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Antioxidant activity and effect of selected herbal remedies on liver and kidney functions of wistar rats

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Abstract
Using industry-standard laboratory techniques for analyzing antioxidant activity and biochemical estimates, the antioxidant and biochemical effects of a few herbal treatments offered in Owerri on Wistar rats in Imo State, Nigeria, were examined. The results showed that all samples A to D contained antioxidants and that the level of inhibition was corresponding. As the number of antioxidants in the herbal medicines (ug/ml) increased, the level of inhibition also increased. To check for toxicity, the animals' ALP, ALT, AST, total bilirubin, and direct bilirubin levels were measured. The four herbal treatments did not harm the rats' livers in any way, according to the results of AST and ALT tests. However, there was a rise in ALP and total bilirubin at doses of 12. 4 mg/kg and 16.32 mg/kg, respectively with the oversight teams. The study's large and little rises in ALP and total bilirubin levels are a clue that the four herbal treatments may have harmed the liver's ability to eliminate bilirubin. When treated with a high dose of sample D of the herbal treatment, the high value of ALP (173.98 82.00) is a clear sign of liver damage. Furthermore, the rat may be experiencing dehydration, hyperventilation, congestive heart valve, or prostatic or other types of urinary blockage due to the high levels of chloride seen in samples A (34.741.94 mEq/L), B (34.865.98 mEq/L and 35.435.17 mEq/L), and D (34.913.71 mEq/L). As a result, it is crucial to raise public knowledge about the security and impending health danger related to the use of herbal products.

Keywords: Antioxidants, herbal remedies, wistar rats, liver, kidney

Introduction
Over the years, the usage of herbs in traditional medicine (TM) has increased and changed (WHO, 2009; Saganuwan 2010; Petrovska, 2012) [31, 17]. According to Inamdar, Edalat, Kotwal, and Pawar (2008) [3], between 70 and 85% of the world's population, primarily in impoverished nations, still rely on herbs as their major form of healthcare today. Because they are readily available, inexpensive, and easy to access, herbs are currently used extensively in both developed and developing countries for healthcare. The use of herbs as medicine has grown mostly because they are more likely to be safe than current medications that contain synthetic ingredients because they come from natural sources (Inamdar, *et al.*, 2008) [3]. Phytochemicals (secondary metabolites) are produced inertly by plants and utilized in a number of chemical processes. The presence of these chemicals, which are also known as "active ingredients" or "bioactive substances," which are known to have therapeutic advantages, is influenced by a variety of elements, including the type of soil, the plant species, the time and season of harvest, the preparation and storage methods, etc (Schreck & Foucault, 2012) [18]. Antioxidants are substances that prevent oxidation, a chemical process that can result in free radicals and cascade events that could harm an organism's cells. When our body's antioxidant defenses are outnumbered by free radicals, we are said to be under oxidative stress. Plants and mammals retain intricate networks of overlapping antioxidants to manage oxidative stress, including glutathione, thiols, and ascorbic acid (vitamin C), which may operate to block. There is currently no suggested daily "total antioxidant" consumption due to the diversity and complexity of antioxidants, despite the fact that they are acknowledged as vital phytonutrients (Kaliora, Dedoussis, & Schmidt, 2006) [6]. Antioxidants, according to protect blood vessel membranes from damage, improve blood flow to the heart and brain, guard against DNA damage that can lead to cancer, and reduce the risk of developing cardiovascular and Alzheimer's diseases. Antioxidants can also stop or delay the oxidative damage connected to a number of disorders, including carcinogenesis, thermogenesis, and aging, according to Jo, Nam, Min, Ahn,... & Lee (2006) [5].

Materials and Method

Procurement of herbal remedies

In Owerri metropolitan (Owerri North, Owerri West, and Owerri Municipal LGA), Imo State, Nigeria, samples for this study were collected in open marketplaces.

Thirty (30) samples of each of the ready-to-use herbal remedies were obtained randomly from selected sale outlets, using standard methods as described. Samples collected included; Sample A (used for malaria), Sample B (used for typhoid), Sample C (used for *Candidiasis*), and Sample D (used for sex enhancement) herbal remedies.

