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Hepatotoxic Effects of Sildenafil Adulterated Herbal Aphrodisiacs (*Attoté* and *Congnons-moussos*), Following Chronic Exposure in Male Wistar Rats

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Abstract

The adulteration of traditional aphrodisiac formulations with phosphodiesterase type 5 inhibitors (PDE5i), particularly sildenafil, represents a significant public health concern due to the toxicological risks associated with their uncontrolled and prolonged use. This study investigated the subchronic hepatotoxic effects of two commercially available aphrodisiac preparations, called *Attoté* (AT) and *Congnons-moussos* (CM), both containing sildenafil (1 mg/g dry matter), in male Wistar rats. Animals were allocated into eight groups and received distilled water (control), pure sildenafil (5 mg/kg b.w.), and increasing doses (50, 100, and 200 mg/kg b.w.) of AT and CM by oral gavage for 90 consecutive days. Hepatic function was evaluated through serum biochemical markers such as Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), total bilirubin, albumin, total proteins, glucose, hepatic tissue enzymes, Alkaline Phosphatase (ALP), Gamma Glutamyl Transferase (GGT), oxidative stress biomarkers Malondialdehyde (MDA), Superoxide Dimutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Reduced Glutathione (GSH), and histopathological examination of liver sections. The results showed a dose-dependent hepatocellular injury, with increased ALT, AST, total bilirubin, glucose, and ALP, accompanied by decreased albumin, total proteins, and GGT activity. Oxidative stress was evident through elevated MDA levels and a marked reduction in antioxidant defenses. Histopathological findings confirmed liver damage, showing vacuolization, sinusoidal congestion, and inflammatory infiltration.

Major Findings: Overall, these findings demonstrate that chronic ingestion of adulterated herbal aphrodisiacs induces hepatotoxicity through oxidative stress and disruption of hepatic homeostasis, showing the health risks associated with their unregulated consumption.

Keywords : Hepatotoxicity, Herbal Aphrodisiacs, Oxidative Stress, Sildenafil Adulteration, Wistar Rats

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1. Introduction

The global consumption of herbal aphrodisiac products has increased in recent decades, accentuated by the widespread perception that plant-based remedies are naturally safer, more accessible, and culturally compatible with traditional healing systems. This trend is particularly pronounced in Sub-Saharan Africa, where limited access to conventional healthcare services, high treatment costs, combined with strong sociocultural beliefs, have contributed to the expansion of informal markets for traditional medicines[1,2]. In this context, many men turn to herbal formulations promoted as « natural » solutions to enhance sexual performance, improve fertility, and treat erectile dysfunction. These products are typically polyherbal decoctions composed of roots, barks, and

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seeds from plants such as *Zingiber officinale*, *Garcinia kola*, *Xylopia aethiopica*, and *Pausinystalia johimbe*, all reputed for their aphrodisiac properties[2,3].

However, the appeal of these products often conceals significant public health risks. Numerous analytical surveys have revealed the clandestine adulteration of traditional herbal aphrodisiacs with synthetic pharmaceutical compounds, particularly phosphodiesterase type 5 inhibitors (PDE5is), particularly as sildenafil, tadalafil, and vardenafil. Recent analytical studies conducted in Africa and Asia have revealed widespread adulteration of herbal aphrodisiacs with undeclared PDE5 inhibitors[4,5]. Originally developed and approved for the clinical management of erectile dysfunction, sildenafil acts by enhancing nitric oxide-mediated vasodilation in the corpus cavernosum. When used under medical supervision, it is generally safe. However, its unsupervised and undeclared addition to over-the-counter herbal formulations represents a serious toxicological concern[6]. The problem is exacerbated by the absence of dosage standardisation, poor quality control, and the lack of pharmacovigilance in informal markets across developing countries[7-9].

Excessive use may lead to damage to the vital organs. The liver, as the principal organ responsible for xenobiotic metabolism and detoxification, is particularly susceptible to injury from adulterated herbal remedies. Hepatic bioactivation of sildenafil, combined with interactions among diverse phytochemical constituents, can trigger oxidative stress, mitochondrial dysfunction, and enzyme dysregulation, ultimately leading to hepatocellular damage[10-12]. Previous reports have documented hepatic injury associated with chronic sildenafil exposure, especially when combined with alcohol or other hepatotoxic agents ; nonetheless, there remains a critical lack of experimental data evaluating the hepatotoxic potential of sildenafil adulterated herbal aphrodisiacs that are commonly sold in West African markets[2,5,13].

To bridge this knowledge gap, the present study investigated the subchronic hepatotoxic effects of two locally marketed herbal aphrodisiac formulations confirmed to contain sildenafil. Adult male Wistar rats were exposed orally to these formulations for ninety consecutive days.

By integrating biochemical, enzymatic, oxidative, and histological endpoints, this study provides novel *in vivo* evidence of the hepatotoxic risk associated with chronic consumption of sildenafil adulterated aphrodisiacs and shows the urgent need for pharmacovigilance and stricter regulation of herbal sexual enhancers in emerging markets.

