Journal of Biotechnology Research
ISSN(e): 2413-3256, ISSN(p): 2413-8878
Vol. 11, Issue. 1, pp: 1-8, 2024
URL: https://arpgweb.com/journal/journal/16
DOI: https://doi.org/10.32861/jbr.111.1.8

Academic Research Publishing Group

Original Research **Open Access**

Assessment of the Effects of TiwanTiwa Herbal Mixture Drink on Oxidative Stress Markers

Usiobeigbe O. S. (Corresponding Author)
Department of Chemical Pathology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria
Email: kennylamai@yahoo.com

Airhomwanbor K. O.
Department of Chemical Pathology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria

Omolumen L. E.
Department of Chemical Pathology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria

Obohwemu K. O.
Department of Health, Wellbeing and Social Care, Global Banking School/Oxford Brookes University, Birmingham, UK.

Oikerhe E. G.
Department of Haematology and Blood Transfusion Science, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo, Nigeria

Asibor E.
Department of Histopathology and Cytopathology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria

Omoviye O. E.
Fellow - Business Development, Sub Sahara Africa. Direct Export Sales Organisation, EMEA. Siemens Healthineers AG, Germany

Kehinde A.
Faculty of Medical Laboratory science, Achievers university, Owo, Ondo state, Nigeria

Lagundoye S. B.
Department of Medical Laboratory Science, Federal Medical Centre, Owo, Ondo State, Nigeria

Aliche P. C.
Department of Nursing Sciences Clifford University Owerrita Abia State Nigeria

Ogunniyi R. O.
Department of Medical Laboratory Science, Lead City University, Ibadan, Oyo State, Nigeria

Article History
Received: 21 August, 2024
Revised: 29 September, 2024
Accepted: 30 October, 2024
Published: 11 November, 2024

Abstract

Introduction: The use of herbal medicine and its effect on health has been established. Recently, there is much interest in therapeutic potential of herbal therapy use as antioxidant in reducing free radical-induced oxidative stress. Method: This study was aimed to determine the effect of herbal mixture on acetaminophen induced oxidative stress in male albino wistar rat. This is an experimental study comprising of total of forty-two (42) wistar rats divided into seven (7) groups of six (6) animals each. The seven groups were Group 1, control group fed with top feed and water only, Group 2 administered with 3000 mg acetaminophen and top feed, Group 3 with 3000 mg of acetaminophen and 140mg sylimarine, Group 4 with 3000 mg of acetaminophen and 50 mg tiwantiwa extract, Group 5 with 3000mg of acetaminophen and 100 mg tiwantiwa extract, Group 6 with 3000mg of acetaminophen and 200 mg tiwantiwa extract and Group 7 with 100 mg of tiwantiwa extract only. The treatment was given daily and lasted for 21 days, animals were sacrificed, and blood sample collected were assayed for oxidative stress; CAT, SOD, and MDA. Result obtained was analyzed with statistical package for social sciences (SPSS) version 20. Results: The result obtained depicts significant increase in CAT and SOD of group 5 (100mg of the extract) when compared to group 2 which is the toxic group induced with 3000mg Acetaminophen. There was also a significant decrease in MDA of group 5 (100mg of extract) when compared to group 2 ($p<0.05$). This implies that dose level (100 mg) of Tiwantiwa herbal mixture in group 5 exhibited the capacity to alleviate 3000 mg of acetaminophen toxicity at a minimal level. This is attributed to phytochemicals present in this extract such as phenolic compounds. Conclusion: 100 mg dose level of Tiwantiwa herbal mixture has a significant effect in alleviating 3000 mg of acetaminophen toxicity, acting as strong antioxidant as evidenced in the study

with results shown in CAT, SOD and MDA bioassays. This is attributed to the presence compounds such as Phenols and Alkaloids which are potent phytochemical constituents in Tiwantiwa herbal mixture with antioxidant property.
Keywords: TiwanTiwa, Herbal, Mixture, Oxidative, Stress.

1. Introduction

The use of herbal remedies all over the world for the management of diseases is increasing [1]. There is a widespread perception that herbal drugs have little or no side effect [2], making them a first consideration in some African and Asian populations for treating many diseases [3, 4]. Traditional remedies are usually made up of only one part of the plant. However, accumulation of therapeutic experience and the search for improved health outcomes by herbal practitioners over time have resulted in a shift from the use single plant or plant parts to combining different plants or plant parts for enhanced therapeutic potentials [4]. Herbals are usually sought after for their health benefits and these have become common medicines in many Nigerian homes [5]. This involves the use of specific proportions of leaves, stem, seeds and roots of different plants in water, alcohol or other non-toxic solvents [4].

