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INFORMATION

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COMMUNITY STAKEHOLDERS' ENGAGEMENT AS A TOOL FOR IMPROVED UTILIZATION OF HEALTH SERVICES IN SELECTED COMMUNITIES IN ONDO STATE NIGERIA

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ABSTRACT

The Alma Ata declaration puts responsibilities on the community as an important stakeholder, including making decisions on planning, organizing, and implementation of health services at primary level. The University of Medical Sciences Ondo (UNIMED) operates a unique and innovative model of Primary Health Care to deliver "Health For All". The objective of this research was to assess the relationship between stakeholders' engagement and community involvement and utilization of maternal and child health services in selected communities of Ondo State in Southwestern Nigeria. The UNIMED model involved a top-bottom approach to community participation and engagement that dovetailed to the selection of 3 local governments, communities and comprehensive health centers. Oke-Igbo community was the target population. The study area is a homogenous community in ward 7 of Ile – Oluji/Oke-Igbo Local government area (LGA), the pattern of chronic diseases seen in the register, with the At-Risk document registering hypertension, diabetes, sickle cell anemia and arthritis and related joint conditions. One year before the intervention, a total of 256 pregnant women attended antenatal care (ANC). This increased to 795 pregnant women attending ANC within a one-year period of implementing community mobilization, giving an increment of 3.1-fold. A marginal increment of 13% was reported in baby deliveries when the data for one year before and after the project was compared. Similarly, one year before the intervention, a total of 744 children got up to Penta 3 vaccine in the community PHC. This increased to 822 children getting up to Penta 3 vaccine within a one-year period of implementing community mobilization, giving another marginal increment of 10.5%. It was concluded that this model that has been worked on should be scaled up to the other communities in the state to improve health outcomes in the State.

Keywords: Community engagement, Primary Health Care, Maternal and child health, Health service utilization, UNIMED care models

Submitted: July 2024; Accepted March 2025

INTRODUCTION

The Alma Ata declaration on Primary Health Care (PHC), regarded as a core strategy to achieving Health For All (HFA), has community participation as one of her principles [1]. Community participation puts people in a position to participate in solving their own health problems, taking appreciable responsibilities including mobilizing local resources and creating demands for health care services at the community level. The community is responsible for planning, organizing, operation and control of primary health care [1] and a participatory approach to health care governance would assist in achieving universal health coverage [2]. Community participation empowers society and guarantees more commitment from stakeholders. The importance of community participation in primary health care has been documented [3, 4].

There are several levels of community participation, ranging from informing to manipulation, therapy of the community to partnership and citizen control. Ideas are usually brought about by any of the stakeholders, but the ability of communities to be carried along determines the success or otherwise of intervention programmes. Traditionally these have roles to play in mobilizing the communities in efforts that will benefit the communities at the primary health care level.

Several challenges face a successful implementation of Primary health care level system in Nigeria, including its local actors such as the ward and health facility development committees. Several challenges are bound to characterize efforts at community participation in health programmes [5,6], ranging from illiteracy, resistance to change, politics, migration, poor political support, to poor planning and implementation by the health care system authorities.

One of the indicators of the success of HFA through community participation at the PHC level is that of maternal health services, most especially access to ANC and health facility delivery. These indicators may improve when barriers toward establishing health as a shared responsibility among the population are removed and careful programme planning and implementation by the health workers in the presence of Government support is ensured.

Authors observed that universities in Nigeria have piloted various schemes of community health programme, but most are not sustainable and could not guarantee HFA due to poor community participation. Most PHC seems to have weak community-based structure, top-bottom management, with mere tokenisms in community engagement and involvement. There has been no previous record of attainment of a PHC with its HFA services in any setting in Nigeria. Hence the conceptualization of the UNIMED/UNIMEDTH-assisted PHC-HFA model. There are five models being planned and these are (1) the full professional community medical and health services, in 3 political *wards (sites), (2) c*ommunity health/PHC services as may best be provided within the given state or region that our department (CM) is physically present in - *(e.g. Epe site, (3) c*ommunity medical assistance and partnership for entire political ward or LGA in which our department has no physical presence e.g. a political ward in Ose LGA, (4) other consultant community medical and health assistances and collaborations to political wards, LGAs, states or beyond outside Ondo State and (5) community medical assistance and partnership for non-statutory community, not a whole (legally existing) country, state/region/province, LGA or political ward as already designated by the govt or country's constitution as a health service unit of any sort, officially

In Nigeria, most PHC have weak community-based structure, top-bottom management, with mere tokenisms in community engagement and involvement. There has been no previous record of attainment of a PHC with its HFA services in any setting in Nigeria. Hence the conceptualization of the UNIMED/UNIMEDTH-assisted PHC-HFA model. There are five models being planned and these are (1) the full professional community medical and health services, in 3 political wards (sites), (2) *c*ommunity health/PHC services as may best be provided within the given state or region that our department (CM) is physically present in - *(*e.g. Epe site, (3) *c*ommunity medical assistance and partnership for entire political ward or LGA in which our department has no physical presence e.g. a political ward in Ose LGA, (4) other consultant community medical and health assistances and collaborations to political wards, LGAs, states or beyond outside Ondo State.

Only the first model is examined in this paper. The conceptual framework for this model includes the implementation activities for this first model in the ward 7 of Oke-Igbo/Ile Oluji LGA being described include appropriate community entry and documented agreement to engage in the programme. The objective of this research was to assess the relationship between stakeholders' engagement and community involvement and utilization of maternal and child health services in selected communities of Ondo State in Southwestern Nigeria

METHODOLOGY

The study area was Ondo State in Southwestern Nigeria, with a population of about 4 million. The University of Medical Sciences (UNIMED) in Ondo town is the first specialized medical University in Nigeria, with a view to providing comprehensive medical education to the citizenry. The University launched an innovative model of Primary Health Care model to deliver

"Health For All" through a community-based approach. The programme was planned to take place in 3 locations in the State, namely an urban, semi-urban and a rural area.

Study design: Descriptive cross sectional
Target population: The stakeholders in the PHC model including Governments, the leadership of the Ministry of Health, Primary Health Care Board, the Hospital Management Board, the Medical Officers of Health (MoHs) and Chairman of the 3 Local Government Areas (where the communities are located), UNIMED academic staff of the Department of Community Medicine, the health care workers in the PHC and members of the selected communities.

Target population: The unit of enquiry was the respective health centers workforce and the communities. Private hospital care workers were excluded.

Sampling methods: This happened through stakeholders' engagement. The criteria were that the PHC to be selected must be able to provide comprehensive and integrated secondary care health services which include preventive, promotive and curative services. Maroko Comprehensive Health Center (CHC) in Ward 1 of Ondo West LGA was selected as the urban area, Oke-Igbo CHC in Ward 8 of Oke-Igbo community in Ile-Oluji/Oke-Igbo LJA was selected as semi-urban while Ilutuntun CHC in Okitipupa LGA was selected as the rural area.

Stakeholder engagement: This was a top-bottom approach to community participation. The processes in the implementation of the first model mentioned above are

- Stakeholders meeting at the ministry of health before the model takes off, to decide on the choice of Senatorial district to locate the 3 PHCs and the LGAs. After exhaustive deliberations, 6 LGAs spanning the 3 senatorial districts were selected using the sampling criteria above.
- Visiting the 6 PHCs and selecting only 3 based on the stated criteria.
- Meeting with the MoHs of the 3 selected PHCs to determine the criteria for ward selection and community engagement.
- Community entry and community gate keepers' engagement. Ward and health facility health committees were sensitized. Community engagement took place thrice in each community until an appreciable community acceptability was achieved.
- Dejure census and PHC numbering of houses in the selected wards was carried out. When the National population in Oke-Igbo and Ilutuntun communities could not give us the population figures as baseline, the community funded the dejure census with minimal financial involvement

controlled by them, and huge community volunteering.

- GIS Mapping of the household in the entire community including administration of a baseline health assessment questionnaire.
- Employment of a community-based nurse midwives volunteered by each community for each community (by the University).
- Conduct of community rounds by the nurse which also includes visit to the identified. The University Clinicians in the Department of Community Medicine provided backing for the nurse via telemedicine.
- Validation of the health status of the community by opening "At Risk" registers and consultations by doctors from the University. Chronic diseases were identified, mapped and assessed.
- Establishment of the birth, death, marriage and divorce registers and the establishment of the rest of the work of the community nurse-midwife
- The nurse provided collaborative services with health care workers in the Primary Health Centres and 2 community volunteers, including referral of clients from communities to health facilities, provision of community-based health services, schools and market visitation and mobilization for improved health care.

Research instruments employed in this model were the designed health assessment questionnaire, the At-Risk Register and the validation questionnaire for chronic diseases in addition to accessing baseline data on service utilization in the health facility. All design instruments were validated by a team of experts raised by the State Primary health care board

Data analysis: Community health problems identified during baseline health assessment and validation of At-Risk Registers were compared with one year health service utilization data, and these were presented in Tables and charts.

RESULTS

Figure 1 shows the enumeration area map of Ward 7 in Ile-oluji/Oke-igbo LGA of Ondo State. The list of activities carried out under the model being described was undertaken in these areas. Table 1 shows some community members listed while placing the At-Risk-Registers. About 1106(45.4% of them were male while the rest are females. A total of 91(3.7%) of them are under 5 in age while 433(17.8%) of them were never married, 528 (21.7%) of them were up to primary school level of education. Table 2 shows the pattern of chronic diseases seen in the register, these include hypertension, diabetes, sickle cell anemia and arthritis and related joint conditions.

Figure 2 shows a chart depicting the reproductive Service Utilization one year before and after community mobilization. One year before the intervention, a total of 256 pregnant women attended antenatal care. This increased to 795 pregnant women attending ANC within a one-year period of implementing community mobilization, giving an increment of 3.1-fold.

One year before the intervention, a total of 123 pregnant women delivered in the community PHC. This increased to 139 pregnant women delivering within a one-year period of implementing community mobilization, giving a marginal increment of 13%.

Similarly, one year before the intervention, a total of 744 children got up to Penta 3 vaccine in the community PHC. This increased to 822 children getting up to Penta 3 vaccine within a one-year period of implementing community mobilization, giving another marginal increment of 10.5%.