Antioxidant Activity of Samples

Free radical (DPPH) scavenging test

This test was performed in accordance with Erhirhie *et al.* instructions (2020) [2]. Methanol was used to create a new 0.6 mM DPPH (1, 1-diphenyl-2-picrylhydrazil) solution. The reaction mixtures contain 0.25 ml of samples that have been diluted by a number of parameters. Ascorbic acid concentrations of 7.82, 15.63, 31.25, 62.5, 125, 250, 500, and 1000 g/ml were used. Samples/ascorbic acid were combined with 0.25 ml of 0.6 mMol DPPH and 2 ml of methanol, and they were then allowed to incubate for 30 minutes at room temperature and in the dark. After that, the samples' 517 nm absorbance was measured using a spectrophotometer. Ascorbic acid is used as a benchmark. A tube with 2.25 ml of methanol and 0.25 ml of DPPH solution was used as a control. Three assays were conducted. Looking for free radicals in the trash.

DPPH scavenging activity=100 x (AC–AS)/AC Eq.3.1

Absorption of control
AS = Sample Absorbance

For ascorbic acid, a graph of percentage inhibition vs concentration was created, and an equation was used to infer the concentration that induced 50% inhibition (IC50). High DPPH radical scavenging activity is shown by low optical density (absorbance) readings.

Biochemical Estimations (Electrolytes/Renal Parameters)

Determination of serum chloride

Serum chloride determination was based on the modified colorimetric method of Skeggs & Hochestrasser (1964) [23].

1500 ml of the chloride reagent were added to test tubes with the labels "blank," "samples," and "standard," respectively. Following this, 10µl of the samples and the standard chloride reagent were added to the sample and standard tubes, respectively. A spectrophotometer was used to measure the absorbance of the combinations against a reagent blank at 480 nm after five minutes of room temperature incubation. The chloride concentration was calculated according to Eq. 3.2.

Calculation

The concentration of chloride in the sample

$$(\text{mEq/L}) = \frac{Abs.of\ sample}{Abs.of\ standard} \text{ x Conc of standard} \quad \text{Eq.3.2}$$

Determination of serum sodium

The determination of serum sodium was done using a modified version of method. One thousand milliliters of sodium filtrate reagent were pipetted into test tubes marked "blank," "standard," and "samples." Following that, 50 µl of the samples and the standard chloride reagent were added to the appropriate tubes and continuously stirred for three minutes. For ten minutes, tubes were centrifuged at 1500 rpm. Then, in a different set of test tubes, 50 ml of the supernatant from the blank, samples, and standards were combined with 1000 ml of sodium acid reagent. Then, 50 µl of color developer reagent was added to the finished combination, and all tubes' absorbances were measured at 550 nm against a water blank. The sodium concentration was calculated according to Eq. 3.6.

Sodium concentration in the sample

$$(\text{mEq/L}) = \frac{Abs.of\ Blank - Abs.of\ Sample}{Abs.of\ Blank - Abs.of\ Standard} \text{ x Conc. Of Standard} \quad \text{Eq. 3.6}$$

Calculation

Sodium concentration in sample = $\frac{Abs.of\ Blank - Abs.of\ Sample}{Abs.of\ Blank - Abs.of\ Standard}$ x Conc. of Standard (mEq/L).

Determination of serum potassium

The method of Terri & Sesin (1958) [25] was used for the determination of serum potassium.

One thousand milliliters of potassium reagent were added to test tubes marked "blank," "samples," and "standard." The following step involved adding and combining 10 µl of the samples with the standard potassium reagent in each test tube. For three minutes, the mixes were incubated at room temperature. Then, using a spectroscope, the absorbance of the samples and the standard were determined against a reagent blank at 500 nm. The potassium concentration was calculated according to Eq. 3.7.

Calculation

Potassium Conc.

$$(\text{mEq/L}) = \frac{Abs.of\ sample}{Abs.of\ standard} \text{ x Concentration of standard (mEq/L)} \quad \text{Eq.3.7}$$

Determination of creatinine

The serum creatinine was measured using the buffered kinetic Jaffe reaction without deproteinization.

A cuvette containing a 50 µl sample and 1000 µl of creatinine reagent was combined for 30 seconds. The first absorbance (A1) was measured at 492 nm at 30 seconds, while the second absorbance (A2) was measured at 492 nm at exactly 2 minutes (Bowers & Wong, 1980) [26].