2. Materials and Methods

2.1 Chemicals and Test Substances

Sildenafil was obtained from Erekta® tablets (50mg, batch no. 7752608, Strides Pharma, India), which were used as a secondary standard. Based on the product specification and standard pharmacopoeial quality, the sildenafil content was assumed to be ≥98% pure. Methanol (100%, Normapure grade, VWR International, France, catalog number 19G194006), Thiobarbituric acid (TBA, Sigma-Aldrich, USA, catalog number T5500), nitroblue tetrazolium (NBT, Sigma-Aldrich, USA, catalog number N6876), hydrogen peroxide (H_2O_2, Merck, Germany, catalog number 107209), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Sigma-Aldrich, USA, catalog number D8130), NADPH (Sigma-Aldrich, USA, catalog number N7505), and all buffer reagents were of analytical grade. Histological reagents, including hematoxylin, eosin, and paraffin, were obtained from Merck (Germany, catalogue numbers 104302, 109844, and 109584, respectively). All chemicals were used without further purification. Solutions were freshly prepared on the day of use and handled according to the manufacturer's guidelines.

Two herbal aphrodisiac formulations were selected for toxicological evaluation. Their selection was based on an exploratory market survey conducted in Ouagadougou (Burkina Faso) to identify the most frequently marketed traditional sexual enhancers in informal settings. To ensure scientific objectivity and avoid any commercial bias, the products were anonymised and coded as AT and CM throughout the study.

Both formulations were obtained in liquid decoction form directly from local vendors. According to the sellers, each product was prepared from a polyherbal mixture traditionally used to enhance sexual performance; however, the exact botanical composition was not disclosed to preserve the proprietary nature of the recipes. The total dry matter content was determined by gravimetric analysis and estimated at 10g per 500mL of solution.

Before *in vivo* administration, each product was analysed using high-performance liquid chromatography (HPLC-UV, Agilent 1200 Infinity system) equipped with an Eclipse Plus C18 column (4.6 × 150mm, 5µm) and a mobile phase consisting of acetonitrile : water (70 : 30, v/v) adjusted to pH 3.0 with orthophosphoric acid, at a flow rate of 1 mL/min and detection wavelength of 230nm. The injection volume was 20µL and the run time was 10 min.

Quantitative analysis revealed the presence of sildenafil in both formulations, at a mean concentration of 1 mg/g of dry matter.

2.2 Experimental Animals

The experiment was conducted using healthy adult male Wistar rats (*Rattus norvegicus*), aged 8 to 10 weeks and weighing approximately 140 g on average. The animals were obtained from the accredited animal care facility of Joseph KI-ZERBO University. They were housed in polypropylene cages under controlled environmental conditions (temperature : 22 ± 3°C ; photoperiod : 12-hour light/dark cycle), with ad libitum access to clean drinking water and a protein-enriched diet containing 29% crude protein.

2.3 Ethical Statement

All experimental procedures involving animals were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee of Université Joseph KI-ZERBO. The protocol complied with the European Union (EU) Directive 2010/63/EU on the protection of animals used for scientific purposes and the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (8th edition, 2011). Ethical approval was obtained prior to the initiation of the study under the code CE-UJKZ/2023-04.

2.4 Experimental Design

A total of forty (40) male Wistar rats, eight weeks old and weighing approximately 180 g, were used in this study. Following a one-week acclimatisation period under standard laboratory conditions, the animals were randomly assigned to eight (8) groups of five (5) rats each, based on the treatment regimen.

- Group 1 (Control) received distilled water.
- Group 2 (Sildenafil) received sildenafil citrate at a dose of 5 mg/kg body weight.
- Groups 3, 4, and 5 were treated with increasing oral doses of the traditional aphrodisiac coded « AT » (50, 100, and 200 mg/kg, respectively).
- Groups 6, 7, and 8 received the second aphrodisiac coded « CM » at the same respective doses.

The dose selection for AT and CM was based on the human daily consumption pattern reported during the exploratory market survey. According to user-reported intake (~5 mL/kg/day of decoction), the corresponding Human Equivalent Dose (HED) was converted to the rat equivalent using the FDA interspecies conversion factor (body surface area normalisation) :

$$Rat\ dose\ (mg/kg) = Human\ dose\ (mg/kg) \times \frac{Km_{human}}{Km_{rat}};$$

$$Km_{human} = 37\ and\ Km_{rat} = 6.2.$$

This yielded approximate rat doses of 50, 100, and 200 mg/kg, representing low, medium, and high exposure levels within the range of possible real conditions of consumption.

All treatments were administered once daily by oral gavage for a period of 90 consecutive days. Doses were adjusted weekly based on individual body weight variations. Throughout the treatment period, animals were monitored daily for clinical signs of toxicity or behavioural abnormalities. Body weight was recorded weekly to assess general health status and treatment-related effects.