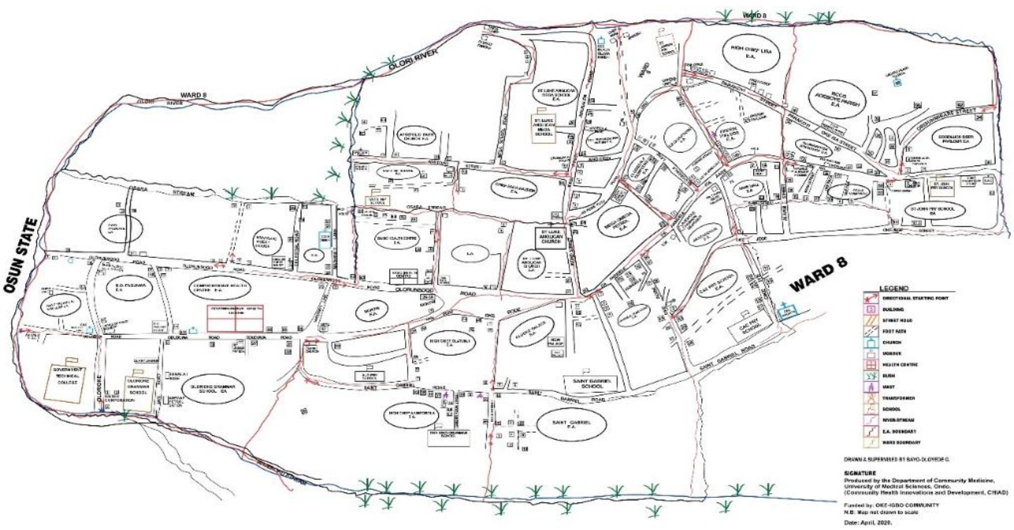

Figure 1: Enumeration Area Map of Ward 7, Ile-oluji / Oke-igbo LGA

Table 1: Socio-demographic Characteristics data of individuals listed (n = 2487)

Variable	Frequency	%
Gender		
Male	1106	45.4
Female	1381	54.6
Age categories		
<1(infants)	16	0.7
Under 5s	91	3.7
15-49(female)	714	28.7
Marital status		
Never married	1433	57.7
Currently married	949	38.1
Others/Not applicable	105	4.2
Highest level of education		
Primary	528	21.7
Secondary	1248	51.2
Tertiary	255	10.5
Others	456	16.6

Table 2: Pattern of Chronic diseases in the At-Risk-Register

Chronic Illness	Agreed periodicity
Hypertension	Twice per annum(minimum)
Diabetes	Twice per annum(minimum)
Arthritis and related conditions	Twice per annum(minimum)
Tuberculosis	Monthly
Sickle cell anaemia	Twice per annum(minimum)
Mental health	Twice per annum (minimum, except called)

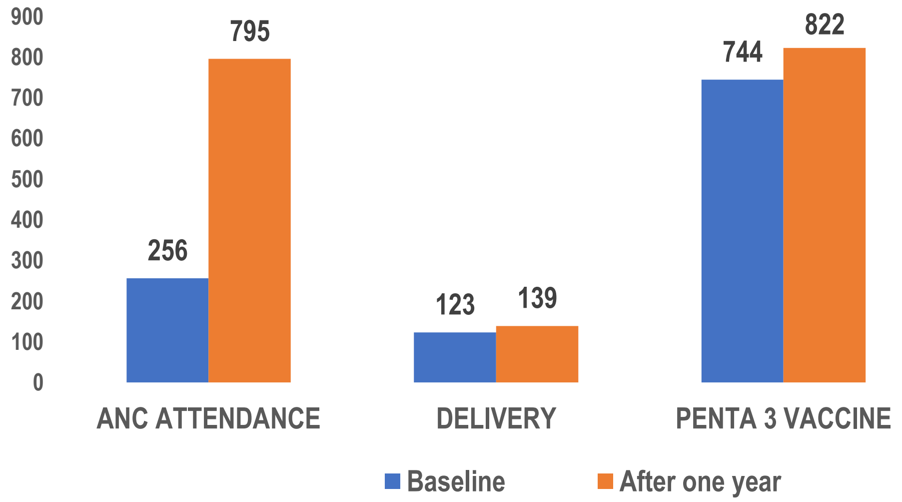

Figure 2: Reproductive Service Utilization one year before and after community mobilization

DISCUSSIONS

The results presented as the preliminary outcome of data generated from only one of the sites show a promising intervention. The role of community mobilization activities thus becomes crucial [1, 7]. Mobilization fosters improved awareness and a possible behavioural change in knowledge and improved access and utilization of health services

In this study, the period one year before and after this innovative community intervention and mobilization approach was compared. There was an appreciable increase in Antenatal attendance after the project following the same pattern of the number of deliveries on comparism. This supports other studies that reported that community participation breeds improved delivery of community-based survival interventions for mother, newborn and children [8. 9. This points to the fact that the degree of community mobilization and participation is a possible measure of ownership and sustainability of programmes.

The community action cycle described led to an appreciable improvement in community awareness, while the rational to use community health resources became more apparent. The entire Ondo State needs to explore the possibilities of incorporating this model of community mobilization in order to improve health outcomes of mother, newborn and children.

In this study, there is a far appreciable increase in the Pentavalent 3 vaccine when compared to the baseline. The role of community mobilization in increasing vaccination uptake has also been reported [10], and in making efforts in solving Community diseases problems [11]. Mothers in ANC or infant welfare clinics have the potential to receive information during mobilization and sensitization that could have led to the observed difference. Along with the line of implementation, researchers observed that communities must be responsive and show interest in programmes that could help them. Oke-Igbo community greatly accepted this idea, and they are already benefitting in the project.

CONCLUSION:

The conceptualization and successful implementation of the UNIMED model of primary health care Health For All concludes that a statutory community-based care is possible in Nigeria with appropriate community engagement and participation. Authors hereby recommend the acceptability of the model of PHC that has the potential of improving health outcomes; it should be allowed and implemented across the various LGAs in the entire Ondo State.

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MELATONIN IMPROVES GLUCO-REGULATORY AND CHONDROPROTECTIVE EFFECTS IN DIABETES-OSTEOARTHRITIS MALE WISTAR RATS VIA ITS ANTI-INFLAMMATORY AND ANTI-APOPTOTIC ACTIVITIES

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ABSTRACT

The present study is aimed to examine the effect of melatonin on diabetes-osteoarthritis (DOA) in male Wistar rats. Thirty male Wistar rats weighing 175-200 g were divided into five groups at random: control, sham, DOA, and treated groups (MEL and metformin). The blood samples and synovial fluid were collected and centrifuged after 21 days of treatment. Biochemical analysis was performed on the supernatants. Values were statistically significant at the $p<0.05$ level, and the data were displayed as mean ± S.E.M. There was a significant increase in fasting blood glucose, insulin resistance, lipid profile, pro-inflammatory markers, caspase-3 levels, and ADAMTS-5 activity in DOA group compared to the control group. However, administration of melatonin significantly reversed the trends. Melatonin improves gluco-regulatory and chondroprotective effects in DOA Wistar rats via anti-inflammatory, anti-apoptotic mechanisms, as well as reducing the activities of cartilage and bone degrading enzyme. Melatonin could be used in the treatment of DOA.

Keywords: Melatonin; Apoptosis; Fasting Blood Glucose; Weight; Lipid Profile

Submitted: April 2025; Accepted: May 2025

INTRODUCTION

The most prevalent chronic degenerative joint disease, osteoarthritis (OA), is primarily defined by gradual joint deterioration [1]. The World Health Organization currently recognizes cancer, heart disease, and osteoarthritis (OA) as the three main diseases that pose a threat to human health, and the prevalence of OA is rising every [2].

OA's pathogenic elements are extremely intricate. Advanced glycation end product (AGE) accumulation in vivo is currently thought to be a molecular basis of age-induced OA, and AGE synthesis and accumulation in vivo is one of the key elements contributing to and hastening the pathological alterations of OA [3]. The mechanism is related to the direct action of its cross- linking with protein and the indirect action caused by its receptor (receptor for advanced glycation end products (RAGE)) binding, thereby activating a series of intracellular signal transduction pathways [4]. Diabetic patients are adversely affected by severe musculoskeletal problems such as bone fracture, joint pain, and OA [5]. It has been suggested that a number of diabetes-related pathways exacerbate the pathophysiological processes of OA. The idea of a diabetes-induced OA (DOA) phenotype was developed on the presumption that DM can also cause OA [6]. Therefore, a number of pathomechanisms are likely involved in the DM-induced exacerbation of OA processes. High extracellular glucose concentrations impair OA chondrocytes' capacity to regulate glucose uptake by downregulating glucose transporters, which leads to glucose accumulation and increased production of reactive oxygen species (ROS), both of which accelerate the development of OA and encourage degenerative changes [7]. AGEs build up in cells and tissues as a result of hyperglycemia, which causes oxidative stress and proinflammatory disorders [7]. Additionally, AGEs increase the synthesis and release of interleukin-6 (IL-6) and trigger inflammatory pathways in human OA chondrocytes through the AGE receptor (RAGE) and toll-like receptor [8]. Additionally, a number of matrix metalloproteinases are enhanced in diabetic OA patients, which accelerates cartilage deterioration [9].

Current DOA therapy approaches are only able to reduce clinical symptoms; they are unable to halt or delay the progression of DOA. Joint arthroplasty is an effective treatment for late-stage DOA [10]. An increasingly significant social and public health issue is the rising prevalence rate of DOA [11]. For the treatment of DOA, it is necessary to create therapeutic agents that are both effective and efficient.

Melatonin, an endogenous hormone, could be one such chemical. In a number of inflammatory autoimmune disorders, melatonin has positive antioxidant and anti-inflammatory properties [12]. Melatonin's binding to high-affinity specific receptors (MT1 and MT2) allows it to stimulate signaling pathways and regulate physiological effects, which in turn facilitates its involvement in important physiological processes in mammals, such as the circadian rhythm, oncogenesis, and immune defense activities [13]. It has been proposed that melatonin influences insulin sensitivity and beta-cell insulin production, which in turn alter glucose metabolism. Melatonin receptors MT1 and MT2 are expressed by pancreatic alpha and beta cells, as is widely known, and there is evidence that melatonin's effect on these receptors regulates insulin secretion and glucagon release throughout the day [14].

There is paucity of data on the effect of melatonin on DOA; hence, this study is aimed to investigate the effect of melatonin on diabetes-osteoarthritis in male Wistar rats.

METHODOLOGY

Animals:

Thirty (30) male Wistar rats weighing between 175 and 200 g were procured from the Animal Household, Biochemistry Department, University of Ilorin, Nigeria. The Bioresources Hub's animal facilities in Ilorin were used to house and acclimatized the experimental animals for a week.

Ethical Approval:

Approval was obtained from the University of Ilorin Ethical Review Committee (UERC/ASN/2024/2907).

Chemicals and Drugs:

Sigma-Aldrich, USA, provided sodium monoiodoacetate and melatonin. Additionally, Nicholas Piramal Ltd. in India was the supplier of metformin. Elabscience Biotechnology Inc., located in Texas, USA, produced kits for interleukin-1β, tumor necrosis factor-alpha, caspase-3, and lipid profile. Bridge Biotechnology Ltd. provided additional materials and chemicals of analytical quality.

Animal Grouping, Induction of Osteoarthritis and Treatment

As indicated in table 1, thirty male Wistar rats were divided into five (5) groups (n=6).

The rats were given a high-fat diet (HFD) for 39 days [15]. Streptozotocin (STZ) (65 mg/kg body weight (b.w.)) and nicotinamide (NAD) (110 mg/kg b.w., i.p.) in normal saline were given intraperitoneally (i.p.) to overnight-fasted rats in a freshly made cold solution of citrate buffer [16]. The Accu-Check Active glucometer was utilized to measure the rats' fasting blood glucose three (3) days later, and rats having a blood glucose level of greater than 135 mg/dl were classified as diabetics [17]. Additionally, as mentioned below, local knee osteoarthritis was induced. The sham rats received 0.5 ml of normal saline intra-articularly, while the control rats received 0.5 ml orally.

Ketamine and xylazine were administered to the rats in 50/10 mg/kg body weight respectively to anaesthetize them, in order to induce osteoarthritis in the knee [18]. 75% ethanol was used to swab and shave the back of the left knee's skin surface. A 27 gauge needle was then used to inject 2 mg of sodium monoiodoacetate intra-articularly into the rats' left knee joint space after it had been mixed with 50 µl of saline [19]. For 21 consecutive days, the MEL-treated group received 100 mg/kg b.w of melatonin per oral, [20], and the metformin-treated group received 180 mg/kg b.w of metformin per oral, [21].

Table 1: Experimental Animal Groups

S/N	Groups (n=6)	Treatment
1.	Control	0.5 ml of normal saline
2.	Sham	0.5 ml of normal saline intra-articularly
3.	DOA untreated	HFD + STZ (65 mg/kg + 2 mg of MIA intra-articularly [19]
4.	DOA + Melatonin	HFD + STZ + 100 mg/kg of Melatonin orally; [20]
5.	DOA + Metformin	HFD + STZ + Metformin 180 mg/kg of metformin orally; [21]

HFD: High fat diet, STZ: Streptozotocin, DOA: Diabetes-osteoarthritis.

Determination of Knee Circumference

With the aid of a Vernier caliper, the area of swelling in the knee joint was precisely measured post-MIA administration.

Sample preparations

All rats received ketamine/xylazine anesthesia (50/10 mg/kg b.w;) [18] after a 21-day treatment period. Blood samples were collected via retro-orbital and centrifuged. Prior to collecting the synovial fluid, a sterile syringe was used to inject 500 µl of phosphate buffer (PBS) into the rat knee articular joint cavity. The fluid was then aspirated out after the PBS was administered and removed five times. Following centrifugation of the synovial fluid, the supernatants were kept cool.