It is believed that the active principles in these plants work synergistically or in combination to produce enhanced therapeutic effect [6]. Furthermore, the general belief that herbal medicines are natural, effective, and without adverse effects has immensely contributed to the upsurge in the patronage of herbal formulations [4, 7]. The popular belief that herbal remedies are without toxic or undesirable effects has however often been proven otherwise [8]. These and other factors form part of several concerns associated with the use of herbal remedies in developing nations. In Nigeria, studies have revealed that the number of Nigerian medicinal plants screened for the validation of biological activity far outweighs the assessment for potential toxic compounds and contaminants [4, 6].

However, the increasing commercial promotion of herbals necessitate the need for assessment of safety and validity of medicinal claims. In recent past, several studies have demonstrated the toxic effects of indiscriminate use of packaged herbals [4, 9].

Another herbal medication that is gaining popularity in Nigeria today is a combination of TiwanTiwa Herbal mixture®. It's a brownish root tincture with a distinct aroma that sets it apart from other alternatives. TiwanTiwa Herbal mixture is commonly known and used in the western part of Nigeria. It is not an alcoholic herbal mixture like the regular herbal mixtures. It is acclaimed by the manufacturer to be potent to treat blood sugar level, cleans up the reproductive system, stimulates sexual appetite in men, treats high blood pressure, treats and prevent cancer e.t.c.

In Nigeria, studies have revealed that the number of Nigerian medicinal plants screened for the validation of biological activity far outweighs the assessment for potential toxic compounds and pro reactive oxygen reactants in leading to oxidative stress in form of lipid peroxidation [10]. Also, many herbal formulations being promoted and marketed in Nigeria lack scientific data to support acclaimed medicinal benefits and may pose great health risk to the numerous unsuspecting consumers [4, 9].

Diseases caused due to '**oxidative stress**' initiated by Reactive Oxygen Species (ROS) is growing at an alarming rate. Several medicinal herbs explored till date were reported to possess numerous pharmacological properties used for combating different diseases [11]. Oxidative stress can be described as an imbalance between oxidant substances (reactive oxygen species and antioxidant defenses) in living organisms. Oxidative stress has a role in various pathologic and physiologic conditions [12].

Free radical production (superoxide, hydroxyl and nitric oxide, and other reactive oxygen species (ROS) occurs as a consequence of the endogenous reactions and plays an important role in the cell. Increased formation of ROS and/or decreased antioxidant defense can be defined as oxidative stress, which is widely recognized as an important feature of many diseases such as diabetes mellitus, cancer, and renal failure. Superoxide dismutase (SOD) and catalase (CAT) are some important endogenous antioxidants, which protect the cells from oxidative stress [13]. Lipid peroxidation (LPO) is one of the most important expressions of oxidative stress induced by ROS. MDA is an indicator of LPO which increases in various diseases.

According to the TiwanTiwa Herbal mixture label, the herbal remedy is indicated for treatments of several ailments which include treating blood sugar level, cleans up the reproductive system, stimulates sexual appetite in men, treats high blood pressure, treats and prevent cancer amongst others. There are no scientific reports on the medicinal relevance of the individual plant constituents of the TiwanTiwa Herbal mixture. However, there are no empirical data to support claims of medicinal benefits or otherwise of TiwanTiwa Herbal mixture. Thus, the study of the effect of the herbal mixture on oxidative straight.

2. Materials and Method

2.1. Materials

Materials used in this study include; Cotton wool, needle and syringe, plain bottle, Lithium heparin, methylated spirit, Gloves, micropipette, Tourniquet, automatic micropipette, pipette tips, spectrophotometer, spectrophotometric cuvette, water bathe, vortex mixer, disposable test tubes, and Eppendorf centrifuge, NADPH reagent, NADPH diluents, assay buffer, hydrogen peroxide reagent, microplates, thermostatic water bath, air displacement pipettes, double distilled water, ascetic acid, Tiwantiwa herbal mixture, acetaminophen and sylimarine.

2.2. Study Design

This study was an experimental study comprising of total of forty-two (42) wistar rats divided into seven (7) groups of six (6) animals each. The seven groups were Group 1, control group fed with top feed and water only,

Group 2 administered with 3000 mg acetaminophen and top feed, Group 3 with 3000 mg of acetaminophen and 140mg sylimarine, Group 4 with 3000 mg of acetaminophen and 50 mg tiwantiwa extract, Group 5 with 3000mg of acetaminophen and 100 mg tiwantiwa extract, Group 6 with 3000mg of acetaminophen and 200 mg tiwantiwa extract and Group 7 with 100 mg of tiwantiwa extract only. The treatment was given daily and lasted for 21 days.