Calculation

$$\text{Creatinine concentration (mg/dL) in sample} = \frac{(A2-A1)\ of\ sample}{(A2-A1)\ of\ standard} \text{ x } 2 \quad \text{Eq. 3.8}$$

Determination of serum urea

It was done with Weather burn's technique (1967). 50 µl reagent 1 was added, mixed, and incubated at 37 0C for 10 minutes with 5 µl samples, the standard, and distilled water. After that, 1.25 ml of each of the remaining two reagents were added, and the mixture was incubated at 37 °C for an additional 15 minutes. At 546 nm, the absorbance values of the samples (A sample) and standards (A standard) were calculated. The sample's urea concentration was calculated according to Eq. 3.9.

Calculation

Urea concentration (mg/dl) = $\frac{A\ sample}{A\ standard}$ x Concentration of standard (mg/dl) Eq. 3.9

Determination of serum total protein

The total protein was calculated using the Tietz (1995) [28] method. 1 ml of total protein reagent was added to 20 µl the sample, stirred, and then left to sit at room temperature for 30 minutes. At 546 nm, sample absorbance was measured against a reagent blank.

The amount of protein in an unidentified sample was determined as follows:

$\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard}$ × Concentration of standard Eq.3.10

Determination of serum albumin

The estimation of serum albumin was performed using the bromcresol green method proposed by Doumas *et al.* in 1971. The working solution (1500 µl) was divided among test tubes marked "blank," "samples," and "standard." Following that, 5 µl of each test tube's standard albumin reagent and sample were combined. The mixtures were incubated at room temperature for five minutes and then using a spectrophotometer, the absorbance of the samples and the standard was measured against a reagent blank at 630 nm.

The following formula was used to determine the amount of albumin (mg/dl) in the sample;

$\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard}$ × Concentration of standard Eq. 3.11

B. Determination of Liver Enzymes

Determination of alkaline phosphatase

The serum alkaline phosphatase was measured using the phosphate thymolpthalein monophosphate technique developed by Roy in 1970.

Alkaline phosphatase substrate reagent in quantities of 250 microliters (µl) was added to test tubes marked "blank," "samples," and "standard." The test tubes were then filled with 25 µl of the samples and the alkaline phosphatase standard reagent, respectively. They were combined and incubated at 37 °C for 10 minutes. Following this, 1250µl of alkaline phosphatase color developer was added to each tube, and the absorbances of the standard and sample tubes were then measured at 590 nm against reagent blanks.

Calculation

Alkaline phosphatase Conc. (u/l)= $\frac{Abs.of\ sample}{Abs.of\ standard}$ x Conc. of standard (mg/dl) Eq.3.12

Determination of serum alanine aminotransferase (ALT)

Reitman and Frankel's approach was used to measure serum ALT (1957).

Samples and blank test tubes have labels. The 20 µl of the sample was then added to the sample tubes. Following that, 100 µl of ALT reagent 1 was added to the test tubes containing the samples and the blanks. The test tubes were then mixed and incubated for 30 min at 37 0C. All tubes received 100 µl of ALT reagent 2 after 30 minutes of incubation, and the mixtures were then incubated for 20 minutes at room temperature. After adding 1000 µl of 0.4 mol/ L sodium hydroxide solution to the mixture, the samples' absorbance was measured at 546 nm after five minutes in comparison to a reagent blank. The ALT calibration curve was used to determine the ALT activity.

Determination of serum aspartate aminotransferase (AST)

Using Reitman & Frankel's approach, serum AST was measured (1957).

Samples and blank test tubes have labels. 20 µl of serum was added to the sample test tube. After that, test tubes containing blanks and samples received 100 µl of AST reagent 1, and all test tubes were incubated for 30 minutes at 37 0C. 100 µl of AST reagent 2 was added after 30 minutes of incubation, and the mixture was then incubated for 20 minutes at room temperature. After adding 1000 µl of a 0.4 mol/L sodium hydroxide solution to the mixture, the samples' absorbance was measured at 546 nm after five minutes against a reagent blank. The AST calibration curve was used to determine the AST activity

Bilirubin

This was done using a colorimetric technique based on Jendrassik & Grof's description (1938).