Determination of Insulin Resistance

Homeostatic model assessment-insulin resistance (HOMA-IR) = Fasting blood glucose (mmol/L) X Fasting serum insulin (pmol/L)/22.5 was the formula used to compute the insulin resistance score.

Determination of Biochemical Parameters

Lipid profile

Triglycerides (TG), total cholesterol (TC), and very low density lipoprotein (VLDL) were determined using enzyme-linked immunosurbent assay (ELISA) kit according to the manufacturer's instructions.

Tumor Necrosis Factor-Alpha

The principle of Sandwich-ELISA was applied. There was a pre-coating of an antibody specific to Rat TNF-α on the micro-ELISA plate included in the kit. The standard, blank, and sample dilutions of 100 µL each were applied to the proper wells. After covering the plate with the sealer included in the kit, it was incubated at 37^0C for 90 minutes. In order to minimize foaming, there was addition of solutions to the base of ELISA plate's well without coming into contact with the inner wall. The liquid was taken out of each well without being cleaned. Each well received 100 µl of the BDA working solution. A fresh sealer was used to cover the plate, and incubation was done at 37^0C for one hour. Wash buffer (350 µL) was introduced after removing each well's solution, it was allowed to soak for one minute, and then the solution was removed and dried by patting it on fresh absorbent paper. This wash phase was carried out three times. After this, the tested strips were used right away. The wells were not allowed to run dry. After adding HRP Conjugate working solution (100 µL) to each well and covering the plate with a new sealer, the plate was incubated at 37^0C for half an hour. The solution was poured out of every well, and the procedure of washing stated above was carried out five times. There was an addition of substrate reagent (90 µL) to every well, and the plate was shielded from light. The reaction time had a minimum of thirty minutes. Before measuring the OD, the micro-plate reader was warmed for roughly fifteen minutes. There was an addition of Stop Solution (50 µL) to each well. The substrate solution and the stop solution were added in the same sequence. Using a micro-plate reader set to wavelength 450 nm, each well's OD value was immediately determined.

Determination of Interleukin-1beta (IL-1β)

The principle of Sandwich-ELISA was applied. There was a pre-coating of an antibody specific to Rat IL-1β on the micro-ELISA plate included in the kit. The sample, blank, and diluted standard wells were identified. The standard, blank, and sample dilutions of 100 µL each were applied to the corresponding wells. After covering the plate with the sealer included in the kit, it was incubated at 37^0C for 90 minutes. In order to minimize foaming, there was addition of liquid to the base of ELISA plate's well without coming into contact with the inner wall. The liquid was taken out of each well without being cleaned. Each well received BDA working solution (100µl). A new sealer was used to cover the plate, and incubation was done at 37^0C for one hour. Wash buffer (350µL) was added after removing each well's solution, it was allowed to soak for one minute, and then the solution was removed and dried by patting it on fresh absorbent paper. This wash phase was carried out for three times. After the wash phase, the tested strips were used right away. The wells were prevented from getting dry.

After adding HRP Conjugate working solution (100µL) to each well and covering the plate with a new sealer, the plate was incubated at 37^{0}C for half an hour. Each well's solution was decanted, and the wash procedure was carried out five times. After adding substrate reagent (90µL) to every well and covering the plate with a new sealer, the plate was incubated at 37^{0}C for approximately 15 minutes while being shielded from light. The reaction time had a minimum of thirty minutes. Before measuring the OD, the micro-plate reader was warmed for roughly fifteen minutes. There was an addition of Stop Solution (50µL) to every well. The substrate solution and the stop solution were added in the same sequence. Using a micro-plate reader set to wavelength 450 nm, each well's OD value was immediately determined.

Determination of Caspase-3

The CLIA kit (Elabscience Biotechnology Inc., Texas, USA) was used to measure the amount of Caspase-3 (CASP-3). In this CLIA kit, the Sandwich-CLIA concept was used. This package included an antibody specific to Rat CASP-3 that was used to pre-coat the micro CLIA plate. After adding 100 µL of the sample or standard to each well, the wells were incubated at 37°C for one hour and thirty minutes. The solution was decanted. The mixture was incubated for one hour at 37°C after 100 µL of BDA was added. Aspiration and washing were done three times. After adding 100 µL of HRP Conjugate, the mixture was incubated for 30 minutes at 37°C. Aspirations and washing were carried out five times. 100µL of the Substrate Mixture Solution was added. At 37°C, the incubation period lasted five minutes. Every well's relative light unit (RLU) value was ascertained right away, and the outcome was calculated.

Determination of A Disintegrin and Metalloproteinase with Thrombospondin-5 (ADAMTS-5)

The wells for the diluted standard, blank, and sample were identified. After adding 100 µL of Standard Working Solution or 100 µL of samples to each well, the plate was covered and incubated for 80 minutes at 37°C. The liquid was then poured out of each well, aspirated, and then washed with 200 µL of 1× wash solution, which was left in each well for 1-2 minutes. The remaining liquid was removed from each well by snapping the plate onto absorbent paper, and the plate was thoroughly cleaned three times. Following the final wash, any leftover Wash Buffer was removed by decanting or aspirating. After being inverted, the plate was blotted against paper that was absorbent. Each well received 100µL of biotinylated antibody working solution, which was then covered with the plate and incubated at 37°C for 50 minutes. Three rounds of washing and aspiration were performed. Each well received 100µL of streptavidin-HRP working solution, which was then sealed with plate sealer and incubated for

50 minutes at 37°C. Aspiration was repeated, and washing was done five times. Each well received 90μL of TMB Substrate Solution. A fresh plate cover was placed over it. The incubation took place in the dark at 37°C for 20 minutes. The addition of TMB substrate solution caused the liquid to become blue. Before measuring the OD, the microplate reader was warmed for roughly fifteen minutes. Each well received 50μL of StopReagent. When the stop reagent was added, the liquid turned yellow. The side of the plate was tapped to mix the liquid. The measurement at 450 nm was then done right away using the microplate reader.

Statistical Analysis

Statistical analysis was carried out on data obtained by using graph pad prism 9.0 version for Windows (GraphPad® Software, San Diego, CA, USA), through which data were subjected to one-way ANOVA and Newman-Keuls *post-hoc*. Two-way ANOVA was used to analyse knee circumference. Data were presented as mean ± standard error of mean (S.E.M). Values were statistically significant at the level of $p<0.05$.

RESULTS

Figure 1 shows the effect of melatonin on knee circumference (KC) of DOA Wistar rats. Prior to treatment (day 1 to 7), there is a significant increase in the KC of all the groups except the control, but from day 4 to 7, the KC of the control also increases. However, a significant decrease in KC is observed in all the treated group from day 7 to 28 which correspond to the treatment period. On the last day of treatment there was a significant decrease in the KC of the melatonin groups compared to the DOA group.

Table 2 shows the effect of melatonin on the fasting blood glucose levels of DOA Wistar rats. There is a significant increase (from the initial to final fasting blood glucose level) in the DOA Wistar rats compared to the control rats, however, a slight decrease is observed in the fasting blood glucose level of the melatonin treated rats.

Table 3 shows the effect of melatonin on the insulin resistance (IR), serum total cholesterol (TC), triglycerides (TG), and very low-density lipoprotein (VLDL) levels in DOA Wistar rats. There is a significant increase in the IR, TC, TG, and VLDL levels of the DOA rats compared to the control rats, however, administration of melatonin significantly reversed the trends.

Table 4 shows the effect of melatonin on the synovial pro-inflammatory markers and serum caspase-3 levels, and serum A Disintegrin and Metalloproteinase with Thrombospondin Motifs (ADAMTS-5) activity in the DOA Wistar rats. There is a significant increase in the TNF-α, IL-1β, and caspase-3 levels, as well as the activity of ADAMTS-5 of the DOA Wistar rats compared to the control. However, the administration of melatonin reversed the trends.

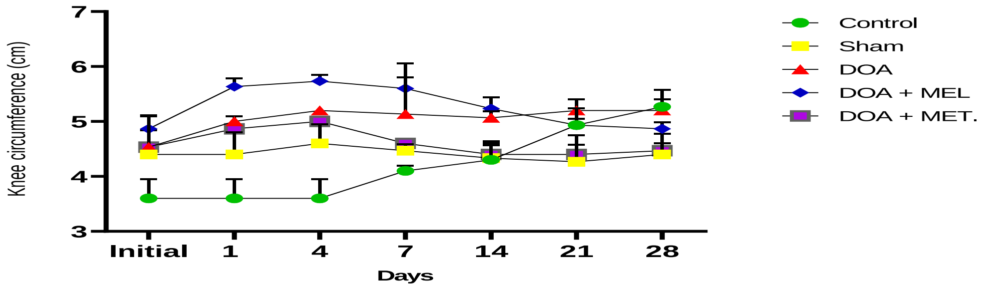

Figure 1: The Effects of Melatonin on Knee Circumference in Diabetes-Osteoarthritis Male Diabetes Wistar rats.
Data are presented as Mean ± SEM, n = 6, DOA, Diabetes-osteoarthritis; MEL, Melatonin; MET, Metformin

Table 2: The Effects of Melatonin on Fasting blood glucose Level in Experimental Osteoarthritis Male Diabetes Wistar rats

Groups	Initial Fasting Blood Glucose (mg/dl)	Final Fasting Blood Glucose (mg/dl)
Control	104.50 ± 0.55	105.50 ± 0.35
Sham	255.50 ± 0.35	278. 00 ± 0.28
DOA	174.50 ± 0.10	305.50 ± 0.25
DOA + Melatonin	290.00 ± 0.15	287.50 ± 0.12
DOA + Metformin	298.50 ± 0.50	190.00 ± 0.30

Data are presented as Mean ± SEM, n = 6, DOA, Diabetes-osteoarthritis

Table 3: The Effects of Melatonin on Insulin Resistance and Lipid Profile Level in Experimental Osteoarthritis Male Diabetes Wistar rats

Groups	Insulin Resistance	Total Cholesterol (mmol/l)	Triglycerides (mmol/l)	Very Low-Density Lipoproteins (mmol/l)
Control	15.13 ± 1.43	1.96 ± 0.09	0.75 ± 0.02	0.15 ± 0.01
Sham	61.60 ± 4.86	2.23 ± 0.05	0.84 ± 0.02	0.18 ± 0.01
DOA	61.67 ± 4.81[a]	2.93 ± 0.02[a]	0.92 ± 0.01[a]	0.19 ± 0.01[a]
DOA + Melatonin	48.57 ± 2.37[b]	1.98 ± 0.09[b]	0.78 ± 0.02[b]	0.16 ± 0.01[b]
DOA + Metformin	29.27 ± 0.73[c]	1.99 ± 0.02[b]	0.73 ± 0.02[b]	0.15 ± 0.01[b]

Data are presented as Mean ± SEM, n = 6; [a]$p < 0.05$ vs. control; [b, c] $p < 0.05$ vs. DOA. DOA, Diabetes-osteoarthritis

Table 4: The Effects of Melatonin on Pro-inflammatory Markers, Caspase-3, and ADAMTS-5 in Experimental Osteoarthritis Male Diabetes Wistar rats

Groups	TNF-alpha (pg/ml)	IL-1β (pg/ml)	Caspase-3 (ng/ml)	ADAMTS-5 (ng/ml)
Control	53.89 ± 0.40	248.70 ± 8.95	0.64 ± 0.04	0.10 ± 0.01
Sham	82.27 ± 5.11	345.10 ± 16.91	3.66 ± 0.07	0.20 ± 0.00
DOA	94.39 ± 3.50[a]	420.30 ± 28.89[a]	4.12 ± 0.07[a]	0.21 ± 0.01[a]
DOA + Melatonin	67.78 ± 3.48[b]	288.90 ± 24.52[b]	2.67 ± 0.26[b]	0.12 ± 0.01[b]
DOA + Metformin	53.89 ±0.40[c]	254.00 ± 10.03[b]	2.47 ± 0.16[b]	0.13 ± 0.00[b]

Data are presented as Mean ± SEM, n = 6; [a]$p < 0.05$ vs. control; [b, c]$p < 0.05$ vs. DOA. DOA, Diabetes-osteoarthritis; TNF-alpha, Tumor necrosis factor-alpha; IL-1β, Interleukin-1 beta; ADAMTS-5, A Disintegrin and Metalloproteinase with Thrombospondin Motifs.

