrobial properties (Imaga and Valentine, 2013; Anionye and Onyeneke, 2016). Yoyo® bitter is a Nigerian polyherbal formulation that contains *Aloe vera*, *Acinos arvensis*, *Citrus aurantifolia*, *Chenopodium murale* and *Cinamomum aromaticum*. It is claimed as antioxidant, hepatoprotective, immunity enhancer and detoxifying agent like other Bitters. However, scientific studies have shown that the antioxidant (Anyasor *et al.* 2017), hypoglycemic (Kale *et al.*, 2018) and hypolipidemic (Imaga and Ogunnusi, 2014) effects of this formulation is linked with varieties of its phytoconstituents (Anionye and Onyeneke, 2016). The present investigation was designed to evaluate the effect of Yoyo® bitter on the haematological and biochemical status of hydrogen peroxide-intoxicated rats.

2. Materials and methods

2.1 Materials

All chemicals used for this study were of analytical grade and were products of Sigma Aldrich. Drugs used for this study were silymarin (Y.S.P. industries (M) Sdn. BHD) and Yoyo® bitters (Abllat Company Nigerian Limited). Reagents used for this study were products of Randox Laboratories Ltd. (USA) and QCA (Germany).

2.2 Phytochemical analyses

The qualitative and quantitative phytochemical analyses were done using the method of Harborne (1973) and Trease and Evans (1989).

2.3 Acute toxicity profile

Twenty-four-hour acute toxicity profile was determined using 16 Wistar mice of body weight

range 25-30 g. After 7 days of acclimatization, the mice were fasted overnight and the body weights were measured. They were then divided into 4 groups of 4 mice each: mice in groups 1-4 were treated with 1, 3, 5 and 10 ml/kg body weight of Yoyo® bitters respectively. The experimental mice were monitored for 24 hours for neurological, behavioural and morphological signs of toxicity. The body weights were also checked after 24 h post-administration of Yoyo® bitters.

2.4 Study design for hepatocurative study

Thirty (30) male Wistar albino rats weighing 170-233 g were obtained from the Animal Breeding Unit, Faculty of Veterinary Medicines, University of Nigeria, Nsukka and acclimatized for 7 days in the Animal House of the Department of Biochemistry, University of Nigeria, Nsukka. They were maintained under standard husbandry conditions of light (12 h) and darkness (12 h), room temperature of $28 \pm 2^\circ\text{C}$, and with free access to commercial rat chow (Vital Feed Nig. Ltd.) and portable water *ad libitum*. The commercial rodent diet used for animal management in this study contains crude protein (14.5 g%), crude fat (4.8 g%), crude fibre (7.2 g%), crude ash (8.0 g%), phosphorus (0.62 g%), lysine (0.6 g%), methionine (0.29 g%), methionine + cysteine (0.52 g%), calcium (0.8 g%), vitamin E (15 mg/100g), vitamin C (50 mg/100g), manganese (30 mg/100g), zinc (30 mg/100g) and sodium (0.15 g%). The animals received humane care throughout the experimental period in accordance with the Ethical Rules and Recommendations of the University of Nigeria Committee on the Care and Use of Laboratory Animals and the revised National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No.85-23, revised 1985). After acclimatization, the rats were divided into 6 groups of 5 rats each. Rats in group 1 served as normal control and received 3 ml/kg b.w. of distilled water only while rats in group 2 served as experimental control and were intoxicated with hydrogen peroxide (H_2O_2) without

treatment. Rats in groups 3-5 were H₂O₂-intoxicated and treated with 1, 2 and 3 ml/kg b.w. of Yoyo® bitters respectively while rats in group 6 were H₂O₂-intoxicated and treated with 100 mg/kg. b.w. of silymarin. Intoxication was done by single intraperitoneal administration of 3 ml/kg b.w of 5% H₂O₂ to rats and drugs treatment was from days 0 to 14. The rats were sacrificed under mild chloroform anaesthesia on day 15 after an overnight fasting and blood samples were collected through the jugular vein and subjected to haematological and biochemical analyses.

2.5 Determination of haematological and biochemical parameters

The haematological indices were determined using methods described by Ochei and Kolhartkar (2008). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in serum were assayed using the method described by Reitman and Frankel (1957). Total bilirubin concentration was determined by colorimetric method as described by Jendrassik and Grof (1938). Activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were assayed using the methods described by Fridovich (1989), Aebi (1983) and Paglia and Valentine (1967) respectively. The concentrations of glutathione, and vitamins A, C and E were determined according to the method of Habig *et al.* (1974), Goodhart and Shils (1973) and Desai (1984) respectively. The malondialdehyde (MDA) concentration was determined using the method of Wallin *et al.* (1993).