Total bilirubin

Sample and Sample Blank were the labels on the test tubes. In both the sample tube and the sample blank tube, 100 microliters (µl) of the sample we re-introduced. Then, 100 µl of reagent 1, 25µl of reagent 2, and 500 µl of reagent 3 were added to sample test tubes whereas blank test tubes for the sample received 500 µl of reagent 3 and 100 µl of reagent. At room temperature, each test tube was incubated for 10 minutes. Following that, 500µl of reagent 4 was added to the test and control test tubes, and they were then incubated at room temperature for an additional 30 minutes. At 578 nm, the sample's absorbance was measured against a blank sample.

Calculation

Total bilirubin (mg/d) = 10^8 x Absorbance against reagent blank at 578 nm.

Direct bilirubin

Sample and Sample Blank were the labels on the test tubes. In both the sample tube and the sample blank tube, 100 microliters (µl) of the sample were introduced. Then, 1000 ml of normal saline, 100 µl of reagent 1, 25 µl of reagent 2, and 1000 ml of reagent 1 were put into sample blank test tubes, while 1000 ml of normal saline, 100 µl of reagent 1, and 25 µl of reagent 2 were added to sample test tubes. At room temperature, each test tube was incubated for 10 minutes. At 546 nm, the sample's absorbance was measured against a blank sample.

Calculation: Direct bilirubin (mg/d) = 14^4 x Absorbance against reagent blank at 546 nm.

Result and Discussions

The antioxidant properties of these herbal remedies

The level of antioxidants in samples A to D and the related level of inhibition is shown in Figures 4.1 to 4.4. In general, the better the inhibition, the higher the concentration of antioxidants in the sample.