2.5 Animal Sacrifice, Blood Sample and Organ Collection

At the end of the 90-day treatment period, animals were fasted overnight and euthanised under light isoflurane anaesthesia, followed by exsanguination via cardiac puncture. Blood samples were collected in dry tubes, allowed to clot at room temperature, and then centrifuged at 3000 rpm for 15 minutes to separate the serum, which was stored at -20°C until biochemical analysis. Immediately after blood collection, the abdominal cavity was opened, and the liver was carefully excised, rinsed in ice-cold saline, blotted, weighed, and sectioned. Portions of liver tissue were either fixed in 10% neutral-buffered formalin for histological processing or immediately frozen in liquid nitrogen and stored at -80 °C for subsequent biochemical and oxidative stress analyses. All procedures were conducted in accordance with institutional ethical

guidelines and international standards for the humane treatment of laboratory animals. CODE AGREEMENT : CE-UJKZ/2023-04

2.6 Serum Biochemical Assays

The following serum biochemical parameters were measured using commercial diagnostic kits from Spinreact S.A. (Girona, Spain), strictly following the manufacturer's instructions. Alanine aminotransferase (ALT ; Cat. No. 1001171) and aspartate aminotransferase (AST ; Cat. No. 1001165) activities were determined by kinetic enzymatic methods using NADH oxidation at 340nm. Total bilirubin levels were assessed colourimetrically based on the diazonium principle (Cat. No. 1001042). Albumin concentration was measured using the bromocresol green dye-binding method (Cat. No. 1001024), whereas total protein levels were evaluated using the Biuret method (Cat. No. 1001295). Serum glucose concentration was determined enzymatically by the glucose oxidase peroxidase technique (Cat. No. 1001191).

2.7 Liver Enzymatic Activities

Liver tissues were washed with cold normal saline to remove residual blood, blotted dry, weighed, and homogenised in ice-cold 0.1 M phosphate buffer (pH 7.4) using a Teflon-glass homogeniser. The homogenates were centrifuged at 10,000 × g for 15 minutes at 4°C, and the resulting supernatants were collected for biochemical analysis.

ALP activity was determined colourimetrically using p-nitrophenyl phosphate as substrate (*Spinreact S.A.*, Girona, Spain ; Cat. No. 1001046), while γ-glutamyl transferase (GGT) activity was measured enzymatically using L-γ-glutamyl-p-nitroanilide as substrate (*Spinreact S.A.*, Girona, Spain ; Cat. No. 1001178). All assays were performed according to the manufacturer's instructions, and enzyme activities were expressed as units per gram of tissue (U/g tissue).

2.8 Liver Oxidative Stress Assessment

Liver tissue samples were also processed for the evaluation of oxidative stress biomarkers. After homogenization in cold phosphate buffer (0.1 M, pH 7.4) and centrifugation at 3000 rpm for 15 minutes at 4°C, the resulting supernatants were analysed as follows :

2.8.1 Malondialdehyde (MDA)

MDA levels, a marker of lipid peroxidation, were assessed using the Thiobarbituric Acid Reactive Substances (TBARS) method. MDA reacts with thiobarbituric acid under acidic and heated conditions to form a pink chromogen measurable at 532nm[14].

2.8.2 Superoxide Dismutase (SOD)

SOD activity was determined based on its ability to inhibit the reduction of Nitroblue Tetrazolium (NBT) by the superoxide anion. Absorbance was read at 560nm, and results were expressed as units per milligram of protein (U/mg prot)[15].

2.8.3 Catalase (CAT)

Catalase activity was measured spectrophotometrically at 240nm by following the decomposition of hydrogen peroxide (H_2O_2). One unit of activity corresponds to the decomposition of 1 µmol of H_2O_2 per minute[16].

2.8.4 Reduced Glutathione (GSH)

GSH levels were determined using the Ellman method, which involves the reaction of GSH with 5,5'-dithiobis (2-nitrobenzoic acid) to produce a yellow chromophore measurable at 412 nm. Samples were deproteinized using sulfosalicylic acid before the assay[17].

2.8.5 Glutathione Peroxidase (GPx)

GPx activity was evaluated by monitoring the consumption of NADPH during the reduction of H_2O_2 in the presence of GSH, glutathione reductase (GR), and NADPH. The decrease in absorbance at 340nm is directly proportional to GPx activity[18].

2.9 Histopathological Analysis of Liver

At the end of the treatment period (90 days), liver tissues from all experimental animals were collected and immediately fixed in 10% neutral buffered formalin for 48 hours. After fixation, samples were dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Sections of 5µm thickness were cut using a microtome and mounted on glass slides. These sections were stained with Hematoxylin and Eosin (H&E) and examined under a light microscope (×400 magnification) for histopathological alterations.

2.10 Statistical Analysis

Data were entered using Microsoft Excel 2016 and expressed as mean ± standard deviation (mean ± SD). Statistical analyses were performed using GraphPad Prism software, version 8.4.3. One-Way Analysis of Variance (ANOVA I) was applied, followed by Dunnett's post hoc test to compare the treated groups to the control group. A significance level of $p < 0.05$ was considered statistically significant.