2.3. Study Site

This study was carried out at the animal house, Department of Medical Laboratory Science, Lead City University Ibadan-Oyo state.

2.3.1. Procurement of *Tiwantiwa* Herbal Mixture

Tiwantiwa Herbal mixture state will be purchased from market in Ibadan. The herbal mixture will be authenticated in the herbarium unit of Botany Department, University of Ibadan, Ibadan, Oyo State, Nigeria.

2.3.2. Experimental Animals

Forty-two (42) healthy male albino wistar rats weighing averagely 130g was purchased. The rats were kept in wooden mesh cages and will be made to acclimatize to animal house condition for one weeks before the commencement of the research and will be fed with a standard commercial pelleted rat feed and clean water. The rats were randomly divided into seven (7) groups of six (6) rats each. The rats will be kept in a well-ventilated room, maintained at 28 ± 2^0 C with a day-night rhythm of 12 hours. The weight of the animals was measured using an electronic analytical and precision balance before the commencement of the study. Cages was be regularly cleaned to avoid infection.

2.3.3. Administration of Tiwantiwa Herbal Mixture

The herbal mixture extract was administered with the aid of oral cannula, twice daily to the appropriate group for the time specified for each group.

2.3.4. Animal Sacrifice and Sample Collection

At the end of the experiment, blood was collected into EDTA (ethylene diamine tetra acetic acid) anticoagulant bottle from brachiocephalic artery of each rat. The rats were immediately ethically sacrificed by cervical dislocation. At the end of the sample collection, the samples were analyzed for oxidative stress parmeters including Malonaldehyde, super-oxide dismutase and catalase.

2.3.5. Determination of Thiobarbituric Acid Reactive Substances (TBARS)

Lipid peroxidation levels were measured by the thiobarbituric acid (TBA) reaction using the method of Ohkawa, *et al.* [14].

This method was used to measure spectrophotometrically the color produced by the reaction of TBA with malondialdehyde (MDA) at 532 nm. For this purpose, TBARS levels were measured using a commercial Malondialdehyde Assay kit according to the manufacturer's instructions.

2.3.6. Principle of the MDA Assay

In the presence of acid, MDA reacts with TBA to produce a colored end product that absorbs light. The intensity of the color at 532 nm corresponds to the level of lipid peroxidation in the sample. Unknown samples are compared to the standard curve.

2.3.7. Superoxide Dismutatse

Principle: Super oxide anions generated as the byproduct of Xanthine Oxidase catalyzed Xanthine oxidation and the anion can oxidize hydroxylamine to nitrite which appears to be amaranth purple in the presence of chromogenic agent and thus the absorbance at certain wavelength can be detected by spectrophotometer. The presence of SOD in the system specifically inhibits the oxidization caused by super oxide anions and because of it, fewer nitrite anions are generated. This would lower the absorbance of the sample tube compared to the reference tube without.

2.3.8. Catalase Assay

Principle: The catalase activity in a sample is determined by measuring the decrease in H_2O_2 concentration observed following an incubation of the analyte sample with an H_2O_2 standard solution.

Using the Megazyme Catalase Assay Kit, two separate reactions must be completed for catalase activity

In reaction A, the catalase sample of interest is incubated with a known concentration (~ 65 mM in assay) of H_2O_2. The reaction is stopped by the addition of 15 mM sodium azide which strongly inhibits catalase.

Reaction A is shown below in the presence of catalase enzymes

$2H_2O_2 \rightarrow 2H_2O + O_2$

In reaction B, the exact concentration of H_2O_2 remaining is measured using an enzyme-linked colourimetric detection method employing 3, 5-dichloro-2-hydroxy-benzenesulfonic acid (DHBS), 4-aminoantipyrine (AAP) and peroxidase. The resulting quinoneimine dye is measured at 520 nm.

$2H_2O_2$ +DHBS + AAP→ quinoneimine dye + $4H_2O$

2.3.9. Statistical Analysis

The SPSS (statistical package for social sciences) software package was used for statistical analysis. Values obtained were expressed as mean ± standard deviation and compared using analysis of variance (ANOVA) and the significance was measured at $p < 0.05$.

