SAR Journal of Medical Biochemistry

Abbreviated Key Title: *SAR J Med Biochem*
Home page: https://sarpublication.com/journal/sarjmb/home
DOI: https://doi.org/10.36346/sarjmb.2024.v05i05.002

OPEN ACCESS

ISSN 2707-7721 (P)
ISSN 2709-6882 (O)

Original Reserach Article

Phytochemical, Growth Performance and Haematological Indices of Aqueous Extract of Aphrodisiac Formulation against Acetaminophen Induced Hepatocyte Injuries in Wistar Rats

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Article History: | Received: 17.09.2024 | Accepted: 22.10.2024 | Published: 24.10.2024 |

Abstract: Zainacin Dadin duniya is known for its antioxidant and liver-protecting properties. This research assesses the phytochemical characteristics and impact of Zainacin Dadin Duniya water extract on growth and hematological factors in Wister rats with acetaminophen-induced liver damage. 60 female and 30 male rats were divided into five groups with each group consisting of 18 rats selected randomly. Group 1 received saline, group 2 received a liver medicine, group 3 received acetaminophen, group 4 received a daily dose of aqueous extract at 500 mg/kg, and group 5 received a daily dose of aqueous extract at 500 mg/kg along with 3 ml/day of acetaminophen, all administered for 28 days. The animals were weighed before and following the experiment. The hematological parameters were determined, including packed Cell Volume (PCV), Red blood Cell (RBC), Haemoglobin (Hb), Mean Cell Volume (MCV), and White Blood Cell (WBC). Findings indicated that the extract contains a considerable quantity of phytochemicals. Findings indicated a notable weight gain ($P<0.05$) in Groups 1, 2, 4, and 5 and weight reduction in Group 3. In Group 3, there was a significant decrease in erythrocyte counts (4.29%), packed cell volume (33.00%), and hemoglobin (6.17%) values, while there was an increase in leukocyte (9.62%) and Mean Cell Volume (54.33%) values compared to the other groups. The presence of acetaminophen in the treatment given to Group 3 rats could lead to a decline in their growth performance and trigger adverse reactions in their blood parameters, potentially impacting their overall development. The segment may act as a strong remedy for acetaminophen-induced hepatocytes by blocking those reactive oxygen species.

Keywords: Acetaminophen, Haematology, Phytochemical, Wister Rats, Zainacin Dadin Duniya.

1.1 INTRODUCTION

The use of herbs in the treatment of erectile dysfunction has been prevalent for centuries, with up to 80% of the global population relying on herbs for primary health care. Despite their natural origins, some herb components have shown potential toxicity. Herbal medicines, containing multiple active ingredients, have various pharmacological effects on the body (Adimoelja, 2000). Sexual dysfunction affects millions of men worldwide, leading many to turn to natural aphrodisiac herbs for enhancement. While synthetic drugs are available, their high cost and adverse effects have led to continued interest in traditional treatments. Scientific data supports the biological activity of some traditional products, necessitating further pharmacological evaluation. Research into the molecular basis of sexual functions is aiding in the identification of effective aphrodisiac substances (Neto, *et al*., 2017). Aphrodisiac herbs, with their ability to alter neurotransmitters and sex hormones, have been used for centuries to improve sexual performance and pleasure (Cui, *et al*., 2018). The widespread use of aphrodisiac herbs without a prescription is a major issue in Nigeria and other countries. Aphrodisiacs are substances that stimulate sexual desire and pleasure, categorized into libido-increasing, pleasure-increasing, and potency-increasing types. In traditional medicine, the use of aphrodisiac herbs to enhance sexual performance is common

Citation: Kabruk Caroline Eziafakaego, Yakubu Ndatsu, Ibrahim Mohammed Ibrahim (2024). Phytochemical, Growth Performance and Haematological Indices of Aqueous Extract of Aphrodisiac Formulation against Acetaminophen Induced Hepatocyte Injuries in Wistar Rats, *SAR J Med Biochem*, 5(5), 50-55.