3. Results

3.1 Effect of Treatments on Relative Liver Weight

After 90 days of treatment, the relative liver weight significantly increased in several treated groups compared to the control group. Rats receiving 200 mg/kg of AT or 100 and 200 mg/kg of CM exhibited a highly significant ($p < 0.01$) increase in liver weight. A moderate but significant ($p < 0.05$) increase was also observed in the group treated with 100 mg/kg of AT. In contrast, no significant changes were noted in animals treated with sildenafil alone or with the lowest doses (50 mg/kg) of either AT or CM (Figure 1).

3.2 Effect of Treatments on Serum Alanine Aminotransferase (ALT) Activity

The results revealed a significant elevation in serum ALT activity in several treated groups compared to the control group. A very highly significant ($p < 0.001$) increase was observed in animals treated with 200 mg/kg of AT, as well as those receiving 100 mg/kg and 200 mg/kg of CM. Additionally, a significant ($p < 0.01$) increase was recorded in the group treated with 100 mg/kg of AT. In contrast, no statistically significant variation was observed in rats administered pure sildenafil (5 mg/kg) or the lowest doses (50 mg/kg) of either AT or CM (Figure 2).

3.3 Effect of Treatments on Serum Aspartate Aminotransferase (AST) Activity

Serum AST activity showed a significant elevation in several treated groups compared to the control group. A highly significant ($p < 0.001$) increase was observed in animals administered AT 200 mg/kg and 200 mg/kg of CM. A significant ($p < 0.01$) increase was recorded in the CM 100 mg/kg group compared to the control (Figure 3).

3.4 Effect of Treatments on Serum Total Bilirubin Levels

Serum total bilirubin concentrations were slightly elevated in several treated groups compared to the control group. Statistically significant ($p < 0.05$) increases were observed in animals treated with 200 mg/kg of AT and 200 mg/kg of CM. Although other treatment groups tended to have increased bilirubin levels, the differences were not statistically significant. Serum albumin concentrations showed a decreasing trend in all treated groups compared to the control, although the differences were not statistically significant. The control

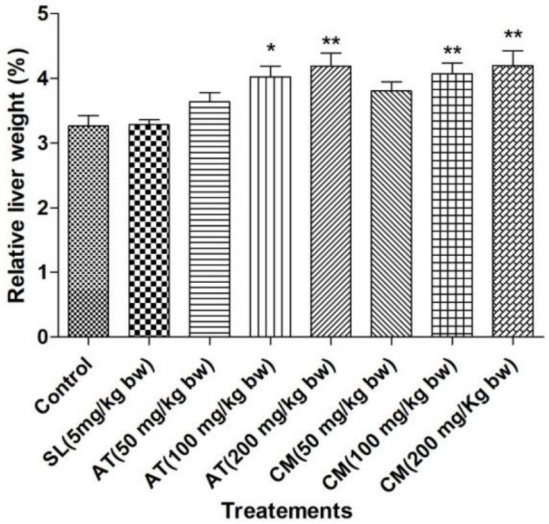


Figure 1. Effect of treatments on relative liver weight. * : $p < 0.05$, ** : $p < 0.01$ in comparison with the control group.

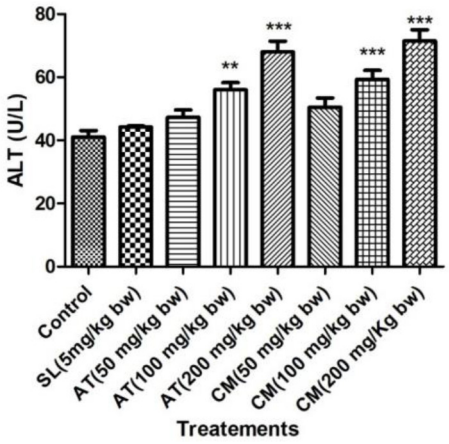


Figure 2. Serum ALT activity in rats. * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$ in comparison with the control group.

group exhibited a mean concentration exceeding 30 g/L, while animals exposed to medium and high doses of AT and CM displayed values ranging between 24 and 28 g/L. Serum glucose concentrations were significantly elevated in several treated groups compared to the control. A very highly significant ($p < 0.001$) increase was observed in the group receiving 200 mg/kg of CM. Highly significant ($p < 0.01$) increases were also noted in animals treated with 200 mg/kg of AT and 50 mg/kg of CM. Additionally, significant ($p < 0.05$) elevations were recorded in the groups treated with 50 mg/kg of AT and 100 mg/kg of CM. No significant difference was observed between the group receiving 100 mg/kg of AT and the control. Serum total protein concentrations were significantly reduced in certain treated groups compared to the control. Specifically, animals administered 100 mg/kg and 200 mg/kg of CM exhibited a statistically significant ($p < 0.05$) decrease in total protein levels. No significant differences were observed in the other groups ; however, a general reduction was noted across several AT and CM-treated groups at various doses (Table 1).