DISCUSSION

The present study investigated the effect of melatonin on diabetes-osteoarthritis male Wistar rats by examining the lipid profile and glucose parameters, and estimating some biochemical parameters.

Osteoarthritis (OA) has been reported to be associated with behavioural alterations [22]. The behavioural alteration observed in this study, such as increase in knee circumference in the DOA-induced rats might be as a result of inflammation [23]. However, the improvement in the behavioural test in the intervention groups observed in this study might be due to the anti-inflammatory properties of MEL [24, 25].

DM has been reported to be characterized by insufficient insulin secretion and insulin resistance [26]. Recent research has shown that OA is linked to systemic metabolic abnormalities frequently observed in type-2 diabetes mellitus (T2DM) [27] as observed in our study. The significant reduction in insulin resistance observed in the melatonin groups might be as a result of ability of the melatonin to protect the beta-cells from apoptosis [28], stimulate hepatic glycogen synthesis [29], and suppresses hepatic gluconeogenesis [30].

DOA is usually associated with a change in the lipid profile with increases in triglycerides, total cholesterol, LDL levels and decrease in HDL level [15, 31]. From this study, the improvement of lipid profile by MEL may be explained by its anti-hyperlipidemia activity; reduction in intestinal absorption of cholesterol and inhibition of the biosynthesis of cholesterol [32].

Increased inflammatory mediator release, such as IL-1β and TNF-alpha observed in DOA-induced rats has been reported to be responsible for the development of proteolytic enzymes such as MMP-13, which cause cartilage damage [33]. Collectively, pro-inflammatory cytokines are powerful catabolic factors in synovial fluid, which activate OA process through up-regulation of catabolic and inflammatory responses and causes cartilage matrix degradation [34]. However, in this study, the decrease in inflammatory mediator release in the melatonin administered group might be due to the anti-inflammatory properties of MEL as well as its anabolic and anti-catabolic effects as reported by Lu et al. [35].

It has been reported that a decrease in the levels of COL-2 act as a predisposing or contributing factor to OA progression [36]. ADAMTS-5 are particularly related to the degradation of COL-2 in articular cartilage [37]. This is evident in this study as there is a significant increase in the ADAMTS-5 activities in the DOA-induced rats. However, the significant decrease in the ADAMTS-5 activity observed in the melatonin group from this study might be as a result of melatonin ability to inhibit the activity of ADAMTS-5 as reported by Lu et al. [35].

Moreover, it has been reported that apoptosis of chondrocytes can cause irreversible damage to the articular cartilage [38]. This could be through ability of ROS to open the mitochondrial permeability transition pore and releasing apoptogenic factors, such as cytochrome c that activate the caspase cascade [39]. In this study, the action of melatonin in mitigating the DOA-induced apoptosis was evidenced by significant down-regulation of caspase dependent apoptosis. This might be due to its anti-apoptotic activities [40, 41].

CONCLUSION

Melatonin improves gluco-regulatory and chondroprotective effects in diabetes-osteoarthritis male Wistar rats by modulating inflammatory and apoptotic processes, and decreasing the activities of cartilage and bone degrading enzyme. Melatonin could be used for the treatment of DOA after its validation through clinical trials.

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FALLS PREVENTION IN HOSPITAL SETTINGS FOR OLDER ADULTS: A NARRATIVE REVIEW

Short Running Title: Falls Prevention in Hospital

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ABSTRACT:
Falls among older adults in hospital settings are a significant concern for patient safety, leading to increased morbidity, prolonged hospital stays, and higher healthcare costs. This narrative review explores current practices and strategies for falls prevention in hospital environments, emphasizing policy development, risk assessment, and multidisciplinary approaches. Risk factors for falls include intrinsic factors such as impaired mobility and cognitive decline, and extrinsic factors such as poor design and environmental hazards. Effective fall prevention begins with structured screening and assessment processes, shifting from predictive scoring tools to individualized risk management.
A safe environment with adequate lighting, non-slip flooring, and ease of access to appropriate assistive devices is important to minimize fall risks. Universal fall prevention strategies such as patient education and proper bed positioning are necessary but should be complemented by targeted interventions for high-risk patients. A multidisciplinary approach incorporating nurses, physiotherapists, occupational therapists, pharmacists, and geriatricians ensures a comprehensive strategy for fall prevention. Hospital-wide policies aligned with international safety standards are also needed to support the standardized implementation of falls prevention initiatives. A proactive and evidence-based approach to falls prevention improves patient safety, optimizes healthcare resource utilization, and enhances the quality of care for hospitalized older adults.

Keywords: aged, falls, inpatients, hospitals, risk assessment

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INTRODUCTION

Falls are the second leading cause of unintentional injury deaths worldwide, with older adults being particularly vulnerable. According to the World Health Organization, up to 35% of those aged 65 years and older fall each year, increasing to 42% for those over 70 years old [1]. Falls and associated injuries occur commonly in older people and are a major cause of pain, disability, loss of independence and death. The impact of falls in hospital settings extends beyond immediate physical harm. While falls can lead to fractures, head injuries and reduced mobility; psychological distress, including fear of falling again further limits a patient's functional independence and put the older person at risk of admission into residential care [2].

Falls-related injuries also contribute to increased hospital costs due to longer hospital stays due to additional treatment and rehabilitation needs [1,3]. A hospital registry found that one-third of falls caused injury, with a large proportion of falls happening at the beginning of the admission into hospital, suggesting a need for early preventive approaches [4]. Hospitals must, therefore, implement comprehensive falls prevention programs to ensure patient safety and optimize healthcare resources.

The Joint Commission International (JCI) Accreditation Standards for Hospitals requires hospitals to achieve International Patient Safety Goal (IPSG) 6 to 'reduce the risk of patient harm resulting from falls. The measurable elements to achieve this goal are to ensure there is a process for assessing falls risk in inpatients and outpatients using appropriate tools, a process for reassessment of inpatients at risk due to existing risk factors or a change in condition, and interventions to reduce fall risk for at-risk patients are implemented and documented in a fall prevention plan [5].

This paper reviews the best practices and strategies for falls prevention in hospitals, emphasizing the need for policy formulation, risk assessment, and multidisciplinary involvement.

Definition and Risk Factors for Falls

A fall is defined as an event that results in a person unintentionally coming to rest on the ground or a lower level, not caused by a major intrinsic medical event such as a stroke [6]. Falls in hospitals are often linked to intrinsic factors, such as impaired mobility, cognitive impairment, medication side effects, and frequent toileting needs. Extrinsic factors include poor hospital design, inadequate lighting, cluttered spaces, and lack of assistive devices. Understanding these risk factors is essential in designing effective falls prevention strategies [7,8].

In one study, falls occurred more frequently in older females in medical wards, with adverse events usually occurring during the night shift [9]. Another study found that a significant proportion occurred at the bedside with around half of all falls related to elimination [10]. A review of falls screening tools, such as STRATIFY, Tinetti, Morse and Hendrich found that the predominant risk factors for falls varies depending on the study and type of patients in the clinical service. Thus, there is a need for each locality to validate the tools and ensure its predictive ability [11]. There is a move away from scoring tools that allocate points to predict an older person's risk for falls; instead, healthcare professionals should identify patients at risk for falls, identify modifiable risk factors and manage them to reduce future falls risk [11].

Falls Risk Screening and Assessment

Effective falls prevention begins with identifying patients at risk. The hospital may incorporate visual triage screening to identify at-risk patients upon arrival. Trained health staff, including front counter personnel and nurses, should be able to observe a person’s gait and highlight those at possible risk of falls. For example, if a staff member observes an abnormal gait or posture, such as ataxia, proactively offering support with assistive devices or referrals to physiotherapy may reduce the risk of subsequent falls [11].

For those without obvious gait abnormalities, a simple screening tool recommended by the Centre for Disease Control and Prevention (CDC) consists of three screening questions:

- Have you fallen in the past year? (a) If yes, how many times? (b) Were there any injuries from the fall?
- Do you feel unsteady when standing or walking?
- Do you worry about falling?

If the person answers ‘Yes’ to any of these questions, an assessment to identify modifiable risk factors will be initiated, including evaluation of gait, strength and balance. Those who had multiple falls or sustained injuries from a fall should be assessed and managed sooner, given their higher risk of subsequent injurious falls [12]. For patients who did not have their gait observed as part of the visual triage screening, the Get-Up-and-Go test can be performed [13]. Essentially, the patient is asked to stand up from a chair or bed, walk a short distance, turn around and return back to the seated position. The assessor should observe for indicators of possibly falling, such as undue slowness, hesitancy, abnormal movements of trunk or limbs, staggering or stumbling [13].

In some settings, it is reasonable to assume that all patients are high falls risk and implement fall prevention interventions as well as assessments for modifiable risk factors. This may include locations such as Geriatrics rehabilitation wards or orthogeriatric wards for patients who sustained neck of femur fractures [14,15], or specific subgroups of patients, such as frailty or delirium [16,17]. While it should be intuitive that patients with neck of femur fractures are high falls risk, audits of patients with neck of femur fractures show that falls assessment are sub-optimally performed [15,18]. Thus, a consistent approach for falls prevention incorporated into a Standard Operating Procedure may be effective in reducing falls risk in these high-risk settings.

Environmental Modifications

Creating a safe hospital environment is important for preventing falls. Key environmental interventions include installing handrails and ramps where necessary, providing wheelchairs at strategic locations, using non-slip flooring, and securing loose rugs or carpets. Marking visible indicators for steps and uneven surfaces, installing emergency call bells within patient reach, and maintaining hospital equipment are also essential measures. Ensuring that hospital furniture, such as beds and chairs, is adjustable to accommodate older adults' needs can further reduce the risk of falls [19].

In addition to structural modifications, staff should be trained to assess the environment regularly and identify hazards that may increase fall risks. Hospitals can implement regular safety checks, ensuring that areas frequently accessed by older adults remain clear of clutter and tripping hazards. Hospital corridors should have clear signage and resting areas with chairs to support older adults who may experience fatigue when moving around.

Environmental audits carried out periodically may be useful to identify providing insight into areas that may cause falls risk. For example, in a hospital that assessed the adequacy of lighting as a safety consideration, it was found that poorly lit areas were potential hazards, such as sinks, toilets and hot water dispensers. In addition, while the wards were well-lit, luminance fell below recommended levels when the curtains were drawn [20]. An audit of bathrooms in medical wards identified that shower chairs had to be repositioned to avoid users unnecessarily reaching out for different items, such as the shower head, soap and clothes [21].

Standard Safety Measures

All older patients, regardless of fall risk, benefit from standard safety measures. Keeping beds at a low height with brakes engaged, ensuring easy access to personal items such as water and mobility aids, and educating patients and caregivers on the importance of fall prevention are necessary. Staff should review the patients' footwear, ensuring they wear non-slip, well-fitted shoes to reduce fall risk. Patients receiving sedation or general anesthesia should be closely monitored, as they may be temporarily unsteady. Universal fall precautions are listed in Table 1.

While universal fall prevention approaches make sense, evidence for their effectiveness is limited. However, the literature supports determining specific risk factors and individualized intervention towards multifactorial fall risk factors [22]. These care plans should incorporate fall prevention strategies tailored to the specific needs of the older adult, including scheduled toileting assistance, use of mobility aids, and physical therapy interventions [23]. Table 2 shows an example of a management plan based on falls risk factors identified.