2.6 Statistical analysis

Statistical analysis of raw laboratory data was performed by one-way ANOVA using statistical products and service solutions (SPSS), version 18 and the results presented as mean \pm standard deviation (SD) in Tables. Differences between means at 95% level of confidence ($p < 0.05$) were considered statistically significant.

3.0 RESULTS AND DISCUSSION

3.1 Phytochemical analyses

Result presented in Table 1 shows that alkaloids (7.50%), steroids (2.50%), glycosides (2.38%) and terpenoids (1.00%) were detected in high amount, saponins (0.30%) and anthocyanins (0.21%) were detected in moderate amount while phenols (0.15%), carotenoids (0.11%), flavonoids (0.10%), tannins (0.03%) and anthraquinones (0.18%) were detected in low amount in the branded herbal drug (BHD). Imaga and Ogunnusi (2014) reported the presence of tannins, flavonoids, phenols and cardiac glycosides in the polyherbal formulation. The presence of these phytochemicals may be responsible for the claimed bioactivities and therapeutic effects of this herbal drug; which are usually attributed to the antioxidant properties of the phytochemicals (Padmanabhan and Jangle, 2012). Some of these phytochemicals such as alkaloids and flavonoids act as antioxidants by removing free radicals, chelating metal catalysts and activating antioxidant enzymes, and hence, prevent damages to cellular components which, if not prevented or repaired, may give rise to diseases (Doughari *et al.*, 2009). Alkaloids have many pharmacological activities including antihypertensive, antiarrhythmic, antimalarial activity, antimicrobial, anthelmintic, anticancer, anti-ulcer and antidiarrhoeal properties (Saxena *et al.*, 2013). Glycosides promote appetite and aid digestion. Tannins have analgesic and anti-inflammatory activities and saponins act as antifungal and antiviral agents (Nandagoapalan *et al.*, 2016). Some of the plant components of this polyherbal drug are sources of ascorbic acid, vitamin E, carotenoids, flavanols and phenolics, which possess the ability to scavenge free radicals (Fajobi *et al.*, 2017). These bioactive constituents may be linked to the claim by the manufacturer that the herbal mixture is used in the treatment of skin disease, dental condition and ulceration.

3.2 Acute toxicity profile

After 24 h of observation, there was no visible sign of toxicity at all the doses evaluated for acute toxicity test. Similarly, there was no significant body weight change in the

experimental mix after 24 h post-administration monitoring. This finding suggests that the branded herbal drug is relatively safe at the doses studied within 24 h of administration.

Table 1: Phytochemical constituents of Yoyo® bitters

| Phytochemicals | Bioavailability | Amount (%) |
|----------------|-----------------|------------|
| Alkaloids | +++ | 7.50 |
| Anthocyanins | ++ | 0.21 |
| Anthraquinones | + | 0.18 |
| Carotenoids | + | 0.11 |
| Flavonoids | + | 0.10 |
| Glycosides | +++ | 2.38 |
| Phenols | + | 0.15 |
| Saponins | ++ | 0.30 |
| Steroids | +++ | 2.50 |
| Tannins | + | 0.03 |
| Terpenoids | +++ | 1.00 |

Key: +++ = high content; ++ = moderate content; + = low content; ND = not detected

3.3 Effects of Yoyo® bitters on the haematological indices of experimental rats

There was a significant ($p < 0.05$) decrease in the packed cell volume (PCV), haemoglobin (Hb) concentration and red blood cell (RBC) of the H₂O₂-intoxicated and untreated rats when compared to rats in normal control (Table 2). The PCV shows the capacity of blood to transport oxygen and absorbed nutrients. Thus, a decreased PCV indicates a poor transportation capacity of the red blood cells (Isaac *et al.*, 2013). A low RBC count often accompanies anaemia, excess body fluid and blood loss (Cheesbrough, 2006). It was evidenced that the anaemia was caused not only by increased destruction of erythrocytes (Kori-Siakpere *et al.*, 2009), but also by decrease in the synthesis and release of erythrocytes into the blood circulation (Vinodhini and

Narayanan, 2008). This could be as a result of the suppression of the activity of haematopoietic tissues and accelerated erythroclasia due to altered membrane permeability by H₂O₂ used as toxicant in this study. The dose-dependent significant ($p < 0.05$) increase in the PCV, HB concentration and RBC count of Yoyo® bitters and silymarin-treated rats when compared to the untreated indicates that the drugs induce the formation of erythropoietin, a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells (Ohlsson and Aher, 2009). A similar trend was observed when *Allium sativum* extract; one of the components of this herbal drug was administered to rats (Iranloye, 2002). Anthocyanin which was detected in a moderate amount in the polyherbal drug has been reported to stimulate erythropoiesis (Oluyemi *et al.*, 2007).