(Thakur, & Dixit, 2008). The term aphrodisiac, also known as "Maganin Burantashi" in the Hausa language, refers to any substance or product that enhances sexual arousal and performance (Ojewole, & Adewole, 2007)**.** Research on aphrodisiac herbs plays a crucial role in understanding their effects on sexual health and overall well-being (Ojewole, & Adewole, 2007)**.** This study focuses on examining the phytochemicals, growth effects, and blood parameters of the aqueous extract of "Zainacin Dadin Duniya" (aphrodisiac herbal) on Wistar rats.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Sample Collection

The sample (ZainacinDadin duniya) was purchased from a popular “kayan mata” seller in Suleja market, Niger state, Nigeria.

2.1.2 Preparation of Plant Extract

The extraction of the plant sample was done using the cold maceration method as explained by Sankeshwari *et al*., (2018), with distilled water being used as the solvent. Around 100g of the specimen will be measured out into sterile 2000 mL conical flasks. 1000 mL of distilled water was poured into the conical flasks and left at room temperature for 72 hours with continuous shaking using a water bath shaker. 72 hours later, the solution was filtered into fresh beakers through Whatman filter paper. The water-based extract is subsequently dehydrated in a water bath until a constant weight is reached. Distilled water was used to reconstitute the extract for this study, providing doses of 100, 200, and 400 mg/kgbw as needed. The liquid extract is dried in a water bath until a consistent weight is achieved. The dehydrated extract was subsequently stored in a sealed container for additional examination.

2.2 Methods

2.2.1 Quantitative Phytochemical Screening

2.2.2 Total Phenol Determination

The method described by Ibrahim *et al*., (2020) was used to determine the total phenolic content in each of the extracts. By using this approach, 0.01 g of all the extracts was dissolved in 10 mL of distilled water, and 0.5 mL was combined with 2.5 mL of 10% Folin-Ciocalteu's reagent for oxidation before being neutralized by 2 mL of 7.5% sodium carbonate. The reaction mixes were left to incubate at a temperature of 45ºC for 40 minutes. Absorbance was measured at 765 nm using a Shimadzu UV spectrophotometer (UV-1800 series) with double-beam technology. The calibration curve was prepared using standard gallic acid.

2.2.3 Total Flavonoids Determination

The total amount of flavonoids in the extracts was measured using the methodology outlined by Ibrahim *et al*., (2020). To a test tube with 1.5 mL of absolute methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water, 0.5 mL of each extract was added and left at room temperature for 30 minutes. The UV-1800 Shimadzu double-beam spectrophotometer was used to measure the absorbance at 415 nm. A calibration curve was prepared using regular Quercetin.

2.3.0 Animals

2.3.1 Experimental Animals

Around thirty (30) healthy (15 male and 15 female) rats of similar age were purchased from the animals housing of the Department of Biochemistry at the Federal University of Technology, Minna, Nigeria. The animals were kept in sanitary plastic enclosures, situated in adequately ventilated indoor environments. They have unlimited access to rat pellets (Premier Feeds, Ibadan, Nigeria) and tap water.

2.3.2 Acclimatization of Animals

Animals were allowed to adapt for two weeks in the animal facility at the Department of Biochemistry and Biotechnology, Ibrahim Badamasi Babangida University, Lapai, Niger State.

2.3.3 Chemicals and Drugs

Acetaminophen in the form of Emzor paracetamol tablets (500 mg) were bought from a pharmacy in Lapai, Niger State, for use in this research. The stock solution was made by mixing 500mg of paracetamol tablets in 50mL of distilled water, following the specified concentration for Wistar rat dosing based on body weight.

2.3.4 Experimental Design

This study utilized 30 Wistar rats, which were divided randomly into five groups consisting of 6 animals each (Table 1). A feeding canular was used to administer leaf extract, paracetamol, and saline.