3. Result

A total of forty-two (42) wistar rats were used in this study comprising of seven (7) groups of six (6) animals each. The seven groups were Group 1, control group fed with top feed and water only, Group 2 as experimental group with administered with 3000 mg acetaminophen and top feed only, Group 3 as experimental group administered with 3000 mg of acetaminophen, top feed and sylimarine, Group 4 as experimental group administered with 3000 mg of acetaminophen, top feed and 50 mg tiwantiwa extract, Group 5 as experimental group administered with 3000mg of acetaminophen, top feed and 100 mg tiwantiwa extract, Group 6 as administered with 300mg of acetaminophen and top feed and 200 mg tiwantiwa extract and Group 7 as administered with 100 mg of tiwantiwa extract and top feed. Mean ± standard deviation of oxidative stress parameters among the study subjects was depicted in table 1 and figure 1, 2 and 3

As shown in table 2, the mean value of CAT and SOD in group 2, 4and 6 was significantly decreased when compared to group A($p<0.05$). Also, there was a significant increase in the MDA mean value of group 2 and 4 when compared to group 1($p<0.05$). However, there was no significant difference in the mean value of CAT and SOD of group 3, 5 and 7 when compared to group 1 ($p>0.05$). Also, there was no significant difference in the MDA mean value of group 3, 6and 7 when compared to group 1($p>0.05$).

As depicted in table 3, there was a significant difference in the mean value of CAT, SOD and MDA of group 3, 5 and 7 when compared to group 2 ($p>0.05$). However, there was no significant difference in the mean value of group 4 and 6 of CAT, SOD and MDA when compared to group 2($p>0.05$).

As shown in table 4, there was a significant difference in the mean value of CAT and SOD of group 6 and 6 when compared to group 3 ($p<0.05$). Also, there was a significant increase in the mean value of MDA of group 4 when compared to group 3. However, there was no significant difference in the mean value of group5 and 7 of CAT and SOD, also there was no significant difference in the mean value of MDA of group 5, 6 and 7 when compared to group 3($p>0.05$).

As shown in table 5, there was a significant difference in the mean value of CAT, SOD and MDA of group 5 and 7 when compared to group 4 ($p<0.05$). However, there was no significant difference in the mean value of CAT, SOD and MDA of group 6 when compared to group 4($p>0.05$).

As depicted in table 6, there was a significant difference in the mean value of CAT of group 6 when compared with group 5 ($p<0.05$). However, there was no significant difference in the mean value of group G CAT, group 6 and 7 SOD and MDA when compared to group 5($p>0.05$). As shown in table 7, there was no significant difference in the mean value of CAT, SOD and MDA of group 7 when compared to group 6($p>0.05$).

Table-1. Mean ± standard deviation of oxidative stress parameters among the study subjects

GROUP	CAT	SOD	MDA
1	110.33±1.53	12.00±1.00	0.87±0.66
2	76.00±3.61	6.00±1.00	2.40±0.53
3	107.44±6.43	12.00±1.00	0.87±0.15
4	77.67±2.52	7.67±0.58	2.07±0.31
5	104.00±4.00	11.00±1.00	0.81±0.16
6	86.00±4.00	8.00±1.00	1.63±0.15
7	102.67±2.51	13.00±1.00	0.83±0.15

Keys: Catalase, CAT; Superoxide dismutase, SOD; Malonaldehyde, MDA

Group 1= Top feed and water only; Group 2= 0.5 mls acetaminophen + top feed only; Group 3= 3000mg of acetaminophen+ top feed+sylimarine; Group 4=3000mg of acetaminophen+ top feed +50mg tiwantiwa extract; Group 5=3000mg of acetaminophen+ top feed +100mgtiwantiwa extract; Group 6= 3000mg of acetaminophen+top feed +200mg tiwantiwa extract; Group 7= 100mg of tiwantiwa extract +top feed only.