3.5 Effect of Treatments on Hepatic Alkaline Phosphatase (Alp) Activity

The results showed a significant increase in alkaline phosphatase (ALP) activity in liver homogenates from several treated groups compared to the control. A highly significant ($p < 0.01$) elevation was recorded in rats treated with 200 mg/kg of AT and 200 mg/kg of CM. Additionally, a significant ($p < 0.05$) increase was observed in the groups receiving sildenafil (5 mg/kg), AT 50 and 100 mg/kg, and CM at 100 mg/kg. The group treated with 50 mg/kg of CM also showed a minor increase in ALP activity, although it was not statistically significant (Figure 4).

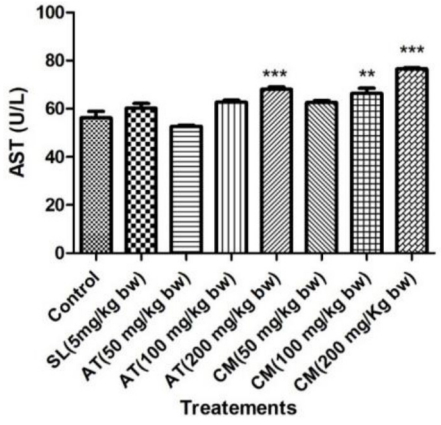


Figure 3. Serum AST activity in treated rats. * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$ in comparison with the control group.

3.6 Effect of Treatments on Hepatic Γ-Glutamyl Transferase (GGT) Activity

In contrast to ALP, hepatic γ-GGT activity was significantly reduced in several treated groups compared to the control. A highly significant ($p < 0.01$) decrease was observed in the group receiving 200 mg/kg of CM. Significant ($p < 0.05$) reductions were also recorded in animals treated with 200 mg/kg of AT, as well as with 50 mg/kg and 100 mg/kg of CM, and 100 mg/kg of AT. No significant changes were noted in the groups treated with pure sildenafil or AT 50 mg/kg (Figure 5).

3.7 Effect of Treatments on Hepatic Oxidative Stress Markers

MDA concentrations, a key indicator of lipid peroxidation, increased significantly in all treated groups in a dose-dependent manner. Significant ($p < 0.05$) increases were recorded in animals treated with 50 mg/kg of AT and CM. Highly significant ($p < 0.01$) elevations were observed at 100 mg/kg for both products, while very highly significant ($p < 0.001$) increases were found at the highest dose of 200 mg/kg for both AT and CM. A marked decrease in SOD activity was observed across all treated groups, with the most pronounced reduction noted at 100 mg/kg of AT. Statistically significant ($p < 0.01$) decreases were also observed at 200 mg/kg of AT, and at 100 and 200 mg/kg of CM. Catalase activity was significantly ($p < 0.05$) reduced in the groups treated with 200 mg/kg of AT and CM. GSH levels were significantly lowered in all groups exposed to either sildenafil or the aphrodisiac formulations. The reduction was particularly marked in the CM 200 mg/kg group ($p < 0.01$) and AT 200 mg/kg group ($p < 0.05$). Finally, a significant ($p < 0.05$) reduction in GPx activity was observed in rats treated with 200 mg/kg of AT, and 100 and 200 mg/kg of CM (Table 2).

3.8 Histopathological Examination of Liver Tissue

Histological evaluation of liver sections revealed progressive structural alterations across the treatment groups. In the control group (A), the hepatic parenchyma appeared well organised, with hepatocyte plates

Table 1. Effects of treatments on serum biochemical parameters in Wistar rats

Parameters	Protein (g/L)	Albumin (g/L)	Bilirubin (mg/dL)	Glucose (g/L)
Control	297.57 ± 53.00	49.22 ± 10.00	0.57 ± 0.02	0.27 ± 0.11
SL 5 mg/kg	239.37 ± 34.00	55.52 ± 6.00	0.61 ± 0.07	0.20 ± 0.09
AT 50 mg/kg	244.70 ± 16.00	49.23 ± 1.41	0.60 ± 0.01	0.31 ± 0.08
AT 100 mg/kg	239.70 ± 12.00	51.80 ± 14.20	0.70 ± 0.01	0.40 ± 0.08 ***
AT 200 mg/kg	248.96 ± 5.00 *	35.00 ± 6.00	0.70 ± 0.10 *	0.20 ± 0.07 ***
CM 50 mg/kg	255.95 ± 5.71	42.10 ± 2.31	0.68 ± 0.07	0.42 ± 0.06
CM 100 mg/kg	256.17 ± 14.00	39.76 ± 0.05	0.68 ± 0.09	0.40 ± 0.07 **
CM 200 mg/kg	258.22 ± 13.00 *	43.40 ± 7.44	0.73 ± 0.04 *	0.68 ± 0.03 ***

Significance compared to the control group : * : $p < 0.05$; ** : $p < 0.01$; *** : $p < 0.001$.