Multidisciplinary Involvement

An effective falls prevention program requires a collaborative approach involving various healthcare professionals. Nurses play a primary role in patient monitoring, risk assessment, and education. Physiotherapists and occupational therapists assist in mobility training and recommend assistive devices. Pharmacists review medications that may contribute to falls risk, such as sedatives and antihypertensives. Geriatricians provide specialized care for older patients at high risk of falls.

Hospital administrators ensure the implementation of hospital-wide policies and staff training programs. In addition, other specialties who care for patients with high falls risk such as post-surgery recovery may need specific discussions and falls prevention plans for their population. For example, diabetes nurse educators encounter patients who may have diabetes-related complications such as peripheral neuropathy and retinopathy, resulting in falls risk [24].

Hospitals should also establish interdisciplinary falls prevention committees to review incidents and periodically update prevention strategies. These committees can meet regularly to discuss challenges in implementation, review fall-related data, and develop targeted interventions to address recurring risk factors [25]. Such discussions that reinforce fall prevention and awareness with clinicians are also useful in reducing falls incidence in hospitals [26]. Engaging caregivers and family members in discussions on falls prevention can also help reinforce hospital measures and improve adherence to safety guidelines post-discharge.

Policy Formulation and Stakeholder Engagement

A structured falls prevention policy is required to ensure consistent hospital-wide practices. Policies should include guidelines for falls risk screening and assessment, standardized safety protocols, training programs for healthcare staff, reporting and documentation procedures for fall incidents, regular audits with quality improvement initiatives. Key stakeholders such as hospital management, department heads, nurses, and allied health professionals should be actively involved in policy development to ensure practicality and adherence [25].

Hospitals should also develop clear protocols for responding to falls when they occur. These protocols should outline immediate medical assessment procedures, documentation requirements, and follow-up interventions to prevent recurrent falls. Staff training programs should include simulation exercises on fall response, enabling healthcare professionals to react swiftly and effectively when managing fall-related incidents [27]. It is also useful to introduce concepts such as fall prevention for healthcare related professionals as early as undergraduate level [28].

Special Considerations for Older Patients

Older adults are particularly vulnerable due to age-related physiological changes such as muscle weakness, impaired balance, and cognitive decline. Additional interventions for elderly patients include encouraging mobility exercises to maintain strength and balance, providing bedside assistance during toileting, ensuring proper hydration and nutrition to prevent dizziness and weakness, and conducting regular medication reviews to minimize falls-related side effects [29,30].

Cognitive impairment further increases fall risk in hospitalized older adults. Patients with dementia or delirium may be unable to recognize hazards, follow safety instructions, or call for assistance when needed. Implementing close observation protocols, designating fall-safe patient areas, and using non-restrictive monitoring technologies can help reduce the incidence of falls in cognitively impaired older adults. Healthcare professionals should also be trained to recognize early signs of delirium and provide interventions that stabilize cognition, such as orientation cues and consistent caregiver assignments [31].

Challenges and Future Directions

Despite extensive research and guidelines on falls prevention, hospitals continue to face challenges in implementation. Barriers include staff workload, resistance to change, inadequate training, and limited funding for infrastructure improvements [32]. Future strategies should focus on integrating digital health solutions, such as sensor-based monitoring systems, implementing artificial intelligence for fall risk prediction, enhancing staff training through e-learning modules, and strengthening community-hospital partnerships to support post-discharge fall prevention. Innovations such as wearable fall detection devices and automated alert systems can provide real-time monitoring and improve response times to fall incidents [33]. Further research is needed to evaluate the effectiveness of emerging technologies and develop cost-effective strategies that hospitals can adopt on a scale.

Table 1: Universal Fall Precautions

Familiarize the patient with the environment.
Have the patient demonstrate call light use.
Maintain call light within reach.
Keep the patient's personal possessions within patient’s safe reach.
Have sturdy handrails in patient bathrooms, rooms, and hallway.
Place the hospital bed in low position when a patient is resting in bed
Raise the bed to a comfortable height when the patient is transferring out of bed.
Keep hospital bed brakes locked.
Keep wheelchair wheel locks in "locked" position when stationary.
Keep nonslip, comfortable, well-fitting footwear on the patient.
Use night lights or supplemental lighting.
Keep the surface of the floor clean and dry. Clean up all spills promptly.
Keep patient care areas uncluttered.
Follow safe patient handling practices.

Table 2: Management Plan for Falls Risk Factors

√	Risk Factor	Management Plan for Falls Risk Factors
	Confusion / Impulsivity	Frequent checks and close to nurses' stations Encourage family to accompany Constant reorientation to time, person, place
	Depression	Assess mood Consider referral to Psychology / Psychiatry if required
	Dizziness / Vertigo	Check postural BP
	Limited mobility	Regular toileting - Schedule 2-4 hourly Supervise/Assist transfer or mobility Walking aid at bedside if needed Environmental Assessment Review for Falls Risks
	Frequent Toileting	
	Visual impairment	
	Medications	Nurses to advise doctor for medication review if patient is on Benzodiazepines, Sedatives and Antiepileptic medications

CONCLUSION

Falls prevention in hospitals is a crucial component of patient safety. Through systematic falls risk screening, environmental modifications, standard safety measures, and multidisciplinary involvement, hospitals can significantly reduce falls incidence and associated adverse outcomes.

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POST MARKETING OBSERVATIONAL STUDY OF FIXED DOSE COMBINATION OF RIFAXIMIN AND METRONIDAZOLE IN THE MANAGEMENT OF ACUTE DIARRHEA IN CHILDREN

Running title: Rifaximin and Metronidazole FDC in Children

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ABSTRACT

Acute diarrhea is a frequent illness in children, especially in developing nations, posing risks of dehydration, malnutrition, and even death. Recently, there's been rising interest in Rifaximin and Metronidazole Fixed Dose Combination (FDC) as a treatment option for pediatric acute diarrhea. The aim of this study was to evaluate the efficiency and tolerability of rifaximin and metronidazole fixed-dose combination in the management of acute diarrhea in children. This was a multicenter, open-label, non-comparative, and non-randomized trial that enrolled 80 patients (age <18 years) experiencing acute diarrhea caused by various factors. This observational study was conducted post-approval of drugs by state Food and Drug Administration (FDA), therefore informed consent and ethics approval were not mandatory. Patients received suspension containing 100mg rifaximin and 200mg metronidazole per 5ml, twice daily for 5 days based on body weight. The primary endpoint assessed was change in frequency of loose/watery stools, fever, nausea, vomiting, abdominal pain, and gas/flatus from baseline to day 5. Statistical analysis using the 'paired t-test' was performed. A total of 64 patients were included in the final analysis with 16 patients lost to follow-up. The average number of watery stools per day decreased significantly from 7.0±1.49 to 2.0±0.66 ($P < 0.001$) after a 5-day therapy. None of the patients experienced fever, nausea, vomiting, abdominal pain or gas/flatus at the end of the study. Rifaximin and Metronidazole FDC demonstrates significant reduction in frequency of watery stools and alleviation of associated symptoms, establishing it as a clinically effective and safe choice for empirically managing acute diarrhea in children stemming from diverse causes.

Keywords: Acute diarrhea, mixed gastrointestinal infection, rifaximin, metronidazole, fixed dose combination.

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INTRODUCTION

Diarrhea is defined as the passage of three or more loose or liquid stools per day and is a leading cause of child mortality and morbidity worldwide, largely due to contaminated food and water [1]. It results in approximately 443,832 deaths annually among children under five and 50,851 among those aged 5 to 9. Access to safe drinking water, sanitation, and hygiene can significantly reduce cases. There are nearly 1.7

billion reported cases of childhood diarrhea each year [1], primarily caused by viruses, bacteria, and parasites. Common bacterial culprits include E. coli, Shigella spp., Salmonella spp., and Campylobacter spp., while Giardia and Entamoeba are notable protozoan parasites [2]. Effective strategies to combat diarrhea include improving water quality, sanitation, hygiene education, and food safety. Treatments like oral and intravenous rehydration therapy have been crucial since the 1970s, and antibiotics are used to alleviate symptoms, reduce illness duration, and prevent transmission [3].

Rifaximin, a semi-synthetic derivative of rifamycin, functions by binding to the β-subunit of bacterial DNA-dependent RNA polymerase, thereby inhibiting bacterial RNA synthesis. This mechanism gives rifaximin a broad spectrum of activity against aerobic and anaerobic Gram-positive and Gram-negative bacteria [4]. It effectively targets enteric pathogens such as Clostridium difficile and Helicobacter pylori. Due to its limited systemic absorption, rifaximin primarily exerts its antimicrobial effects in the gastrointestinal tract, which reduces the risk of developing antimicrobial resistance and systemic side effects. Rifaximin is well-tolerated across various patient groups, including young children. Notably, bacterial resistance to rifaximin during treatment has shown minimal potential and, if present, is typically unstable within the intestinal flora. Additionally, rifaximin minimally impacts both Gram-positive and Gram-negative flora in the colon [4].

Metronidazole is an antimicrobial agent with a longstanding history in clinical use spanning over 45 years. It has proven effective in treating protozoal infections like amoebiasis and giardiasis. The drug demonstrates potent activity against gram-negative anaerobic bacteria such as Bacteroides fragilis and gram-positive anaerobic bacteria like Clostridium difficile. Metronidazole is recognized for its favourable pharmacokinetic and pharmacodynamic profiles, robust efficacy against anaerobic pathogens, minimal adverse effects, and cost-effectiveness [5]. Based on the evidence presented, the present study suggests that a FDC of rifaximin and metronidazole is a promising treatment option for effectively managing acute diarrhea and its related symptoms in children.

METHODOLOGY

Study design and Patients

This study, conducted in 8 clinics across diverse regions of India, was an open-label, non-comparative, non-randomized, multicenter trial involving 80 patients. Children (<18 years) were eligible if they were experiencing acute diarrhea, while exclusion criteria encompassed known or suspected hypersensitivity to trial-related medications, dysentery, colitis, gastrointestinal bleeding, renal or liver failure, cardiac conditions and pregnancy or lactation. Final analysis comprised 64 patients, with 16 lost to follow-up. The enrolled patients ranged in age from 3 to 15 years, with a mean age of 6.75 ± 2.81 years.

Ethical Considerations:

This was an observational study. The study was conducted after approval of the drug by the state FDA; therefore, ethical approval was not mandatory.

Treatment and duration of treatment:

Patients were administered Rifaxigyl-M suspension (Medley Pharmaceuticals Ltd.) containing Rifaximin 100 mg and Metronidazole 200 mg per 5 ml twice daily for 5 days. Dosage was based on their body weight at a dose of Rifaximin 10 mg/kg/day and Metronidazole 20 mg/kg/day.

Evaluation of the primary outcome measure

The following parameters were evaluated at baseline, day 3 and day 5 of the study. Number of soft or watery stools, Body temperature, Nausea, Vomiting, Abdominal pain and Gas/flatus.

Evaluation of the secondary outcome measure

Global assessment by the investigator on a three-point scale marked as Excellent/Good/Poor was used for efficacy and tolerability evaluation. Adverse events were recorded and the action taken was documented.

Statistical analysis

Statistical analysis was performed using the 'paired t test' for number of soft or watery stools per day to assess the change from baseline on day 5. The minimum level of significance was fixed at the 95% confidence limit and $P < 0.05$ was considered significant. All statistical analysis was performed using Graph Pad Prism 9 version 9.5.1.

RESULTS

A total of 64 patients were included for final analysis, with 16 patients lost to follow-up. The enrolled patients ranged in age from 3 to 15 years, with a mean age of 6.75 ± 2.81 years. Baseline and post-treatment clinical symptoms are shown in Table 1.

Number of soft or watery stools:

The number of stools per day was recorded at the start and end of the trial. Statistically significant reduction in the number of bowel movements was reported with rifaximin plus metronidazole fixed dose combination compared to baseline. The number of stools per day reduced from 7.0±1.49 to 2.0±0.66 ($P < 0.001$) which are depicted in Figure 1.