Figure-1. Mean ± standard deviation of CAT parameters among the study subjects

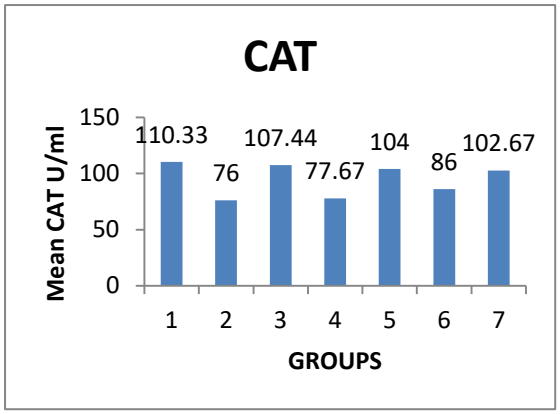

Figure-2. Mean ± standard deviation of SOD parameters among the study subjects

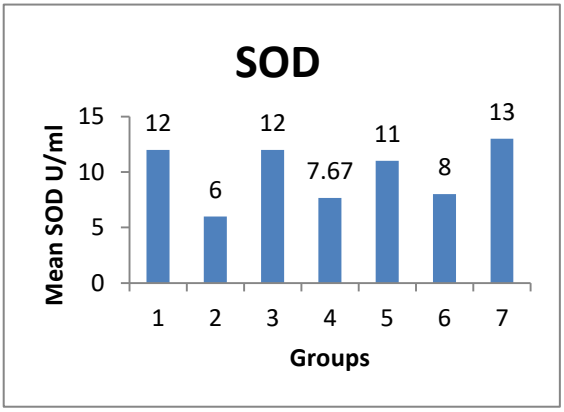

Figure-3. Mean ± standard deviation of MDA parameters among the study subjects

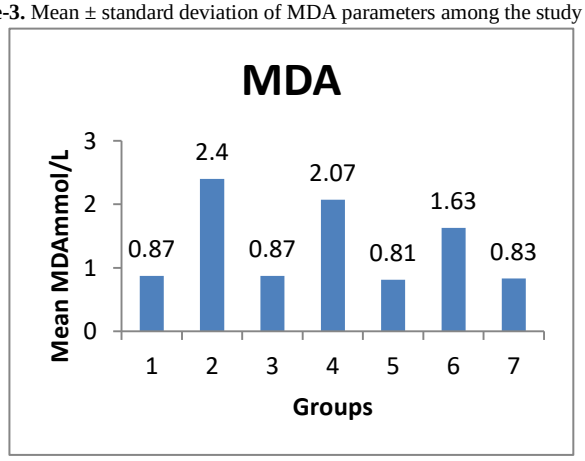

Table-2. Comparison of oxidative stress of Group 1 with Group 2, 3, 4, 5, 6 and respectively

GROUP	CAT	SOD	MDA
1	110.33±1.53	12.00±1.00	0.87±0.66
2	76.00±3.61*	6.00±1.00*	2.40±0.53*
3	107.44±6.43	12.00±1.00	0.87±0.15
4	77.67±2.52*	7.67±0.58*	2.07±0.31*
5	104.00±4.00	11.00±1.00	0.81±0.16
6	86.00±4.00*	8.00±1.00*	1.63±0.15
7	102.67±2.51	13.00±1.00	0.83±0.15

Data presented as mean ± standard deviation
*means Significant at p-value <0.05
Keys: Catalase (CAT); Superoxide dismutase (SOD); Malonyaldehyde, (MDA)

Table-3. Comparison of oxidative stress of Group 2 with Group 3, 4, 5, 6, and 7 respectively

GROUP	CAT	SOD	MDA
2	76.00±3.61	6.00±1.00	2.40±0.53
3	107.44±6.43*	12.00±1.00*	0.87±0.15*
4	77.67±2.52	7.67±0.58	2.07±0.31
5	104.00±4.00*	11.00±1.00*	0.81±0.16*
6	86.00±4.00	8.00±1.00	1.63±0.15
7	102.67±2.51*	13.00±1.00*	0.83±0.15*

Data presented as mean ± standard deviation
*means Significant at p-value <0.05
Keys: Catalase, CAT; Superoxide dismutase, SOD; Malonaldehyde, MDA

Table-4. Comparison of oxidative stress of Group 3 with Group 4, 5, 6 and 7 respectively

GROUP	CAT	SOD	MDA
3	107.44±6.43	12.00±1.00	0.87±0.15
4	77.67±2.52*	7.67±0.58*	2.07±0.31*
5	104.00±4.00	11.00±1.00	0.81±0.16
6	86.00±4.00*	8.00±1.00*	1.63±0.15
7	102.67±2.51	13.00±1.00	0.83±0.15

Data presented as mean ± standard deviation
*means Significant at p-value <0.05
Keys: Catalase, CAT; Superoxide dismutase, SOD; Malonaldehyde, MDA

Table-5. Comparison of oxidative stress of Group 4 with Group 5, 6 and 7 respectively

GROUP	CAT	SOD	MDA
4	77.67±2.52	7.67±0.58	2.07±0.31
5	104.00±4.00*	11.00±1.00*	0.81±0.16*
6	86.00±4.00	8.00±1.00	1.63±0.15
7	102.67±2.51*	13.00±1.00*	0.83±0.15*

Data presented as mean ± standard deviation
*means Significant at p-value <0.05
Keys: Catalase, CAT; Superoxide dismutase, SOD; Malonaldehyde, MDA

Table-6. Comparison of oxidative stress of Group 5 with 6 and 7 respectively

GROUP	CAT	SOD	MDA
5	104.00±4.00	11.00±1.00	0.81±0.16
6	86.00±4.00*	8.00±1.00	1.63±0.15
7	102.67±2.51	13.00±1.00	0.83±0.15