Sample A

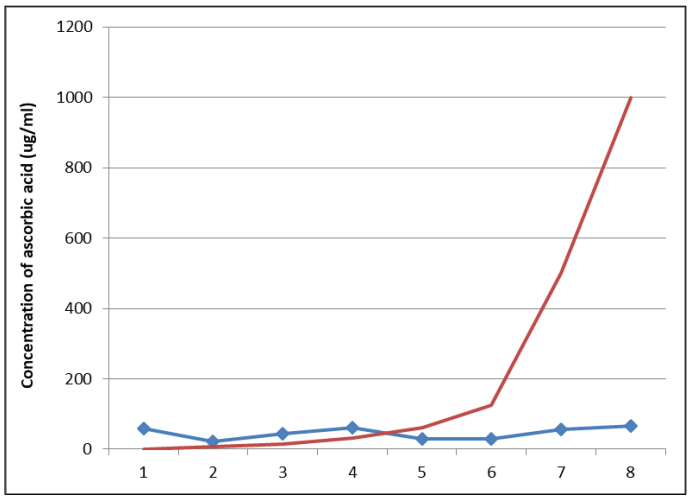

Fig 1: Concentration of antioxidants in sample A

Sample B

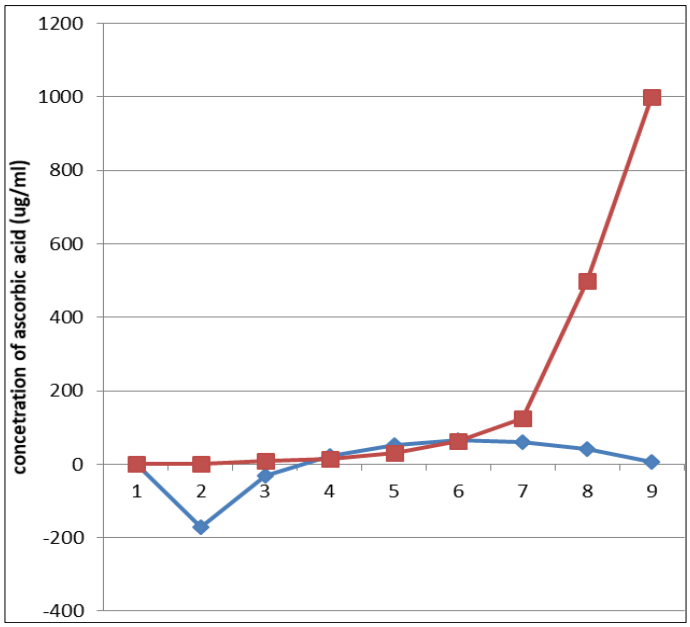

Fig 2: Concentration of antioxidants in sample B

Sample C

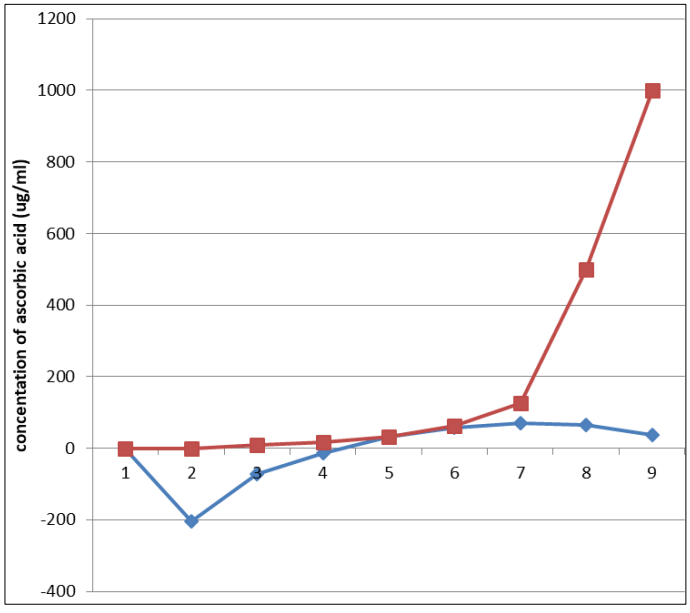

Fig 3: Concentration of antioxidants in sample C

Sample D

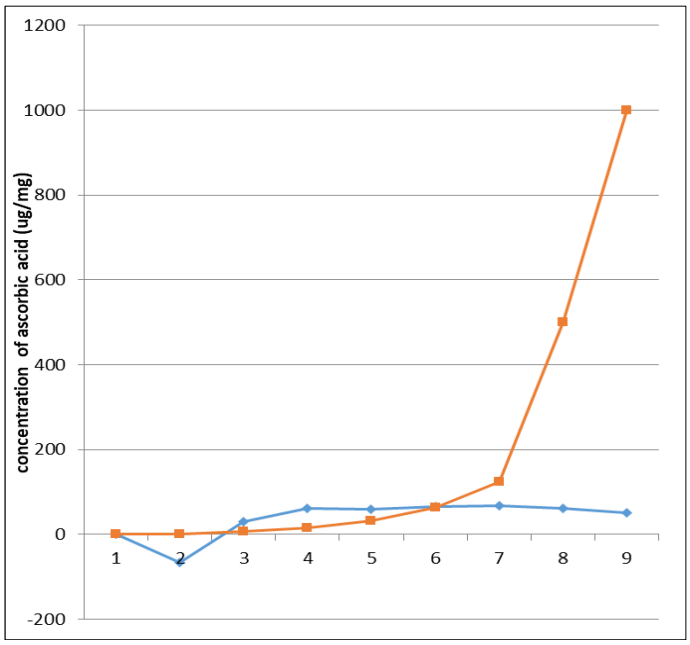

Fig 4: Concentration of antioxidants in sample D

The hepatotoxic effect of the herbal remedies on Wistar rats: The impact of the herbal treatments on the examined liver enzymes is shown in Table 1. The outcome demonstrates that there was no significant difference (p>0.05) between the groups of herbal remedy treatments for Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Direct Bilirubin when compared to Control. However, compared to the control group (104.97 12.13 IU/L), the high dose of sample D showed a significantly higher level of alkaline phosphatase (ALP) at 173.98 82.00 IU/L. When compared to the control, the ALP levels of the other groups were not statistically different. Additionally, compared to the control group's level (0.05 0.03 mg/dl), the high dose of sample B had a substantially higher level of total bilirubin (0.09 0.02 mg/dl). Other groups' total bilirubin levels did not differ significantly from the control group. The impact on albumin and total protein is seen in Table 2.