Leukocytes are responsible for clearing off injured or dead cells and tissues in body and fighting infection and their counts are increased during infection and decreases during inflammation. There was a significant ($p < 0.05$) increase in the white blood cell count and a significant ($p < 0.05$) decrease platelets counts in the H_2O_2 -intoxicated and untreated rats when compared to rats in control. A decrease in the platelet count of H_2O_2 -intoxicated and untreated rats, which was also recorded in the intoxicated and Yoyo® bitters-treated rats could be caused by chemo-toxic effect of the herbal product (Debled *et al.*, 2007). It has been described that high level of H_2O_2 is cytotoxic to a wide range of plant, animal and microbial cells' culture; although the mode of cell death induced (apoptosis or necrosis) depends on the cell type, its physiological state, length of exposure to H_2O_2 and concentration of H_2O_2 used (Halliwell and Gutteridge, 1999). Therefore, the increase in the white blood cell count in rats administered the Yoyo® bitters supported the induction of systemic or localized inflammatory response by the ingestion of the herbal bitters. It has been reported that saponins, a phytoconstituent of the Yoyo® bitters have both stimulatory effects on the components of specific immunity and non-specific immune reactions such as inflammation (Bedir *et al.*, 2000). It was also observed that

treatment of intoxicated rats with the different doses of Yoyo® bitters in groups 3, 4 and 5 caused, in a dose-dependent manner, a significant ($p < 0.05$) decrease in the platelet count of the rats when compared with groups 1 and 2. This may be linked with a decreased level of tissue healing processes or systemic inflammatory response. This is so because innate immune cells (macrophages and neutrophils) in response to toxicants, usually stimulate the production of blood platelets via increased production of adenosine diphosphate (ADP) and the inhibition of nitric oxide (NO) production when there is tissue injury or damage (Ford and Giles, 2000). However, the recorded decrease in platelet count in this study could be as a result of H_2O_2 toxicity that may have overwhelmed the innate immune cells, consequently, preventing it from stimulating blood platelets production as well as mobilization of existing platelets for healing of tissue injuries generated by H_2O_2 . In the same vein, treatment with Yoyo bitters further decreased the platelets count. This agrees with result of a recent study by Kosoko *et al.* (2018) which demonstrated that administration of higher doses of the polyherbal drug for long duration elicits clastogenic, hematotoxic and genotoxic potentials in rats.

Table 2: Effects of Yoyo® bitters on the haematological indices of experimental rats

| Groups | PCV (%) | HB (g/L) | RBC count ($\times 10^9$ cells/L) | TWBC count (cells/mm ³) | Platelets count ($\times 10^6$ cells/L) |
|---------|--------------------------------|-------------------------------|------------------------------------|-------------------------------------|--|
| Group 1 | 42.50 \pm 2.08 ^c | 14.08 \pm 0.83 ^d | 302.50 \pm 23.27 ^b | 7750.00 \pm 157.43 ^e | 180.00 \pm 9.13 ^d |
| Group 2 | 30.75 \pm 0.96 ^a | 10.58 \pm 0.34 ^a | 236.25 \pm 27.20 ^a | 5300.00 \pm 113.25 ^a | 171.25 \pm 8.54 ^d |
| Group 3 | 35.50 \pm 1.91 ^b | 11.83 \pm 0.67 ^b | 331.25 \pm 6.29 ^c | 6200.00 \pm 432.05 ^{bc} | 131.25 \pm 9.31 ^c |
| Group 4 | 41.50 \pm 1.29 ^c | 13.83 \pm 0.43 ^c | 373.75 \pm 17.88 ^d | 6450.00 \pm 106.23 ^c | 126.25 \pm 11.09 ^b |
| Group 5 | 45.50 \pm 1.00 ^d | 15.17 \pm 0.30 ^e | 412.25 \pm 11.90 ^e | 7150.00 \pm 251.66 ^d | 110.75 \pm 8.16 ^a |
| Group 6 | 43.00 \pm 2.58 ^{cd} | 13.82 \pm 0.87 ^c | 363.75 \pm 12.50 ^d | 5850.00 \pm 251.66 ^b | 125.00 \pm 6.46 ^b |