Other Parameters:

Of 64 patients, 35.93% reported fever, 23.43% reported nausea, 48.43% reported vomiting, 45.31% reported abdominal pain and 23.43% reported gas/flatus at baseline. None of the patients reported fever, nausea, vomiting, abdominal pain or gas/flatus at the end (day 5) of the trial.

Safety Evaluation

No adverse events were reported that led to the withdrawal of a patient from the study.

Global assessment of efficacy and tolerability:

According to investigators assessment of efficacy and tolerability of Rifaximin and Metronidazole FDC, 87.5% of the patients reported excellent and 12.5% of the patients reported good efficacy as well as tolerability.

DISCUSSION

Diarrhea commonly stems from complex gastrointestinal infections with diverse causes. Effective management of these infections often requires a combination therapy that includes a broad-spectrum antibiotic and an antiprotozoal medication. Even today, cost remains a significant factor, leading doctors to avoid frequent stool examinations. Instead, they often recommend empirical treatment targeting both bacteria and protozoa, common culprits due to prevalent unhygienic conditions in the country. This approach not only cuts down on therapy expenses but also minimizes the days of work missed and subsequent loss of wages due to diarrhea.

Rifaximin has shown efficacy and tolerability in several clinical studies and systematic reviews that included patients with traveler’s diarrhea [6], small intestinal bacterial overgrowth (SIBO) [7], diarrhea-predominant irritable bowel syndrome [8] and hepatic encephalopathy [9]. Rifaximin has also demonstrated promise in treating Clostridium difficile infection (CDI), particularly in cases of recurrent CDI [10]. Good evidence supports rifaximin use as a chemoprophylactic agent in traveler’s diarrhea, especially in people at high risk of severe complications from acute infectious diarrhea [11]. A short treatment course (3 days on average) with rifaximin oral suspension when compared to neomycin and bacitracin combination yielded highly satisfactory results in children suffering from severe episodes of bacterial diarrhea [12]. Similarly, another study compared oral pediatric suspension of rifaximin with paromomycin for the oral treatment of pediatric infectious diarrhea. The study concluded that rifaximin rapidly exerts its antimicrobial action, shows very good clinical effectiveness and can be used with complete safety, particularly for the treatment of younger children [13].

In cases of mixed infections, 73% of patients were predominantly diagnosed with protozoa such as Giardia lamblia, Entamoeba histolytica, and Cryptosporidium spp. [14]. Metronidazole remains effective against protozoal infections, supporting its use in treating conditions like trichomoniasis, amoebiasis, and giardiasis [5]. Therefore, it should be considered as a complement to rifaximin in the treatment of mixed infections. A similar study that evaluated the combination of rifaximin and metronidazole in adult patients with acute diarrhea concluded that the combination significantly reduces diarrheal episodes and associated symptoms [15]. A study reported by Gawronska et al showed similar effectiveness of metronidazole and rifaximin treatments for Clostridium difficile infection in pediatric patients (age between 12 and 18 years) with IBD [16].

In the present study, the FDC of rifaximin and metronidazole significantly reduced the number of soft or watery stools. At the end of the study, none of the patients reported fever, nausea, vomiting, abdominal pain or flatus. None of the patients withdrew from the study due to adverse events. Thus, based on clinical studies literature and present clinical data, rifaximin and metronidazole FDC suspension is a safe and effective treatment option for the empirical management of acute diarrhea in pediatric patients due to mixed infections.

Limitation of the study:

While the combination showed a notably positive response, a major limitation of the study was the absence of stool examinations for patients. Stool examinations were omitted due to the common mixed nature of gastrointestinal infections, and the additional cost to patients, particularly significant in households with low per capita income. Conducting routine stool examinations could have provided more unbiased study results, particularly in ruling out diarrhea caused by exclusive viral infections, which are often self-limiting illnesses.

CONCLUSION

Addressing acute diarrhea in children poses a substantial challenge in India. Seeking a more effective and safer treatment approach, the fixed-dose combination of rifaximin and metronidazole suspension emerges as a promising addition to the therapeutic arsenal. This combined therapy has demonstrated a significant decrease in diarrhea frequency and associated symptoms, showcasing outstanding efficacy and tolerability. Consequently, the FDC of rifaximin and metronidazole stands out as an innovative, safe and efficacious treatment option for managing episodes of acute diarrhea in children.

Table 1: Baseline and Post-treatment Clinical Symptoms			
Clinical symptoms	Number (%)		
	Baseline	Day 3	Day 5
Fever	23 (35.93%)	4 (6.25%)	0 (0%)
Nausea	15 (23.43%)	2 (3.125%)	0 (0%)
Vomiting	31 (48.43%)	8 (12.5%)	0 (0%)
Abdominal Pain	29 (45.31%)	8 (12.5%)	0 (0%)
Gas/Flatus	15 (23.43%)	6 (9.37%)	0 (0%)

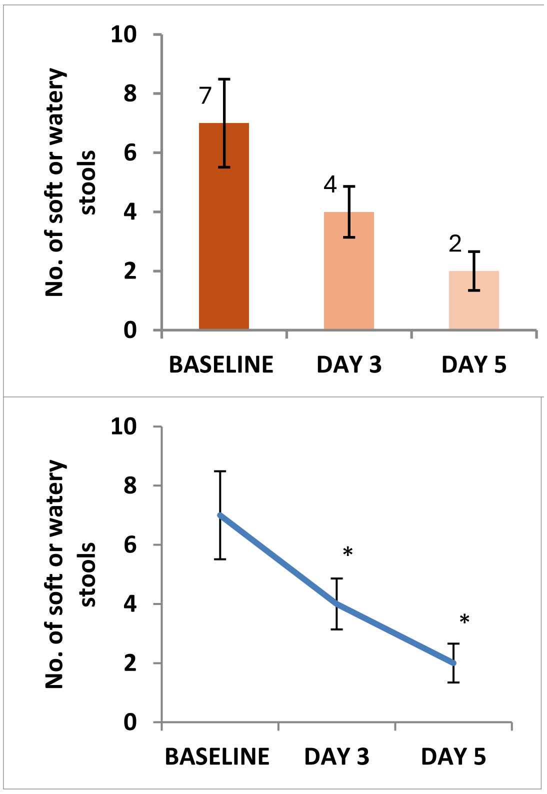

Figure 1: Mean reduction in the number of watery stools. *P < 0.001

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REVIEW OF PATHOPHYSIOLOGY OF RUSSELL'S VIPER ENVENOMATION AND CLINICAL UTILITY OF SERUM LACTATE DEHYDROGENASE AS A PREDICTOR OF SEVERITY AND OUTCOME

Short Running Title: Russell's viper envenomation pathophysiology

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ABSTRACT:

Russell's viper (Daboia russelii) envenomation is a major public health problem in South and Southeast Asia, often resulting in life-threatening complications such as acute kidney injury (AKI), disseminated intravascular coagulation (DIC), and shock. Serum lactate dehydrogenase (LDH), an accessible and cost-effective biochemical marker, has been proposed as a surrogate indicator of tissue injury, haemolysis, and severity of systemic envenomation. This review explores the role of LDH in the context of Russell's viper bite, examining the pathophysiological basis of envenomation and its complications, the mechanism of LDH elevation, and clinical studies correlating LDH levels with severity and outcomes. The findings suggest that elevated LDH may assist in early recognition of severe envenomation, guide timely interventions, and serve as a valuable adjunct to clinical and laboratory assessment in resource-limited settings.

Keywords: envenomation, haemolysis, lactate dehydrogenase, snake bites, viperidae

INTRODUCTION:

Snakebites are a major issue in Myanmar, commonly caused by the Russell's viper [1]. Complications from viper bites include acute kidney injury (AKI), disseminated intravascular coagulation (DIC) and shock, which are associated with high risk of mortality. Clinical risk factors such as reduced urine output, prolonged bite to needle time, uncoagulable blood status, are associated with adverse outcomes and high mortality [2]. The combined use of both clinical and laboratory parameters is recommended to prognosticate outcomes from viper bites [3]. Serum biomarkers such as Lactate Dehydrogenase (LDH) are gaining interest as a biomarker, given its availability and low cost, which may be used to tailor treatment for patients with viper bite. In this paper, the pathophysiology of Russell's viper envenomation is described, the biochemical basis of LDH increase in envenomation and the association with envenomation severity.

Pathophysiology of Russell's Viper Envenomation:

There are three main venom components relevant to the complications of envenomation: phospholipase A2, coagulant enzymes and hyaluronidase. The major lethal toxin is phospholipase A2 (PLA2), also called daboiatoxin (DbTx), with enzymatic activity against cell membrane lipids [4]. Around 30% of total venom protein have PLA2 activity, which causes the release of mediators such as histamine, serotonin and slow reacting substance. This results in a wide range of effects, contributing to cardiotoxicity, haemolysis, increased vascular permeability, rhabdomyolysis, hepatic necrosis and platelet damage. Coagulant enzymes are enzymes of proteolytic nature, which activate Factors X and V on the coagulation cascade, leading to coagulopathy. Anticoagulant activity only occurs at very high concentrations of venom. Hyaluronidase breaks hyaluronic acid which is a major glycosaminoglycan in tissues. This enzyme is one of the spreading factors promoting the spread of venom and increases the extent of tissue damage [5].

Venom bites lead to local reactions, with swelling and bruising on the bitten limb due to increased vascular permeability induced by protease, phospholipase, membrane damaging polypeptide toxins, hyaluronidase and endogenous autacoids present in the venom. However, local signs of envenomation from Russell's viper bite may be mild compared to the severity of systemic envenomation [6].

Haemostatic disturbances may occur, with severe envenomation resulting in DIC. A haemostatic study showed that all confirmed Russell's viper bite cases with a positive 20-minute clotting test had most of the laboratory features of DIC. In Russell's viper bite patients with DIC, clotting factors V and X are more depleted than factors VIII, IX and VII. The two major procoagulant factors in venom activate clotting factors V and X, with a fall in active clotting factor XIIIA subunit levels due to activation by thrombin and active clotting factor XA. The production of cross-linked fibrin may obstruct small blood vessels in vulnerable organs such as the kidneys and anterior pituitary. PLA2 also contributes to the thrombin induced platelet aggregation in envenomed patients. When the patient's blood becomes defibrinated and incoagulable, haemorrhagin activity and platelet function abnormalities may lead to spontaneous systemic haemorrhage [7].

Fibrinolysis in Russell's viper envenomation also occurs due to physiologic activation of the fibrinolytic system and a direct venom toxic effect. Fibrinolytic activation results in degradation products. Except in fulminant DIC, the fibrinolytic system is capable of rapidly lysing large amounts of fibrin as it is formed, which maintains vascular patency. Fibrin degradation products (FDP) inhibit thrombin and fibrin polymerization, binding to the platelet membrane. Some patients with severe DIC may not have increases in fibrin degradation products due to a poor fibrinolytic response. These patients tend to have extensive fibrin deposition, which is associated with a poor prognosis [8]. A study showed that intense fibrin-olytic activity was detected in all cases with marked elevation of FDP, a large proportion of which were cross-linked. The monospecific Myanmar anti-venom was shown to be effective in neutralizing the venom procoagulants and restoring blood coagulability [9].

Complications of envenomation:

Viper envenomation disrupts the circulatory system by affecting haemostasis, resulting in complications due to unrestrained bleeding. It also induces myotoxicity, nephrotoxicity and serious local manifestations, such as myonecrosis, dermonecrosis, extracellular matrix (ECM) degradation, blistering, haemorrhage and oedema [10]. A study showed that viper bites may lead to debilitating secondary or long-term complications, such as thrombocytopenia, renal abnormalities, hypopituitarism, persistent local tissue degradation and infertility [11].

Incoagulable blood due to defibrination and consumptive coagulopathy is a common complication of systemic envenomation. Patients with clottable blood one or two hours after a bite may develop severe defibrination four to eight hours later. Spontaneous systemic haemorrhage may occur, manifesting as gum bleeding, gastrointestinal bleeding, epistaxis, subconjunctival haemorrhage, frank or microscopic haematuria, skin or mucous membrane bleeding as purpura or ecchymosis, bleeding per vagina or from venepuncture and incision sites [12].