Table 1: Experimental design showing the groups of Wistar rats and the treatments they were given

Group	No. of Animals	Treatment
Grp1	6	2mL of normal saline alone
Grp2	6	2mL of silymarin alone
Grp3	6	2mL of saline + 3ml of acetaminophen alone
Grp4	6	500kg/kg/day of aqueous extract alone
Grp5	6	500kg/kg/day of aqueous extract + 3ml/day of acetaminophen

2.3.5 Samples Dosage Administration and Collection

The extracts were orally administered and continued for a total of twenty-eight (28) days. On the 29th day, the rats were weighed in their designated groups, and blood samples were taken for hematological analysis in an EDTA bottle (Ibrahim *et al*., 2022).

2.3.6 IN Vivo Haematological Indices Analysis

The Packed cell volume, Red blood cell, Haemoglobin, Mean corpuscular volume, and White blood cell of the experimental animals were examined with the SYSMEX KX21 automated hematologic analyzer from Sysmex Corporation, Japan (Dacie and Lewis, 2002).

Statistical Analysis of Data

Mean ± SD values were expressed and subsequently analyzed using ANOVA with SPSS version 17.0 by SPSS Inc. located in Chicago, Illinois. Statistical significance was deemed significant at a threshold of $\rho < 0.05$.

3.0 RESULTS

3.1 Phytochemical Analysis

Values are reported as the average ± SEM of six replicates. Differences in values within the column are statistically significant at a level of $p < 0.05$.

The sample contained a large amount of phenols (531.69mg/100g) and a minimal amount of Flavonoids (84.31 mg/100g) phytochemicals.

3.2 Growth Performance

Values are reported as the average ± SEM of six replicates. Differences in values within the column are statistically significant at a level of $p < 0.05$.

3.3 Hematological Indices Analysis

Values are reported as the average ± SEM of six replicates. Differences in values within the column are statistically significant at a level of $p < 0.05$.

Table 2: Displays the quantified phytochemicals present in the aqueous extract

Sample	Phenols (mg/100 g)	Flavonoids (mg/100 g)
Extract	531.69±2.20^{b}	84.31±1.27^{a}

Table 3: Mean weight (g) changes in Wister rats in various treatment groups (G1 – G5)

Group	Body weight (g)		
	Initial	Final	Change in weight
G1	135.33±3.48^{a}	164.783±2.92^{c}	29.453
G2	137.603±3.28^{a}	152.833±3.25^{b}	15.23
G3	136.473±3.15^{a}	119.913±2.24^{a}	-16.563
G4	138.623±3.05^{a}	165.783±3.91^{c}	27.16
G5	138.853±3.77^{a}	154.543±3.40^{b}	15.69

Table 4: Haematological parameters of Wister rats in various treatment groups (G1 – G5)

Group	PCV	RBCs	Hb	MCV	WBC
G1	38.67±0.88^{b}	6.81±0.19^{bc}	8.79±0.33^{b}	48.67±0.88^{a}	7.26±0.21^{ab}
G2	38.33±0.88^{b}	6.29±0.36^{bc}	7.88±0.14^{b}	49.33±0.88^{a}	7.84±0.51^{b}
G3	33.00±0.58^{a}	4.29±0.24^{a}	6.17±0.21^{a}	54.33±0.88^{b}	9.62±0.32^{c}
G4	38.67±0.88^{b}	7.12±0.52^{c}	10.09±0.47^{c}	47.67±0.88^{a}	6.39±0.23^{a}
G5	38.67±0.33^{b}	5.84±0.34^{b}	8.16±0.53^{b}	50.00±1.15^{a}	7.32±0.56^{ab}

Table 3 displays the hematological results of grouped Wister rats subjected to different treatments. The mean values of PCV, RBCs, and Hb decreased significantly ($P<0.05$) in group 3 treatment, whereas MCV and WBC increased compared to the other groups treatments. The PCV in group 3 was significantly ($P<0.05$) lower than in groups 1, 2, 4, and 5, while the RBC count in group 3, significantly ($P<0.05$) lower than in group 5, was not statistically different from RBC counts in groups 1, 2, and 4. The average MCV count of group 3 was significantly greater ($P<0.05$) than that of groups 1, 2, 4, and 5, but there was no statistical difference between groups 1, 2, 4, and 5. There was a significant increase in WBC levels ($P<0.05$) in group 3, whereas a significant increase in Hb levels was observed in group 4. There was no significant difference in WBC levels between groups 4 and 5 treatments, but Hb levels significantly decreased in group 3 compared to groups 1, 2, and 5 treatments, while significantly increasing in group 4 treatment.