Data are mean \pm standard deviation (SD) ($n = 5$). Values with different superscripts down the groups are significant at $p < 0.05$. PCV = Packed cell volume, Hb = Haemoglobin, RBC = Red blood cell, TWBC = total white blood cell

3.4 Effects of Yoyo® bitters on the antioxidant enzyme activities and lipid peroxidation status of experimental rats

Enzymatic antioxidants are capable of stabilizing, or scavenging free radicals, preventing their damaging effect to cellular components by donating some of their electrons to free radicals, thereby causing them to become stable (Krishnamurthy and Wadhvani, 2012). The intoxication of rats with H₂O₂ significantly ($p < 0.05$) decreased the antioxidant enzyme, SOD activity of the rats in group 2 which represent the untreated H₂O₂-intoxicated rats when compared with the normal control. The SOD activities of rats in the intoxicated and treated groups were significantly ($p < 0.05$) higher, in a dose-dependent manner, when compared with the intoxicated and untreated groups (Table 3). This suggests that some antioxidant principles in Yoyo® bitters have stimulatory effect on SOD activity. A similar result was obtained when the SOD activity of rats in group 6 (intoxicated and treated with silymarin) when compared to the intoxicated and untreated rats. SOD is the antioxidant enzyme that catalyzes the dismutation of the highly reactive superoxide anion to O₂ and to the less reactive species H₂O₂ (Mates *et al.*, 1999). Similarly, the intoxication of rats with H₂O₂ significantly ($p < 0.05$) decreased the CAT activity of the rats in group 2 rats when compared with the normal control. This is probably due the overwhelming stress caused by the H₂O₂ which inactivates the enzyme. The CAT activities of rats in the intoxicated and treated groups were significantly ($p < 0.05$) higher in a dose-dependent manner compared with the untreated groups. Catalase converts H₂O₂ to water and molecular oxygen; and with H-donors (such as methanol, ethanol, formic acid, or phenols); it has peroxidase activity (Krishnamurthy and Wadhvani, 2012). Similarly, observation was recorded for GPx activity of the experimental rats. Sen *et al.* (2010) stated that at high concentrations, reactive oxygen species (ROS) can be important mediators to damage cellular nucleic acids, lipids and proteins. They

also have the capability to decrease the activity of natural antioxidant defense system such as SOD, CAT and GPx activities. The intoxicated and treated groups had significantly ($p < 0.05$) higher GPx activity, in a dose-dependent manner when compared with the untreated groups. GPx catalyzes the reduction of hydroperoxides using GSH, thereby protecting mammalian cells against oxidative damage (Nimse and Pal, 2015). CAT, GPx and SOD show synergistic effect in the scavenging of reactive species such as superoxide anion radical ($\cdot\text{O}_2^-$) (Nimse and Pal, 2015). This could be linked to the reduction in activities of the three enzymes in intoxicated rats; the enzymes may have been mobilized to scavenge reactive species produced by H₂O₂.

There was a significant ($p < 0.05$) increase in concentration of MDA (an index of lipid peroxidation that is implicated in many disease states) in H₂O₂-intoxicated and untreated rats when compared with the normal control. Malondialdehyde is a product of lipid peroxidation, a destructive action of free radicals on lipids. Increase in activity of MDA shows that lipid peroxidation and oxidative stress is taking place in the system. Rats in the intoxicated and treated groups had significantly ($p < 0.05$) lower MDA concentration compared with that of intoxicated and untreated rats. The decrease in MDA concentration upon treatment with herbal drug was dose-dependent. Phytoconstituents with antioxidant potentials act as radical scavengers and inhibitors of lipid peroxidation (Sen *et al.*, 2010). The antioxidant phytoconstituents present in Yoyo® bitters may be responsible for the reduced MDA concentration in the treated groups when compared with the untreated. This finding agrees with the report of Alabi *et al.* (2013) that Yoyo® bitters possesses antioxidant and anti-lipid peroxidative effects.