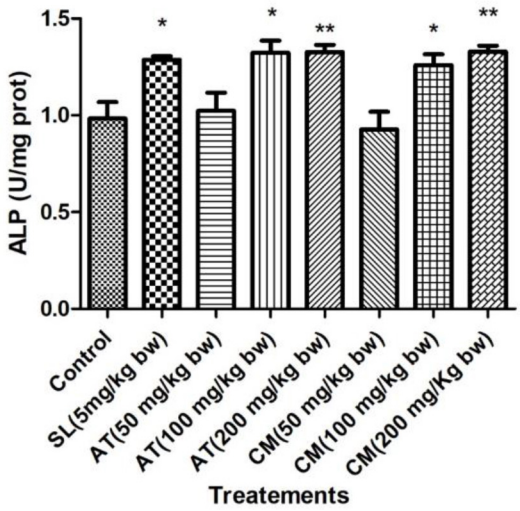


Figure 4. Hepatic ALP activity in liver homogenates. * : $p < 0.05$, ** : $p < 0.01$ in comparison with the control group.

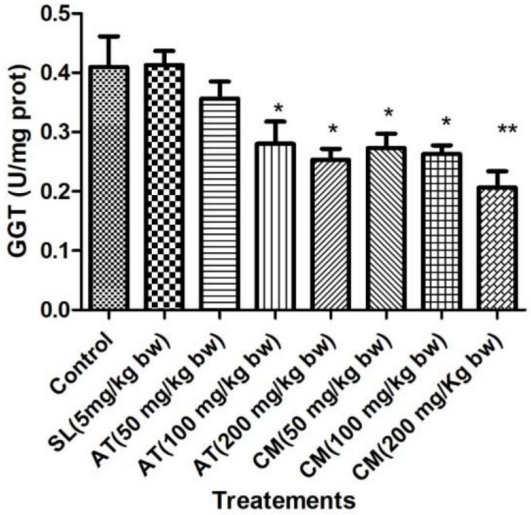


Figure 5. Effect of treatments on hepatic GGT activity. * : $p < 0.05$, ** : $p < 0.01$ in comparison with the control group.

Table 2. Oxidative stress biomarkers (MDA, SOD, CAT, GSH, GPx) in liver homogenates of rats treated with sildenafil or herbal aphrodisiac formulations (AT and CM) for 90 days

Parameters	MDA (nmol/mg prot)	SOD (U/mg prot	CAT (U/mg prot)	GSH (µmol/mg prot)	GPx (U/mg prot
Control	36 ± 0.02	89 ± 0.05	29 ± 0.04	53 ± 0.03	6.70 ± 0.44
SL 5 mg/kg	44 ± 0.03	75 ± 0.08	23 ± 0.06	41 ± 0.03*	5.90 ± 0.62
AT 50 mg/kg	48 ± 0.05*	78 ± 0.18	23 ± 0.04	45 ± 0.04	5.93 ± 0.25
AT 100 mg/kg	53 ± 0.03**	65 ± 0.03	21 ± 0.03	43 ± 0.04*	5.17 ± 0.70
AT 200 mg/kg	56 ± 0.03***	53 ± 0.08**	21 ± 0.03*	43 ± 0.06*	4.53 ± 0.70**
CM 50 mg/kg	47 ± 0.05	72 ± 0.09	27 ± 0.03	46 ± 0.03	5.80 ± 0.95
CM 100 mg/kg	54 ± 0.05**	56 ± 0.08**	22 ± 0.03	42 ± 0.03*	4.80 ± 0.66*
CM 200 mg/kg	60 ± 0.03***	59 ± 0.10**	20 ± 0.02*	41 ± 0.04**	4.77 ± 0.61*

* : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$ in comparison with the control group.