DISCUSSION

Table 2 demonstrates that the existence of secondary metabolites in different plant extracts has been and remains the basis of treatment for a variety of conditions such as diabetes, liver diseases, and asthma (Bhandary *et al*., 2012). The most abundant metabolite in the aqueous extract of Zainacin Dadin duniya leaf, phenol and flavonoid, is the active compound responsible for treating chemically induced hepatitis (Shin *et al*., 2005). The protective properties of the extract may be linked to the antioxidant effects, anti-

blood clotting, and prevention of cell growth in the blood vessel walls of the polyphenols. According to Thamizh *et al*., (2015), phenol group compounds and flavonoids have been found to have hepatoprotective effects. Therefore, the presence of these secondary metabolites in the extract could play a role in the liver-protective effects observed in this study.

Table 3 indicates a notable rise ($p<0.05$) in the body weight of rats in most groups, except group G3, which decreased. The study summarized the overall effects of different treatments on various groups by presenting their weight changes in Table 3. The noteworthy decrease in weight seen in Wister rats treated with paracetamol (G3) could be due to the adverse biochemical impact caused by the induced oxidative stress, whereas the noticeable weight variations in the other treatment groups show the overall impact of paracetamol on oxidative stress.

Table 4 haematological indices reflect how treatments affect animals based on the quality of feed and nutrients they consume to meet their physiological needs. In this study, lower values of RBC, PCV, and Hb were observed in group 3, which were correlated with the concentration of paracetamol given to the animals, leading to the lowest values for those parameters in the animals fed with paracetamol. The average RBC count in group 3 of Wister rats was lower compared to the other treatment groups, but all groups remained within the normal ranges of 4.35-5.65 million cells/mcL for males and 3.92-5.13 million cells/mcL for females, as stated by Mitruka and Rawnsley (1977) for young adult Wister rats. A Red Blood Cell Count (RBC) is a medical examination to determine the quantity of Red Blood Cells (RBC) in an organism (Wikipedia, 2013b). Almost always, the CBC includes the RBC count. The examination can assist in identifying anemia and other red blood cell-related conditions (Gernsten, 2009; Bunn, 2011). Red blood cell indices are laboratory tests that offer details on the hemoglobin amount and dimensions of red blood cells. Unusual results show the existence of anemia and specify which type of anemia it is (Gernsten, 2009). They are utilized to assist in determining the reason behind anemia, a condition characterized by a decreased number of red blood cells. Anaemia is characterized by the size of red blood cells (MCV) and the level of hemoglobin (MCH). Haemoglobin is carried by red blood cells to transport oxygen. Awodi *et al*., (2005) and Chineke *et al*., (2006) state that the main role of erythrocytes is to act as carriers of heamoglobin. The levels of hemoglobin, a protein containing iron that transports oxygen and carbon dioxide in the body, were notably lower in rats treated in group 3 than in the other groups, indicating a decrease in respiratory function in the rats in group 3. The findings also indicated that rats given paracetamol in group 3 treatment experienced a notable impact on the production and levels of red blood cells (erythrocytes). The Packed Cell Volume (PCV) represents the percentage of red blood cells in the blood (Purves *et al*., 2004) or the percentage of blood volume taken up by red blood cells (Wikihow, 2013). The origin of the term hematocrit can be traced back to the Greek words Hema, which translates to 'blood', and criterion. In 1891, Magnus Blix introduced the term hematocrit at Uppsala (Hedin, 1891; Raser, 1981). Animals' anemia status can be identified by determining their Packed Cell Volume (PCV). The typical heamatocrit value of a Wistar rat falls within the range of 37.48%. The animals in group 3 showed a 33% decrease in PCV, indicating that they were anemic. White Blood Cell Count is a procedure that calculates the quantity of white blood cells. White Blood Cells (WBC) or leucocytes are cells of the immune system that protect the body from infections, disease, and foreign substances (WebMD, 2012; Medline Plus, 2012; Wikipedia, 2013h). The grouped animals (grp 1-5) had circulating WBC counts within the normal physiological range of 5.20 - 10.6×10^9/L for leukocyte counts of young adult Wistar rats as reported by Mitruka and Rawnsley (1977). The findings show that the animals placed in groups with varying treatments did not show any effects from leukocytosis. As stated by Coles (1986), intoxications such as metabolic disorders can lead to leukocytosis. There are five distinct and diverse types of leucocytes, as stated by Lafleur-Brooks in 2008, all originating from a multi-potent cell in the bone marrow called hematopoietic stem cell. White blood cells can be located throughout the body, in both the bloodstream and the lymphatic system (Maton *et al*., 1997). The amount of white blood cells in the bloodstream is frequently a sign of illness. Typically, there are around 7000 white blood cells in every microliter of blood. In a healthy adult animal, they account for around 1% of the total volume of blood (Alberts, 2005). Leucocytosis is when there is a higher number of leucocytes than the upper limit, while leucopenia is when there is a lower number than the lower limit. An elevated white blood cell count may indicate various issues like infection, stress, inflammation, trauma, allergy, or specific diseases; therefore, further investigation is needed for a high number of white blood cells. Valencia (2012) and Braun (2013) found that an elevated white blood cell count may result from infection, immune system problems, stress, and other factors. Other research has found that elevated levels of white blood cells could be linked to conditions like anemia, bone marrow cancer, infectious diseases, inflammation, intense physical strain, tissue injuries (such as burns), and more (Bagby, 2007; Dinaiers, 2008; Dugdale, 2011). Having a low white blood cell count, also known as leucopenia, means there are fewer disease-fighting cells (leucocytes) circulating in an animal's body (Mayo, 2013; Kumar, 2010; Kilegman, 2011; Marx, 2010). A few white blood cells can result from bone marrow issues, liver or spleen disease, radiation, or certain infections, tumors, or scar tissue (Bagby, 2007; Dinaver, 2008; Dugdale, 2011). The MCV levels of animals in groups 1-5 were lower than the normal range (80-100fL) with group 3 having the highest MCV value at 54.33% and group 4 having the lowest MCV value at