3.5 Effects of Yoyo® bitters on the glutathione and antioxidant vitamins concentrations of the experimental rats

The intoxication with H₂O₂ significantly ($p < 0.05$) decreased the concentration of glutathione in the untreated H₂O₂-intoxicated rats compared with that of the control. Treatment of intoxicated rats with Yoyo® bitters as seen in groups 3, 4 and 5 significantly ($p < 0.05$) increased the reduced glutathione (GSH) levels in a dose-dependent manner when compared with the intoxicated and untreated rats. Krishnamurthy and Wadhvani (2012) documented that glutathione can directly scavenge free radicals or act as a substrate for GPx and glutathione-S-transferase (GST) during the detoxification of hydrogen peroxide, lipid hydro-peroxides and electrophilic compounds. In general, the major protective roles of glutathione against oxidative stress is that it can act as a co-factor for several detoxifying enzymes, participate in amino acid transport across plasma membrane, scavenge hydroxyl radical and singlet oxygen directly, and regenerate vitamins C and E back to their active forms (Masella *et al.*, 2005). On the other hand, GSH concentration of rats in group 6 (intoxicated rats treated with silymarin) was significantly ($p < 0.05$) lower when compared with that of normal

control but higher when compared with that of group 2. Intoxication of rats with H₂O₂ lead to decrease in concentrations of vitamins A, C and E in rats intoxicated and untreated when compared with that of normal control. Treatment of H₂O₂-intoxicated rats with Yoyo® bitters and silymarin resulted in elevated concentrations of the antioxidant vitamins compared to the untreated group (Table 4). These suggest the presence of oxidative stress in the intoxicated rats leading to the depletion of antioxidant vitamins that were mobilized to scavenge free radicals produced by H₂O₂. This finding is in line with the report of Oyewo *et al.* (2017) that the polyherbal drug possesses ameliorative effects on the haematological and biochemical aberrations induced by arsenite toxicity in rats. Similarly, Adeyemi *et al.* (2017) after 28 days oral administration of Yoyo® bitters to rats reported that the polyherbal product showed have hypolipidaemic, hypoglycemic, immunity-boosting, choleric, hepatoprotective, antihypertensive, as well as antioxidant activities and was also protective against heart and related vascular diseases.

Table 3: Effects of Yoyo® bitters on the activities of antioxidant enzymes and malondialdehyde concentration of experimental rats

| Groups | SOD (IU/L) | CAT (IU/L) | GPx (IU/L) | MDA (mmol/l) |
|---------|---------------------------|--------------------------|---------------------------|---------------------------|
| Group 1 | 10.52 ± 0.26 ^b | 0.93 ± 0.15 ^b | 12.50 ± 0.88 ^c | 4.70 ± 0.70 ^b |
| Group 2 | 8.80 ± 0.22 ^a | 0.59 ± 0.28 ^a | 3.68 ± 0.88 ^a | 6.63 ± 0.10 ^d |
| Group 3 | 10.75 ± 0.37 ^b | 1.06 ± 0.07 ^b | 8.94 ± 0.49 ^b | 5.38 ± 0.25 ^c |
| Group 4 | 11.75 ± 0.41 ^c | 1.27 ± 0.13 ^c | 11.63 ± 1.22 ^c | 4.23 ± 0.36 ^{ab} |
| Group 5 | 12.53 ± 0.39 ^d | 1.91 ± 0.08 ^d | 15.39 ± 1.30 ^d | 3.93 ± 0.13 ^a |
| Group 6 | 10.33 ± 0.66 ^b | 0.99 ± 0.10 ^b | 11.63 ± 1.22 ^c | 4.30 ± 0.23 ^b |

Data are mean ± standard deviation (SD) (n = 5). Values with different superscripts down the groups are significant at $p < 0.05$. MDA = Malondialdehyde, CAT = Catalase, SOD = Superoxide dismutase, GPx = Glutathione peroxidase

3.6 Effects of Yoyo® bitters on the liver status markers of experimental rats

The liver marker enzymes assayed after 14 days of treatment showed a significant ($p < 0.05$) dose-dependent increase in the activities of aspartate aminotransferase (AST) and alanine

aminotransferase (ALT) in intoxicated and untreated rats when compared with control. However, treatment of intoxicated rats with graded doses of Yoyo® bitters showed significant ($p < 0.05$) dose-dependent decrease in the activities of AST and ALT when compared

with intoxicated and untreated rats. Studies have reported that *Allium sativum* (Ajayi *et al.*, 2009; Ilyas *et al.*, 2011) and *Citrus aurantifolia* (Gokulakrishnan *et al.*, 2009), constituents of the Yoyo® bitters possess hepatoprotective effects. Similarly, Nigam and Paarakh (2011) reported hepatoprotective effects of *Chenopodium album*, another constituent of Yoyo® bitters. Persistent increase in serum AST and ALT activities and total bilirubin levels are reliable markers for hepatotoxicity (Singh *et al.*, 2011; Seif, 2016). In other words, the results of AST and total bilirubin activities showed that the polyherbal drug had a curative effect on the hepatic injury produced by the toxicant. The hepato-curative effects of Yoyo® bitters might be by reducing hepatic permeability and thus the ability for the hepatocytes to be prone to seepage of liver enzymes. Similarly, treatment of intoxicated rats with silymarin significantly ($p <$