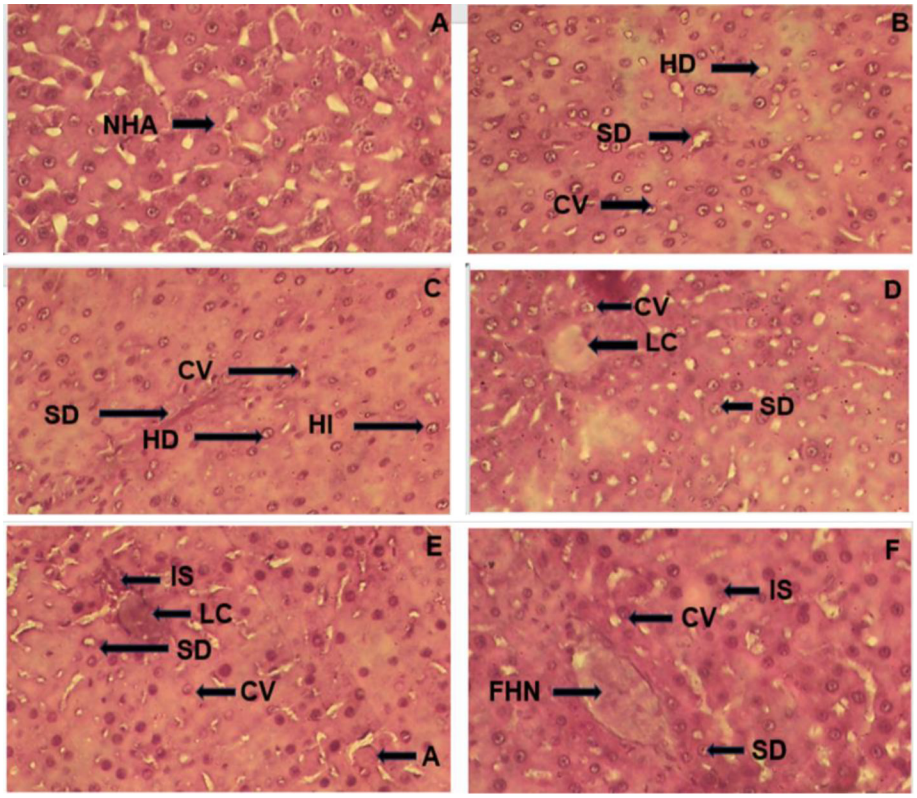


Figure 6. Representative photomicrographs of renal tissue across treatment groups.**A** : Control ; **B** : Sildenafil 5 mg/kg ; **C** : AT100 mg/kg ; **D** : CM100 mg/kg ; **E** : AT200 mg/kg ; **F** : CM200 mg/kg stained with hematoxylin and eosin (H&E). Observed histopathological features include : NHA : Normal Hepatic Architecture ; HD : Hepatocellular Degeneration ; SD : Sinusoidal Dilatation ; CV : Congested Vessels ; LC : Lipidic Cytoplasmic Vacuolization ; IS : Inflammatory Sites ; FHN : Focal Hepatocellular Necrosis ; HI : Hepatic Infiltrates. Images were captured at ×400 magnification.

arranged radially and sinusoids intact, confirming a Normal Hepatic Architecture (NHA) and the absence of pathological changes.

In the group treated with sildenafil at 5 mg/kg (B), the liver tissue showed mild Hepatocellular Degeneration (HD), Sinusoidal Dilatation (SD), and Vascular Congestion (CV). The AT 100 mg/kg group (C) exhibited more pronounced histopathological changes, including HD, SD, CV, and the presence of Hepatic Infiltrates (HI). In the CM 100 mg/kg group (D), histological sections revealed lipidic cytoplasmic vacuolization (LC). These lesions reflect disturbances in lipid metabolism and hepatic perfusion. The liver architecture in the AT 200 mg/kg group (E) displayed more severe damage, marked by extensive LC, prominent inflammatory cell infiltration (IS), sinusoidal dilatation, vascular congestion, and focal apoptotic changes (A). These alterations point toward substantial hepatocellular injury involving both inflammatory and degenerative mechanisms. Finally, the CM 200 mg/kg group (F) demonstrated the most severe lesions, characterised by Focal Hepatocellular Necrosis (FHN), pronounced sinusoidal dilatation, dense inflammatory sites, and widespread vascular congestion (Figure 6).

4. Discussion

The findings of this study demonstrate that subchronic oral administration (90 days) of two traditional aphrodisiac formulations adulterated with sildenafil (AT and CM) induces hepatic dysfunction in Wistar rats, evidenced at biochemical, enzymatic, oxidative, and histological levels. These results raise concerns about the potential risks associated with the unregulated use of consumption of herbal aphrodisiacs from informal markets.

Significant elevations in serum ALT and AST were recorded in animals exposed to the highest doses of AT and CM. These enzymes are well-established indicators

of hepatocellular injury, released into the bloodstream following membrane disruption and cytolysis of hepatocytes. Such alterations have also been described in rats exposed to high doses of sildenafil or phytochemical-rich preparations with hepatic bioactivation potential[1,10]. The concurrent increase in total bilirubin further reflects impairment of hepatic conjugation or bile excretion, consistent with hepatobiliary dysfunction[19,20]. The marked reduction in serum albumin and total proteins in treated groups suggests a decline in the synthetic function of the liver, a hallmark of chronic hepatotoxicity[21]. Hypoalbuminemia can result from compromised protein synthesis or oxidative damage to endoplasmic reticulum structures responsible for protein folding. These disturbances were accompanied by hyperglycemia, particularly in the CM200 group, suggesting interference with hepatic carbohydrate metabolism. Hepatic oxidative stress and mitochondrial dysfunction are known to disrupt insulin signalling and glucose regulation, promoting metabolic dysregulation[6,22,23]. In liver homogenates, the divergent enzymatic response between ALP and γ-GGT indicates a mixed pattern of hepatotoxicity. Elevated ALP activity reflects canalicular membrane perturbation or cholestatic stress, whereas reduced GGT activity suggests enzyme depletion under sustained oxidative conditions[10]. Such patterns are consistent with the hypothesis of dual hepatocellular and cholestatic injury induced by chronic exposure to xenobiotic mixtures[22]. The oxidative stress profile provides mechanistic insight into the observed biochemical disturbances. The significant rise in MDA indicates enhanced lipid peroxidation, while the depletion of SOD, CAT, GPx, and GSH points to impaired antioxidant defences. This imbalance confirms a shift in the hepatic redox state, where Reactive Oxygen Species (ROS) generation exceeds the capacity of enzymatic and non-enzymatic detoxification systems[23]. Similar redox disturbances have been reported in rats treated with adulterated herbal formulations or isolated PDE-5 inhibitors, implicating oxidative stress as a central mechanism of liver injury[7,24]. The herbal formulations investigated in this study, although marketed as "natural," are complex polyherbal decoctions that likely contain bioactive compounds such as alkaloids, tannins, and polyphenols, known to modulate xenobiotic metabolism. While such phytoconstituents may exhibit intrinsic therapeutic properties, their interaction with sildenafil, a phosphodiesterase type 5 inhibitor (PDE5i) metabolised primarily by cytochrome P450 isoenzymes, can potentiate oxidative stress, enzyme induction, or metabolic inhibition. This could explain the more pronounced hepatocellular damage observed with the CM formulation, which may contain phytochemicals acting as Cytochrome P450 (CYP) inhibitors or redox modulators[23].