Data presented as mean ± standard deviation
*means Significant at p-value <0.05
Keys: Catalase, CAT; Superoxide dismutase, SOD; Malonaldehyde, MDA

Table-7. Comparison of oxidative stress of Group 6 and 7

PARAMETER	GROUP 6	GROUP 7	P-value
CAT	86.00±4.00	102.67±2.51	0.007
SOD	8.00±1.00	13.00±1.00	0.075
MDA	1.63±0.15	0.83±0.15	0.080

Data presented as mean ± standard deviation
*means Significant at p-value <0.05
Keys: Catalase, CAT; Superoxide dismutase, SOD; Malonaldehyde, MDA

4. Discussion

Plants have been used for the treatment of various diseases from ancient times. There are many evidences that the generation of free radicals inside the body causes damage to DNA and lead to the development of different diseases and if these free radicals are neutralized by the antioxidants from different medicinal plants, then it prevents diseases from taking place. Several studies have shown that plant derived antioxidant scavenge free radicals and modulates oxidative stress [15, 16].

Treatment by herbal medicines may have some advantages over treatment by single purified chemicals in the sense that herbal medicine are the mixtures of more therapeutic or preventive components, and so might have more activity than single products alone [4]. The antioxidant and anti-cancerous effects of extracts from various herbs and medicinal plants have been proved experimentally and clinically over time [17].

Superoxide dismutase (SOD) acts as a first defense system against oxidative stress. Catalase (CAT) is one of the most important intracellular enzymes in the detoxification of the oxidant hydrogen peroxide in the liver. In our study, there was significant decrease ($p<0.05$) in the activities of CAT and SOD enzymes in group 2 (positive control) administered with 0.5mls of acetaminophen when compared to group 1 (negative control). This outcome was attributed to the increased utilization of antioxidant enzymes in scavenging and neutralizing the free radicals and

lipid peroxidation (MDA) [18]. This outcome is also consistent with similar data obtained by Akgun, *et al.* [19] who administered 500mg/kg and Matić, *et al.* [20] who administered 2g of acetaminophen respectively.

In this study, we observed a significant increase in antioxidant enzymes CAT and SOD ($p<0.05$) of group 3 when compared to the group 2. Herbal mixture contains phenolic compound which has the ability to scavenge free radicals [3, 21]. Our findings showed that there was no significant difference in CAT, SOD and MDA level of group 4(50mg of extract) and group 6 (200mg of exract) when compared to the group 2 (positive control).

In the present study, there was a significant increase in CAT and SOD of group 5(100mgof the extract) when compared to group 2 which is the toxic group induced with 3000mg Acetaminophen. There was also a significant decrease in MDA of group 5(100mg of extract) when compared to group 2 ($p<0.05$). This implies that dose level (100 mg) of Tiwantiwa herbal mixture in group 5 exhibited the capacity to alleviate 3000mg of acetaminophen toxicity at a minimal level. This is attributed to phytochemicals present in this extract such as phenolic compounds.

This corroborate with a similar study by Chilvery, *et al.* [22] who stated that co-treatment of *Morinda lucida* extracts (containing quercetin and other phytochemicals), to acetaminophen induced injury significantly restored oxidative stress effect and improved the basal antioxidant status of the cell.

Similar research showed that 125 and 250 mg/kg of aqueous and methanolic extracts of *Cuscuta campestris* were found to notably exert hepatoprotective and antioxidant effects on acetaminophen induced oxidative stress Chang, *et al.* [23]. The hepatoprotective and antioxidant properties could be attributed to polysaccharide, saponins, quinic acid, flavonol, and flavonoids in the plant extract [24].

Our findings indicated that100mg of Tiwantiwa herbal mixture administered in group 5 and 7 has phytochemicals such flavonoids and tannin which suppresses ROS/RNS synthesis by inhibiting specific enzymes or chelating trace metals involved in free radical generations. One of the attributes for improved mitochondrial function and hepatic protection of the plant extract is likely related to preventing the excessive generation of NAPQI with concurrent improvement in the mitochondrial antioxidant defense system, thereby protecting crucial nucleophilic sites on the enzymes once more. Additionally, a slight uncoupling of oxidative phosphorylation in the liver is related to a protective effect against oxidative stress and decreased production of ROS [25]. This membrane-stabilizing effect may be attributed to the phytoconstituents such as flavonoids and glycosides present in the plant extract [26]. This present study suggests that Tiwantiwa herbal mixture protects and improve the integrity cells and tissue against toxicity induced by acetaminophen. Oxidative stress is a known mechanism in acetaminophen toxicity, and therefore this study depicts that Tiwantiwa herbal mixture may be associated with a reduction of oxidative stress level.