0.05) decreased the activities of AST and ALT as well as levels of total bilirubin when compared with intoxicated and untreated rats. Silymarin is a flavonolignan (polyphenolic fraction) extracted from the seeds and fruits of *Silybum marianum*. It is composed of mainly silybin, isosilybin, silydianin and silychristin all of which possess structural similarity to steroids and could be linked to their protein synthesis stimulating effects (Lien *et al.*, 2016). It is reported to have antioxidant, anti-inflammatory, antifibrotic, anti-lipid peroxidative, membrane stabilization and liver regenerating activities (Vargas-Mendoza *et al.*, 2014). The mechanism of action includes inhibition of hepatotoxin binding to receptor sites on the hepatocyte membrane, and stimulating the ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration (Pandey and Gupta, 2014).

Table 4: Effects of Yoyo® bitters on reduced glutathione and antioxidant vitamins concentrations of the experimental rats

| Groups | Reduced (mmol/l) | glutathione | Vitamin A (mg/dl) | Vitamin C (mmol/l) | Vitamin E (mmol/l) |
|---------|--------------------------|-------------|--------------------------|--------------------------|--------------------------|
| Group 1 | 6.73 ± 0.17 ^c | | 8.88 ± 0.51 ^c | 3.90 ± 0.08 ^c | 0.51 ± 0.01 ^c |
| Group 2 | 2.68 ± 0.78 ^a | | 5.28 ± 0.91 ^a | 1.50 ± 0.75 ^a | 0.13 ± 0.01 ^a |
| Group 3 | 5.93 ± 0.13 ^b | | 7.53 ± 0.80 ^b | 2.30 ± 0.52 ^b | 0.39 ± 0.02 ^b |
| Group 4 | 7.58 ± 1.50 ^d | | 7.78 ± 0.95 ^b | 3.63 ± 0.24 ^c | 0.63 ± 0.01 ^d |
| Group 5 | 9.93 ± 0.56 ^e | | 8.88 ± 0.79 ^c | 4.38 ± 0.13 ^d | 1.15 ± 0.01 ^e |
| Group 6 | 5.30 ± 0.50 ^f | | 7.53 ± 0.84 ^b | 2.20 ± 0.18 ^b | 0.34 ± 0.01 ^b |

Data are mean ± standard deviation (SD) (n = 5). Values with different superscripts down the groups are significant at $p < 0.05$.

Table 5: Effects of Yoyo® bitters on the markers of liver function of experimental rats

| Groups | AST (IU/L) | ALT (IU/L) | T. Bil (mg/dl) |
|---------|-----------------------------|----------------------------|--------------------------|
| Group 1 | 86.75 ± 2.87 ^a | 54.25 ± 2.75 ^a | 1.38 ± 0.15 ^b |
| Group 2 | 192.00 ± 3.92 ^e | 87.75 ± 3.10 ^d | 2.53 ± 0.10 ^d |
| Group 3 | 137.00 ± 4.08 ^d | 53.75 ± 4.43 ^c | 1.50 ± 0.14 ^c |
| Group 4 | 126.75 ± 3.10 ^c | 59.25 ± 2.06 ^{bc} | 1.38 ± 0.10 ^b |
| Group 5 | 113.50 ± 3.00 ^b | 64.50 ± 3.32 ^b | 1.23 ± 0.10 ^a |
| Group 6 | 103.50 ± 10.34 ^b | 62.50 ± 2.38 ^{ab} | 1.18 ± 0.19 ^a |

Data are mean ± standard deviation (SD) (n = 5). Values with different superscripts down the groups are significant at $p < 0.05$. H₂O₂ = Hydrogen peroxide, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, T. Bil = Total bilirubin

4. Conclusion

Findings from this study have shown that Yoyo® bitters possess hepatoprotective, antioxidant and erythropoietic effects. It was also demonstrated to be safe at doses up to 10 ml/kg b.w. and to be rich in phytochemicals. However, further studies are required to evaluate the long term safety of its use and to assess if any toxicant such as heavy metals and polycyclic aromatic hydrocarbons exist in the herbal product. These will guarantee the possible safety of its use in disease management.

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