Histopathological analysis supported the biochemical and oxidative data. Liver sections from treated animals revealed cytoplasmic vacuolization, sinusoidal congestion, inflammatory infiltration, and lobular disorganisation, particularly at higher doses of CM. These alterations reflect progressive hepatocellular degeneration and inflammatory response, typical of oxidative and drug-induced liver injury. The more pronounced lesions in CM-treated rats may suggest synergistic interactions between sildenafil and certain phytochemicals, leading to inhibition of cytochrome P450 enzymes, accumulation of reactive metabolites, and mitochondrial damage[25,26]. Overall, these results corroborate previous analytical and toxicological studies demonstrating the presence and adverse effects of undeclared PDE-5 inhibitors in herbal aphrodisiacs marketed across Africa. Chronic ingestion of such adulterated preparations exposes consumers to cumulative hepatotoxic risks, which may progress to irreversible hepatic injury. The lack of regulatory control, absence of quality assurance, and misconception that "natural" implies safety remain key contributors to this emerging public health concern[3,5].

Lastly, these findings underscore the hepatotoxic potential of so-called natural aphrodisiacs adulterated with sildenafil. Several recent market analyses have identified undeclared PDE-5 inhibitors in such products, sometimes at concentrations exceeding therapeutic recommendations[4,27,28]. The absence of quality control and regulatory oversight exposes consumers to serious adverse effects, especially under long-term use. Taken together, the present findings provide experimental evidence that chronic exposure to sildenafil-adulterated herbal aphrodisiacs induces dose-dependent hepatotoxicity mediated primarily through oxidative stress and hepatic metabolic disruption. These data call for urgent pharmacovigilance, analytical monitoring, and public awareness to regulate and discourage the uncontrolled use of unverified aphrodisiac products in developing countries.

5. Conclusion

This study provides compelling experimental evidence that chronic oral exposure to traditional aphrodisiac

formulations adulterated with sildenafil (AT and CM) induces dose-dependent hepatotoxicity in male Wistar rats. The biochemical alterations, including elevated serum transaminases, disrupted protein synthesis, and hyperglycemia, along with oxidative stress and histopathological lesions, collectively confirm hepatocellular degeneration and oxidative imbalance as the primary mechanisms of toxicity. The hepatotoxic effects were more pronounced with the CM formulation, suggesting either a higher sildenafil content or synergistic phytochemical interactions that may potentiate oxidative damage and impair hepatic detoxification pathways. These findings emphasise the urgent need for rigorous quality control, analytical surveillance, and strict regulatory oversight of aphrodisiac products, especially those marketed as "natural" but containing undeclared synthetic pharmaceuticals. From a public health perspective, the results highlight the hidden toxicological risks associated with long-term consumption of such adulterated products and call for enhanced pharmacovigilance, public awareness, and national regulation to govern their sale and ensure consumer safety.

In conclusion, the present work provides scientific evidence that uncontrolled use of sildenafil-adulterated herbal aphrodisiacs can result in severe hepatic injury, underscoring the necessity of integrating analytical monitoring with toxicovigilance strategies to prevent health hazards linked to unregulated traditional medicine markets.

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7. Data Availability Statement

Data are available from the corresponding author on reasonable request.

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9. Conflict of Interest

The authors declare no conflict of interest related to this study.

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Methodology, Experimental investigation, Data curation. Elisabeth OUEDRAOGO : Biochemical analysis, Validation. T. Jean Luc KABORE : Statistical analysis, Data interpretation, Writing, review and editing. Abdel Aziz KOUSSOUBE : Histological analysis, Visualisation, Method validation. Dramane SANA : Reagents preparation, Technical support, Data acquisition. Souleymane BENGYENDE : Laboratory assistance, Quality control, Resources management. Balé BAYALA : Project administration, Conceptualisation, Supervision, Funding acquisition, Writing - final review and approval.

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