5. Conclusion

About 100 mg dose level of Tiwantiwa herbal mixture has a significant effect in alleviating 3000mg of acetaminophen toxicity, acting as strong antioxidant as evidenced in the study with results shown in CAT, SOD and MDA bioassays. This is attributed to the presence compounds such as Phenols and Alkaloids which are potent phytochemical constituents in Tiwantiwa herbal mixture with antioxidant property.

Tiwantiwa herbal mixture should be recommended as a strong antioxidant as evidenced in our study and administered at a dose level of 100mg to alleviate oxidative stress. Fifty (50) mg of this herbal mixture represents low dose, and 200mg represents high dose.

5.1.1. Conflict of Interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

5.1.2. Funding

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

5.1.3. Authors' Contributions

The entire study procedure was conducted with the involvement of all writers.

Acknowledgements

The authors would like to acknowledge the management and all the technical staff of St Kenny Research Consult, Ekpoma, Edo State, Nigeria for their excellent assistance and for providing medical writing/editorial support in accordance with Good Publication Practice (GPP3) guidelines.

References

[1] Johnson, J. T. and Okafor, E. O., 2021. "Effects of Various Alcoholic Bitters on the Hepatological Indices of Albino Wistar Rats." *European Journal of Biomedical*, vol. 8, pp. 293-298.

[2] Kale, O. E., Akinpelu, O. B., Bakare, A. A., Yusuf, F. O., Gomba, R., Araka, D. C., and Odutola, O., 2018. "Five traditional Nigerian Polyherbal remedies protect against high fructose fed, Streptozotocin-induced type 2 diabetes in male Wistar rats." *BMC complementary and alternative medicine*, vol. 18, pp. 1-11.

[3] Umaru, I. J., Shuaibu, S. I., Adam, R. B., Habibu, B., Umaru, K. I., Haruna, D. E., and David, B. C., 2020. "Effect of herbal medicine and its biochemical implication." *Int. J. Adv. Biochem. Res.*, vol. 4, pp. 46-57.

[4] Iyevhobu, K. O., Airefetalor, A. I., Osagiede, E. K., Omolumen, L. E., Ikede, R. E., and Ken-Iyevhobu, B. A., 2022. "Effects of some herbal plants and herbal drink on selected micro-organisms using ditch and agar methods." *South Asian Research Journal of Applied Medical Sciences*, vol. 4, pp. 14-19.

[5] Akande-Sholabi, W., Iluyomade, A., Ilesanmi, O. S., and Adisa, R., 2020. "Perception and use of herbal medicines among clients visiting selected community pharmacies in Ibadan, Nigeria." *African Journal of Biomedical Research*, vol. 23, pp. 147-153.

[6] Abba, C. C., Nsofor, C. B., Udeozor, C. C., Orjiakor, J. E., Ajah, U. K., Adione, N. M., and Eze, P. M., 2024. "In-vitro comparative study of solvent effect on antioxidant properties of leaf extracts of Anthocleista djalonensis." *Magna Scientia Advanced Biology and Pharmacy*, vol. 11, pp. 072-079.

[7] Emam, R., Ghanem, M., Abdel-Raoof, Y., and Elkhyat, H., 2020. "Evaluation of some hepatoprotective preparations in experimentally induced hepatopathy in Baladi goats." *Benha Veterinary Medical Journal*, vol. 38, pp. 17-25.

[8] Aboraya, D. M., El Baz, A., Risha, E. F., and Abdelhamid, F. M., 2022. "Hesperidin ameliorates cisplatin induced hepatotoxicity and attenuates oxidative damage, cell apoptosis, and inflammation in rats." *Saudi Journal of Biological Sciences*, vol. 29, pp. 3157-3166.

[9] Adeyemi, O. S., Shittu, E. O., Akpor, O. B., Rotimi, D., and Batiha, G. E. S., 2020. "Silver nanoparticles restrict microbial growth by promoting oxidative stress and DNA damage." *EXCLI Journal*, vol. 19, p. 492.

[10] Shamohamadi, M., Pooyanmehr, M., Maleki, A., and Haghnazari, L., 2021. "Biochemical modulatory and protective effects of the hydroalcoholic extract of scrophularia striata on the hepatotoxicity of silver nanoparticles in the rat model." *Iranian Journal of Veterinary Medicine*, vol. 15, pp. 311-324.

[11] Vasanth, K., Minakshi, G. C., Velu, K., Priya, T., Kumar, R. M., Kaliappan, I., and Dubey, G. P., 2022. "Anti-adipogenic β-sitosterol and lupeol from moringa oleifera suppress adipocyte."