The effects of the investigated herbal treatments on sodium, potassium, urea, creatinine, and chloride are shown in Table 3. Although all herbal remedy groups had higher urea levels than the control group, these high values were not statistically different (p>0.05) from the control group. When compared to the control group, the levels of creatinine, salt, and potassium in the herbal treatment were not significantly different. These herbal treatments had a substantially greater impact on chloride levels than the control group (24.553.92 mEq/L) in samples A (high dose), B (low and high doses), and D (high dose). Despite the fact that the chloride levels in the other group were greater than those in the control group, they were not statistically significant.

Table 1: Effect of herbal remedies on liver enzymes, total bilirubin and direct bilirubin of Wistar rats

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
Control	9.64 ± 1.65	36.23 ± 4.17	104.97 ± 12.13	0.05 ± 0.03	0.04 ± 0.02
Sample A, LD	11.28±2.06^{ns}	37.19±7.03 ^{ns}	93.72±24.75 ^{ns}	0.09± 0.03^{ns}	0.05±0.02 ^{ns}
Sample A, HD	10.80±2.59 ^{ns}	35.53±7.68 ^{ns}	100.22±24.97^{ns}	0.07±0.02 ^{ns}	0.03± 0.01 ^{ns}
Sample B, LD	10.56± 4.24 ^{ns}	29.28±5.20 ^{ns}	127.19±4.83 ^{ns}	0.06± 0.03 ^{ns}	0.02±0.02 ^{ns}
Sample B, HD	12.16± 2.87 ^{ns}	32.29±3.64 ^{ns}	111.48±27.75^{ns}	0.09± 0.02*	0.03±0.01 ^{ns}
Sample C, LD	11.48±5.87 ^{ns}	39.46±8.92 ^{ns}	112.50±15.67 ^{ns}	0.07± 0.02 ^{ns}	0.02±0.01 ^{ns}
Sample C, HD	8.61 ± 1.61 ^{ns}	26.46±8.55 ^{ns}	110.53±26.95 ^{ns}	0.04±0.02 ^{ns}	0.05± 0.02 ^{ns}
Sample D, LD	8.61 ± 2.97 ^{ns}	31.57±11.33^{ns}	97.95±16.02 ^{ns}	0.03±0.01 ^{ns}	0.03±0.02 ^{ns}
Sample D, HD	8.74 ± 5.93 ^{ns}	34.44±9.38 ^{ns}	173.98±82.00*	0.03±0.03^{ns}	0.02± 0.01^{ns}

Values are presented as mean ± Standard deviation. LD (Low dose), HD (High dose).

Table 2: Effect of herbal remedies on albumin and total protein of Wistar rats

Group	Albumin (g/dl)	Total protein (g/dl)
Control	2.39±0.85	6.55±0.26
Sample A, LD	3.19±0.39^{ns}	6.14±0.65 ^{ns}
Sample A, HD	2.84 ±0.74 ^{ns}	6.66±0.51 ^{ns}
Sample B, LD	3.05± 0.51^{ns}	6.38±0.60 ^{ns}
Sample B, HD	3.66± 0.62*	6.99±0.79 ^{ns}
Sample C, LD	3.44±0.52 ^{ns}	6.467±0.56 ^{ns}
Sample C, HD	3.21 ± 0.37 ^{ns}	6.84±0.56 ^{ns}
Sample D, LD	3.38 ± 2.97 ^{ns}	6.50±0.65^{ns}
Sample D, HD	2.77 ± 0.44 ^{ns}	5.55±0.38 ^{ns}

Values are presented as mean ± Standard deviation. LD (Low dose), HD (High dose).

Table 3: Effect of herbal remedies on urea, creatine, and electrolytes of Wistar rats