Hypotension, with a systolic blood pressure below 80 mmHg was observed in 25% of systemic envenomation. Shock may occur at any time during the first week after the bite. Signs of increased capillary permeability may be detected within twelve hours after the bite, observed as conjunctival, periorbital or facial oedema, serous effusion and radiological and clinical evidence of pulmonary oedema [6,13]. Conjunctival oedema may progress within 24 hours to subconjunctival haemorrhage, indicating a further breakdown of permeability barriers. Chemosis and facial oedema are unique features of the Myanmar Russell's viper bite. Capillary leak syndrome is defined as a packed cell volume (PCV) above 50%, retinal oedema, late-onset hypotension (twelve hours after bite) with or without acute respiratory distress syndrome [14].

Russell's viper bites may also lead to endocrine complications. During the acute phase, hypoglycaemia and shock may be due to adrenal haemorrhage [15]. Several months to years after the bite, some patients may exhibit features of panhypopituitarism due to pituitary infarction, such as weakness, lack of secondary sexual characteristics, amenorrhea, testicular atrophy and hypothyroidism [16].

Acute kidney injury (AKI) occurs in 55% of patients with Russell's viper bite [17]. While there is a lack of consensus regarding the mechanism for developing AKI, viper venom induces several abnormalities such as bleeding, hypotension, circulatory collapse, intravascular haemolysis and DIC. Ischaemia, haemorrhage, direct nephrotoxic actions of the venom and 'pigment nephropathy' associated with rhabdomyolysis and intravascular haemolysis may contribute to the development of acute tubular necrosis and bilateral cortical necrosis [18].

The presence of fibrin thrombi in renal microvasculature, particularly glomerular capillaries and the finding of microangiopathic haemolytic anaemia and thrombocytopenia in patients with cortical necrosis suggest that DIC may be involved in a major pathogenetic role. However, urinary N-acetylglucosaminidase levels are also abnormal in Russell's viper patients without DIC, indicating a direct toxic effect of venom on the kidneys [19]. Evidence supporting direct nephrotoxicity is a dose dependent decrease in inulin clearance and an increase in fractional excretion of sodium in the isolated perfused rat kidney following Russell's viper envenomation. Renal histology shows predominantly acute tubular or cortical necrosis. A number of glomerular changes have been described but their significance is unknown [20].

Acute tubular necrosis is the predominant lesion seen in 70-80% of patients with AKI. Although the blood vessels appear normal under light microscopy, ultrastructural abnormalities are observed in both large and small calibre vessels. Medullary vessels are the most affected, with markedly swollen, focally necrotic, endothelial cells obliterating the lumen. Smooth muscle cells show cytoplasmic vacuoles, which are empty or are filled with granular material. The severe vascular lesions, distal tubular apoptosis, presence of mast cells, eosinophils, and active fibroblasts in the interstitium are features not previously observed in acute tubular necrosis due to other causes [21].

Fibrin thrombi within the arterioles are a prominent feature. A narrow subcapsular rim of cortex often escapes necrosis, with the underlying area showing necrosis of glomerular and tubular elements. The necrotic zone is often bordered by an area of hyperaemia and leukocytic infiltration. Calcification of necrotic areas may occur at later stages. The number of glomeruli affected varies in those with patchy cortical necrosis. With healing, fibroblastic proliferation and organization of thrombi may also be seen [22].

AKI may occur rapidly within a few hours or up to several days after the bite. Rapid administration of anti-snake venom within one to two hours may not be able to prevent AKI in some cases [23].

Lactate dehydrogenase (LDH) in envenomation:

LDH is a ubiquitous enzyme found in muscle, liver, kidney, and red blood cells. LDH catalyzes the conversion of lactate and nicotinamide adenine dinucleotide (NAD) to pyruvate and NADH (NAD and hydrogen). LDH isoenzymes show tissue-specific patterns: LDH1 in cardiac and red blood cells, LDH2 in the reticuloendothelial system, LDH3 in the lungs, LDH4 in the kidneys, placenta and pancreas, and LDH 5 in the liver and striated muscle [24].

LDH is present throughout the body in small concentrations, with the highest concentration in the heart, liver, muscle, kidney, lung and red blood cells. Marked LDH elevations are observed in megaloblastic anaemia, untreated pernicious anaemia, Hodgkin’s disease, carcinoma and shock. Moderate to slight LDH increases have been observed in myocardial infarcts, pulmonary embolism, leukaemia, haemolytic anaemia, hepatitis and pancreatitis. As red blood cells contain more LDH than serum, in-vitro haemolysis may also raise serum LDH, such as transportation via pneumatic tube, vigorous mixing and traumatic venepuncture [25]. LDH activity increases within 18 hours of cell injury, peaks between 48 and 72 hours and remains elevated for about a week [24].

There are several mechanisms for LDH elevation in envenomation, especially direct venom-induced haemolysis, rhabdomyolysis, tissue necrosis, coagulopathy and microangiopathy. The myotoxic activity of snake venom leads to skeletal and cardiac muscle injury and the release of myoglobin leading to myoglobinaemia and myoglobinuria. Accumulation of myoglobin in renal tubules may lead to nephrotoxicity and AKI. Haematotoxins lead to haemolysis due to direct or indirect effects on red blood cell membranes by PLA2 enzymes. The conversion of fibrinogen to cross-linked fibrin may obstruct small blood vessels leading to thrombotic microangiopathy. When red blood cells pass through strands of fibrin and clotting factors in blood vessels, they are partially or completely destroyed, resulting in haemolysis [25].

PLA2 inhibits the extrinsic pathway of coagulation cascade, causes haemolysis and directly damages tissues, which trigger the release of LDH, and may serve as a useful marker of tissue damage after a snake bite. There are several studies that indicate that LDH correlates well with the presence and severity of envenomation, degree of AKI and haemolysis, antivenom requirements and risk of cardiotoxicity at 24 hours [26].

A study in India compared 30 snake bite patients and 30 healthy subjects and measured blood haemoglobin (Hb), serum creatinine, serum aspartate transaminase (AST), serum alanine transaminase (ALT) and LDH. Patients with haemolysis had lower Hb and increased LDH with elevated creatinine for those with AKI. Serum AST, ALT and LDH activity were significantly higher in patients with viper bite and rhabdomyolysis. As AST and ALT are present in all body cells, they were found to be less specific, while increases in serum LDH provided a better indication of rhabdomyolysis, haemolysis, extent of renal failure and prognosis in snake bite patients [27].

Another study from India divided patients who presented within six hours of a snake bite into two groups: one with signs and symptoms of envenomation, and one without signs or symptoms of envenomation. The envenomation group had a significantly elevated LDH level at days one to three, while there were no significant elevations of AST in the envenomated group. This study found that early LDH increases in snake bite patients was significantly associated with systemic envenomation [28].

In a study of 65 snake bite patients divided into no, mild, moderate and severe envenomation group based on a pre-determined clinical scale, the correlation of initial LDH and grade of envenomation was evaluated. All patients with severe envenomation had increased LDH, with a significant difference in LDH between no envenomation and severe envenomation. The severely envenomed viper bite patients with raised LDH also required 30 vials or more of anti-snake venom [26]. Another study found that elevated LDH at 24 hours after admission was also associated with cardiotoxicity [30].

CONCLUSION:

Serum LDH shows promise as a supplementary biomarker in Russell's viper envenomation, correlating with haemolysis, AKI, and overall severity. Its accessibility and low cost make it a valuable tool in early risk stratification and management. However, LDH is non-specific and may be elevated in other conditions, such as myocardial infarction and haemolytic anaemia. Clinical correlation remains essential and further prospective studies are needed to validate its prognostic role.

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PROTECTIVE ROLE OF OMEGA H3 IN PROPOXUR-INDUCED TESTICULAR TOXICITY: INSIGHTS INTO OXIDATIVE STRESS AND INFLAMMATORY PATHWAY MODULATION

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ABSTRACT

Propoxur, a widely used carbamate pesticide, used in agriculture and household pest control has raised concerns with respect to its potential toxicity towards non-target organisms. Studies from previous research show that propoxur exposure can cause testicular toxicity, potentially affecting male reproductive function. This study investigates omega H3 antioxidant and anti-inflammatory response in mitigating propoxur exposure-induced testicular toxicity. A total of 24 male rats were exposed to propoxur via inhalation. The study evaluated its impact on oxidative stress markers, inflammatory mediators and testicular histology. Oxidative stress parameters measured were Superoxide Dismutase (SOD) and Malondialdehyde (MDA). Inflammatory markers were on Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-Kb) and P53 gene with Ki67 protein. The study found that omega H3 had a protective effect on oxidative stress markers, with lower MDA levels ($p<0.05$) and higher SOD levels ($p>0.05$) in treated groups compared to the propoxur-only group. Propoxur exposure led to substantial structural damage in the seminiferous tubules and alterations to the testicular basal membrane which were mitigated by omega H3. Propoxur exposure triggers oxidative stress and inflammatory responses that lead to dysregulation of P53, which was ameliorated by omega H3 intervention. In conclusion, this study suggests that Omega H3 could serve as a potential therapeutic agent; mitigating the adverse effects of propoxur exposure- induced testicular toxicity.

Keywords: Propoxur, testicular toxicity, omega H3, oxidative stress, anti-inflammatory
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INTRODUCTION

Propoxur (PPX), a carbamate pesticide is a known agricultural agent that causes reproductive toxicity partly through induction of cellular oxidative stress [1]. Pesticides have been identified as one of the factors that can result in reduced male fertility due to their role in male reproductive organs and germ cells toxicity [2]. Exposure to *PPX* insecticide has obvious deleterious effects on testicular micro-structure and reproductive hormones using rat model [3]. Studies have raised concerns about potential toxic effects of propoxur exposure, particularly on the male reproductive system. Previous research has shown that propoxur can induce oxidative stress, inflammation, and apoptosis (cell death) in testicular tissues, leading to impaired fertility [4].

The widespread use of carbamate insecticides, like propoxur, for agricultural and household uses has raised concerns resulting from their potential toxic effects, especially on the male reproductive system [5]. Pesticide exposure has been associated with testicular toxicity, characterized by oxidative stress, inflammation, and apoptosis in testicular tissues, ultimately leading to impaired fertility and hormonal imbalances [6, 7].

There is a growing interest in identifying compounds that can ameliorate or mitigate the effects of propoxur exposure on the testes. Omega-3 fatty acids, such as those found in fish oil supplements, have gained attention due to their antioxidant and anti-inflammatory properties [8]. In particular, a supplement, Omega H3, a synthetic derivative designed to enhance the bioavailability and potency of omega-3s, has shown promising results in preliminary studies. A study showed that omega-3 supplementation results in higher antioxidant activity in human seminal fluid also enhancing sperm count, motility, and morphology [9].

Several studies have demonstrated the potential of omega-3 fatty acids in attenuating oxidative stress and inflammation in various pathological conditions [4]. Specifically, in the context of testicular toxicity, Omega H3 supplementation has been reported to reduce lipid peroxidation and restore antioxidant enzyme activities in rats with induced testicular toxicity [10].

The proposed study aims to further investigate the potential protective effects of Omega H3 against propoxur-induced testicular toxicity. Specifically, it evaluates whether Omega H3 supplementation can reduce oxidative stress, mitigate inflammation, and inhibit apoptosis in testicular cells exposed to propoxur. Understanding the mechanisms by which Omega H3 may ameliorate these toxic effects could pave the way for developing interventions to safeguard male reproductive health in agricultural workers and populations at risk of propoxur exposure.