47.67fL. MCV measures the typical size of red blood cells and is included in a standard blood test called a complete blood count. The mean corpuscular volume (MCV) is determined by dividing the overall volume of packed red blood cells by the total count of red blood cells. After that, the number obtained is multiplied by 10. In animals experiencing anemia, the MCV measurement determines if it is microcytic, normocytic, or macrocytic anemia based on MCV levels. Normocytic anemia is typically characterized by the lack of a change in cell volume in the bone marrow. It sometimes happens in acute situations, specifically in cases of blood loss and hemolysis. The contrast between the effects of various treatments on the grouped animals' heamatological parameters in this study differed from the findings of Ogunlade *et al*., (2004).

CONCLUSION

According to the results of this research, it can be deduced that the aqueous extract of Zainacin Dadin duniya contains a significant amount of phenol and flavonoid phytochemicals that play a role in their biochemical characteristics. This study showed that giving rats 2ml of saline and 3ml of acetaminophen daily for 28 days can decrease growth performance and change blood parameters, potentially impacting rat growth and development adversely. Findings indicated a substantial increase in weight ($P<0.05$) in Groups 1, 2, 4, and 5, with Group 3 experiencing weight loss. Group 3 showed a significant decrease in erythrocyte counts (4.29%), packed cell volume (33.00%), and hemoglobin (6.17%) values ($P<0.05$), while an increase was observed in leukocyte (9.62%) and Mean Cell Volume (54.33%) values compared to the other Groups. Furthermore, the current findings offer compelling proof that combining Zainacin Dadin duniya extract can both hinder and improve the impact of acetaminophen-induced Hepatocyte effects. This function may be mediated by the extract's ability to scavenge free radicals or inhibit their generation. Additional research is needed to evaluate the plant extract's biochemically hepatoprotective properties and its pharmacological role in protecting against acetaminophen toxicity to verify these protective effects.

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