[12] Kıran, T. R., Otlu, O., and Karabulut, A. B., 2023. "Oxidative stress and antioxidants in health and disease." *Journal of Laboratory Medicine*, vol. 47, pp. 1-11.

[13] Abdel-Daim, M. M., Abushouk, A. I., Alkhalf, M. I., Toraih, E. A., Fawzy, M. S., Ijaz, H., and Bungau, S. G., 2018. "Antagonistic effects of Spirulina platensis on diazinon-induced hemato-biochemical alterations and oxidative stress in rats." *Environmental Science and Pollution Research*, vol. 25, pp. 27463-27470.

[14] Ohkawa, H., Ohishi, N., and Yagi, K., 1979. "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction." *Analytical Biochemistry*, vol. 95, pp. 351–358. Available: https://doi.org/10.1016/0003-2697(79)90738-3

[15] Akbari, B., Baghaei-Yazdi, N., Bahmaie, M., and Mahdavi Abhari, F., 2022. "The role of plant-derived natural antioxidants in reduction of oxidative stress." *BioFactors*, vol. 48, pp. 611-633.

[16] Engwa, G. A., Nweke, F. N., and Nkeh-Chungag, B. N., 2022. "Free radicals, oxidative stress-related diseases and antioxidant supplementation." *Alternative Therapies in Health and Medicine*, vol. 28,

[17] Khan, M. I., Bouyahya, A., Hachlafi, N. E., Menyiy, N. E., Akram, M., Sultana, S., and Elebiyo, T. C., 2022. "Anticancer properties of medicinal plants and their bioactive compounds against breast cancer: a review on recent investigations." *Environmental Science and Pollution Research*, vol. 29, pp. 24411-24444.

[18] Chukwuebuka, N. B., Elias, D. T. M., Ijego, A. E., Peggy, O. E., Ejime, A. C., and Omeru, O., 2021. "Changes in antioxidant enzymes activities and lipid peroxidase level in tissues of stress-induced rats." *Biomedical and Pharmacology Journal*, vol. 14, pp. 583-596.

[19] Akgun, E., Boyacioglu, M., and Kum, S., 2021. "The potential protective role of folic acid against acetaminophen-induced hepatotoxicity and nephrotoxicity in rats." *Experimental Animals*, vol. 70, pp. 54-62.

[20] Matić, M. M., Paunović, M. G., Milošević, M. D., Ognjanović, B. I., and Saičić, Z. S., 2021. "Hematoprotective effects and antioxidant properties of β-glucan and vitamin C against acetaminophen-induced toxicity: an experimental study in rats." *Drug and chemical toxicology*, vol. 44, pp. 302-309.

[21] Savych, A. and Mazur, O., 2021. "Antioxidant activity in vitro of antidiabetic herbal mixtures." *PharmacologyOnLine*, vol. 2,

[22] Chilvery, S., Yelne, A., Khurana, A., Saifi, M. A., Bansod, S., Anchi, P., and Godugu, C., 2023. "Acetaminophen induced hepatotoxicity: An overview of the promising protective effects of natural products and herbal formulations." *Phytomedicine*, vol. 108, p. 154510.

[23] Chang, L., Xu, D., Zhu, J., Ge, G., Kong, X., and Zhou, Y., 2022. "Herbal therapy for the treatment of acetaminophen-associated liver injury: recent advances and future perspectives." *Frontiers in Pharmacology*, vol. 11, p. 313.

[24] Lin, J., Xiong, C., He, X., Lu, Z., Zhang, X., Chen, X., and Sun, W., 2018. "Using SSR-HRM to identify closely related species in herbal medicine products: a case study on licorice." *Frontiers in Pharmacology*, vol. 9, p. 407.

[25] Karkucinska-Wieckowska, A., Simoes, I. C., Kalinowski, P., Lebiedzinska-Arciszewska, M., Zieniewicz, K., Milkiewicz, P., and Wieckowski, M. R., 2022. "Mitochondria, oxidative stress and nonalcoholic fatty liver disease: A complex relationship." *European Journal of Clinical Investigation*, vol. 52, p. e13622.

[26] Islam, M., Prottay, A. A. S., Sultana, I., Al Faruq, A., Bappi, M. H., Akbor, M. S., and Islam, M. T., 2023. "Phytochemical screening and evaluation of antioxidant, anti-inflammatory, antimicrobial, and membrane-stabilizing activities of different fractional extracts of Grewia nervosa (Lour.)." *Panigrahi. Food Bioscience*, vol. 54, p. 102933.