METHODOLOGY

Experimental Animals

Twenty-four (24) adult-male Wistar rats (*Rattus norvegicus*) weighing (160g - 180g) were procured and used for the experiment. They were housed in the animal house of the Faculty of Basic Medical Sciences, college of health sciences, university of Ilorin, at room temperature and maintained under 12 hours dark cycle. The rats were randomly divided into four (4) groups with each group comprising of six (6) rats. Then the rats were acclimatized in animal houses for two weeks, fed a rat diet and tap water. Ethical approval was obtained from the University of Ilorin Ethical Committee. The duration of the experiment was eight weeks. The protocol used for the study is summarized in Table 1.

Table 1: Shows the Grouping of experimental animals and the Dosage of drugs administered

GROUPS	DOSAGE	ROUTE OF ADMINISTRATION	DURATION
PROPOXUR (PPX) (Group 2)	3ppm/KgBW of Propoxur (PPX) for 30 Mins	Inhalation	56 DAYS
OMEGA H3 (Group 3)	300mg/KgBW of Omega H3	Orally (by gavage)	56 DAYS
PPX + OMEGA H3 (Group 4)	3ppm/KgBW of Propoxur (PPx) for 30mins 300mg/KgBW of Omega H3	Propoxur by Inhalation Omega H3 Administered Orally	56 DAYS
CONTROL (Group 1)	0.5ml/KgBW Distilled Water	Orally	56 DAYS

HISTOLOGICAL ANALYSIS

At the completion of treatment, the animals were sacrificed with an anesthetic agent using sodium pentobarbital (50 mg/kg, *i.p.*) as a chemical method of euthanasia. The blood sample was collected by cardiac puncture into heparinized tube. The samples were centrifuged at 704 g (5 min, room temperature). Plasma was stored (−80 °C) until the period of biochemical analysis. Routine histological processing was performed using the hematoxylin and eosin staining procedure. Isolated testicles had been properly fixed in Bouin's solution, and the testicles were then dehydrated with ascending grades of alcohol, cleared in xylene, and infiltrated with molten paraffin wax before being embedded in molten paraffin wax to form a tissue block. The rotary microtome was used to section the paraffin block containing the tissue at a thickness of 4 μm. After floating in a water bath at 40 ° C, the sections were then transferred to a glass slide and stained with hematoxylin and eosin stains.

The slides were examined under a light microscope (magnification 200x).

Immunohistochemistry

The expression of P53 and Ki67 was determined using immunohistochemical staining. Tissue sections were deparaffinized in xylene and rehydrated through graded alcohols to water. Antigen retrieval was performed by heating sections in citrate buffer (pH 6.0) in a microwave oven for 15 minutes. Endogenous peroxidase activity was quenched by incubating sections in 3% hydrogen

Photomicrography

The slides were mounted on the microscope with the cover slip facing the objective lenses and secured on the stage using the stage clips. The coarse and fine adjustment knobs were used to focus and resolve the specific region of the tissue for viewing. An Amscope camera was inserted into one of the ocular lenses and connected to a laptop. The micrographs were captured on the laptop using the Amscope software application at magnifications of X200. The histological sections were examined to observe any changes in the structure of the testes.

Qualitative analysis

Histological slides prepared were observed under a Microscope at low and high magnifications and photographed using an amscope and then comparisons were made between the control and treated groups.

Statistical analysis

All quantitative analysis were analyzed using GraphPad (version 8) software and presented as mean and standard error of mean (mean±SEM) to show statistical significance and comparison between each groups using the analysis of variance (ANOVA). Comparison was done between all experimental groups and differences were determined using $p<0.05$, giving credence to the statistical significance of the comparisons.

RESULTS ANALYSIS

Body Weight

At the end of the 56-day treatment period, the mean value of animals administered Propoxur only showed a significant loss in weight compared to the control. Animals administered Omega H3 only showed significant body weight gain compared to control. However, animals administered Propoxur then Omega H3 showed no significant change in body weight compared to all other groups. The results obtained are summarised in Table 2.

Table 2: Table showing the initial and final weights, and weight differences of CONTROL, PPX, OMEGA H3 and PPX + OMEGA H3 groups.

GROUPS	INITIAL WEIGHT(g)	FINAL WEIGHT(g)	RELATIVE WEIGHT(g)
CONTROL (Group 1)	154.2 ± 3.277	256.2 ± 5.472	39.81 ± 1.264
PROPOXUR (Group 2)	150.6 ± 3.458	211.0 ± 2.702	38.62 ± 2.848
OMEGA H3 (Group 3)	156.6 ± 1.720	270.6 ± 4.490	42.13 ± 1.784
PROPOXUR + OMEGA H3 (Group 4)	152.2 ± 3.277	258.3 ± 5.472	41.08 ± 1.264

Data are presented as mean and standard error of mean (mean ± SEM). Analysis of variance (ANOVA) was used to analyze the data at $p \leq 0.05$.

OXIDATIVE STRESS PARAMETERS

SOD (Superoxide Dismutase):

Table 3 below indicates values used in a Tukey post hoc test, which revealed a significant decrease ($p < 0.05$) in the Propoxur only group compared to the control group, Omega H3 group. Also, there is no significant differences between the Propoxur + Omega H3 group, Omega H3 only group and the control group. Analysis of variance (ANOVA) with mean ± SEM was used.

Malonialdehyde (MDA):

Table 4 below indicates values used in a Tukey post hoc test, which revealed a significant decrease ($p < 0.05$) in MDA is observed in Control group compared to animals administered Propoxur, Propoxur + Omega H3. MDA level is reduced in animals administered Omega H3 compared to animals administered Propoxur.

INFLAMMATORY MARKER

Nuclear Factor-kappa B (NFkB)

Table 5 below indicates values used in a Tukey post hoc test, which revealed a significant decrease ($p < 0.05$) in NFkB is observed in Propoxur group compared to animals in the control group and the Omega H3 group. NFkB level is increased in animals administered Propoxur + Omega H3 compared to animals administered Propoxur only.

Table 3:

CONTROL (U/ml)	PROPOXUR (U/ml)	OMEGA H3 (U/ml)	PROPOXUR + OMEGA H3 (U/ml)
1.771428571	1.580901	1.223468	1.956726
1.675573	1.524398	1.8	1.8
1.750994	1.539006	1.9	1.7
1.802127	1.4	2	1.5

Table 4:

CONTROL (μM)	PROPOXUR (μM)	OMEGA H3 (μM)	PROPOXUR + OMEGA H3 (μM)
0.467789	0.68516129	0.459877	0.724013
0.501218	0.657852	0.6	0.6
0.489756	0.599871	0.65	0.65
0.3	0.690613	0.5	0.5

Table 5:

CONTROL (μM)	PROPOXUR (μM)	OMEGA H3 (μM)	PROPOXUR + OMEGA H3 (μM)
47.483	35.773	32.988	44.786
46.878	37.243	33.256	45.243
48.143	38.311	31.992	43.988
47.321	38.285	32.679	44.587

DISCUSSION

Oxidative stress parameters

The protective impact of Omega H3 is further supported by the oxidative stress indicators examined in this study, such as malondialdehyde (MDA) and superoxide dismutase (SOD). When comparing the Propoxur-only group to the control, the SOD levels were considerably lower, indicating considerable oxidative stress. On the other hand, rats given Omega H3 treatment had a marked rise in SOD levels, indicating improved antioxidant defense systems. Similarly, the group that only received Propoxur had much higher MDA levels, an indication of oxidative damage and lipid peroxidation, whereas the group that received Omega H3 had significantly lower MDA levels. This is in line with the study of Mohammad *et al*. [12].

Effect of Omega H3 on Body Weight

Omega H3, a supplement known for its rich composition of essential fatty acids, vitamins, and minerals, has been observed to have a positive impact on the overall health and well-being of laboratory animals, including Wistar rats. In the context of this study, Omega H3 administration to the Wistar rats led to a stabilization or slight increase in body weight. This effect can be attributed to the nutritional and antioxidant properties of Omega H3, which support metabolic functions and improve the nutritional status of the animals.

The essential fatty acids in Omega H3 are crucial for maintaining cell membrane integrity and function, and the vitamins and minerals aid in various biochemical processes. This nutritional support helps in maintaining an optimal body weight, as the rats receiving Omega H3 did not exhibit the significant weight loss often associated with oxidative stress and toxicity. The overall health benefits provided by Omega H3 contribute to the maintenance of body weight, reflecting its role in promoting general well-being and mitigating stress-related weight changes. These findings were similar to those reported by Bays *et al.* [13].

Effect of Propoxur on Body Weight

The oxidative stress induced by Propoxur leads to the production of reactive oxygen species (ROS), which can damage cellular structures and disrupt metabolic processes. This metabolic disruption can impair nutrient absorption and utilization, leading to weight loss. Additionally, the toxic effects of Propoxur may cause gastrointestinal distress and reduced appetite, further contributing to the observed decrease in body weight. The weight loss observed in the Propoxur-exposed rats is a clear indicator of the systemic toxicity exerted by the pesticide.

Effect of Propoxur Plus Omega H3 on Body Weight

The combined treatment of Propoxur and Omega H3 provided a significant protective effect against the weight loss typically induced by Propoxur exposure. Rats treated with both Propoxur and Omega H3 showed a less pronounced reduction in body weight compared to those treated with Propoxur alone. This suggests that Omega H3 mitigates the adverse effects of Propoxur on body weight.

Omega H3's antioxidant properties play a crucial role in counteracting the oxidative stress induced by Propoxur. By reducing the levels of ROS and enhancing the body's antioxidant defense mechanisms, Omega H3 helps in maintaining cellular integrity and metabolic functions. This protective effect likely contributes to better nutrient absorption and utilization, thus preventing the severe weight loss observed in the Propoxur-only group.

Additionally, the nutritional benefits provided by Omega H3 support overall health and metabolic efficiency, helping to counterbalance the toxic effects of Propoxur. This results in a more stable body weight, indicating that Omega H3 can effectively ameliorate some of the systemic toxic effects of Propoxur.

Apoptosis and Cellular Integrity

The effects of Propoxur and Omega H3 on apoptotic markers and testicular cellular integrity were also investigated in this study. Biochemical indicators demonstrated that exposure to propoxur was linked to increased apoptosis and damage of cellular integrity. Treatment with omega H3 attenuated these effects by lowering apoptosis and maintaining cellular architecture. This finding aligns with the established anti-apoptotic characteristics of Omega H3, which contribute to preserving cell viability and functionality in hazardous environments [4, 14].

Comparative Analysis with Control Groups

A comparative study of the control, Propoxur-only, and Omega H3-treated groups demonstrated how effective Omega H3 is at preventing damage caused by Propoxur. Function of the control group were normal, but the Propoxur-only group displayed notable departures from the norm. The protective effects of Omega H3, however, were highlighted when the structural and functional integrity of the group treated with Omega H3 was nearly identical to that of the control group.

Oxidative Stress and Gene Regulation in Propoxur-Induced Testicular Toxicity

In the context of investigating omega-3 fatty acids' potential to mitigate propoxur exposure-induced testicular toxicity, the roles of P53 and Ki67 are crucial. Propoxur exposure triggers oxidative stress and inflammatory responses that lead to dysregulation of P53, a protein central to controlling apoptosis and managing cellular stress [11]. Elevated P53 levels reflect increased apoptosis in response to the cellular damage caused by propoxur. Concurrently, Ki67 expression, a marker of cell proliferation, often rises as a compensatory mechanism to repair the damaged testicular tissue, potentially exacerbating the overall toxicity if the repair is ineffective [15]. Understanding these dynamics helps elucidate how omega-3 fatty acids could counteract propoxur-induced oxidative and inflammatory stress, potentially normalizing P53 and Ki67 expression and thus reducing testicular damage and apoptosis [16, 17].

Conclusion

In conclusion, this study provides evidence that Omega H3 significantly reduces Propoxur-induced testicular toxicity. The data indicates that Omega H3 boosts antioxidant defenses, decreases oxidative stress and inflammation, and inhibits apoptosis, preserving testicular structure and function. These findings show the potential of Omega H3 as a therapeutic agent for controlling testicular toxicity caused by exposure to toxicants such as Propoxur.

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