Group	Urea (mg/dl)	Creatinine (mg/dl)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)
Control	16.99±10.34	1.45 ± 0.14	137.93± 8.54	8.39 ± 3.37	24.55± 3.92
Sample A, LD	22.65±12.23^{ns}	2.02±0.46 ^{ns}	135.57±8.39^{ns}	7.10±0.37^{ns}	29.84±4.91^{ns}
Sample A, HD	26.90 ±7.12^{ns}	1.63±0.26^{ns}	139.89±5.46^{ns}	8.83±3.03^{ns}	34.74±1.94*
Sample B, LD	26.42 ± 6.54^{ns}	1.70±0.74 ^{ns}	138.91±2.47 ^{ns}	9.24±1.55^{ns}	35.43±5.17*
Sample B, HD	24.07±2.83 ^{ns}	1.84±0.19 ^{ns}	140.79±4.58 ^{ns}	7.18±2.68 ^{ns}	34.86±5.98*
Sample C, LD	28.31± 4.62 ^{ns}	1.56±0.72 ^{ns}	139.40±5.52 ^{ns}	10.01±0.59^{ns}	28.44±1.95 ^{ns}
Sample C, HD	26.05± 3.10 ^{ns}	2.47±1.86 ^{ns}	140.54±5.62 ^{ns}	8.83± 1.87 ^{ns}	31.94±7.03^{ns}
Sample D, LD	20.39 ±3.10 ^{ns}	1.50±0.48 ^{ns}	136.37±5.13 ^{ns}	9.89±2.65^{ns}	32.16±5.98^{ns}
Sample D, HD	24.91 ±5.06 ^{ns}	1.44±1.36 ^{ns}	140.54±10.62^{ns}	7.67±2.76^{ns}	34.91±3.71*

Values are presented as mean ± Standard deviation. LD (Low dose), HD (High dose).

Discussion

Effects of herbal remedy on liver enzymes and kidney analysis

The use of herbal remedies as a complement to conventional medicine has increased, resulting in higher demand (Mythilypriya, Shanthi, & Sachdanandam, 2007) [11]. In rural areas of poor nations, the sole use of herbal medications to treat a variety of disorders is still highly popular. These medications are most frequently made and administered by herbalists who lack official training. The efficacy and safety of herbal products must therefore be determined by experimental screening methods, which are currently done on animals (Sushruta, Satyanarayana, Srinivas, & Sekhar, 2006) [22]. The liver is crucial to metabolism, especially when it comes to detoxification and getting rid of foreign toxins. Through hepatocellular and extracellular mechanisms, herbal preparations or synthetic drugs can harm the liver, and these pathways may include bile duct injury. Despite the fact that this enzyme is also found in the kidney and bone, ALP levels that are above normal are mostly linked to blocked bile ducts. ALP is excreted by the liver through bile. When liver function is impaired, the hepatocytes' ability to excrete bile decreases, which causes the blood ALP levels to rise (Rajesh & Latha, 2004) [16]. However, when compared to the control groups, an increase was seen at dosages of 12.4mg/kg ALP and 16.32mg/kg total bilirubin. The four herbal remedies did not inhibit the liver's ability to eliminate bilirubin, as seen by the considerable and slight increase in ALP and total bilirubin levels seen in this study. Alkaline phosphatase levels in the serum are elevated during liver disease.

Due to their capacity for filtration, secretion, and reabsorption, the kidneys are more likely to be exposed to harmful compounds than other organs (Boroushaki *et al*., 2014) [33]. As a result, the kidneys play a crucial role in the removal of toxic substances. An essential stage in ensuring the security of treatment is the toxicological study of a compound in animal models to ascertain the possible risk to human life. Numerous processes are carried out by the kidneys in animals, including homeostasis, acid-base balance, control of blood electrolyte balance, elimination of metabolic waste products, the release of certain hormones and enzymes, metabolism, and osmoregulation. Any modification or anomaly involving the kidneys may result in underperformance.

Chloride is crucial for preserving the cation/anion equilibrium in intracellular and extracellular fluids. To maintain appropriate hydration, osmotic pressure, and acid/base equilibrium, this electrolyte is crucial. With severe burns, considerable vomiting, intestinal blockage, nephritis, metabolic acidosis, and Addisonian crisis, low serum chloride levels are observed. Dehydration, hyperventilation, congestive heart valve, and prostatic or other types of urinary blockage can all cause elevated serum chloride readings. (Tietz & Saunders, 1976) [19]. This study's high concentrations of chloride in samples A (high dose), B (high and low doses), and D (high dose) suggest that the rats may be experiencing hyperventilation, dehydration, congestive heart valve disease, and prostatic or other types of urinary blockage.

Conclusion

When used in small dosages, herbal medicines were essentially risk-free, non-toxic, and well-tolerated. Free radicals can be neutralized by antioxidants, preventing both acute and chronic illnesses including liver and kidney damage. Higher therapeutic doses and prolonged use, however, may cause hepatobiliary, hepatic, and renal problems.

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