Research Article

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Therapeutic Effects of Melittin Against Zearalenone Induced Renal Failure in Rats

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Abstract: Zearalenone is a toxic mycotoxin that is frequently present in foods and feeds. The accumulation of zearalenone in the kidney caused proteinuria and glomerular distortion. The main biological and pharmacological component of honeybee venom is called melittin. This study aimed to examine the therapeutic effects of melittin against rat renal failure induced by zearalenone. Three groups of animals (N = 10) have been generated: control, Zearalenone group, and Zearalenone + Melittin group. Zearalenone was administered orally to rats at a dose of 2.7 mg/kg body weight whereas melittin was administered at a dose of 40 µg/kg body weight. Three groups' values of biochemical alterations were recorded, and statistical analysis was done. Zearalenone reduced kidney weight and increased renal biomarker levels. Renal biomarkers were reduced by melittin, however, all changed biomarkers and renal weight were restored. Biochemical abnormalities were confirmed by histopathological changes. Melittin, therefore, had therapeutic effects that were adaptable to renal dysfunction and damage induced by zearalenone.
Keywords: melittin, zearalenone, renal failure, mycotoxin.

1. Introduction

One of the most toxic mycotoxins is zearalenone (ZEA), which is mainly produced by the Fusarium fungi, including F.graminearum, F.culmorum, and F.cerealis [1], and is capable of causing disease and death in humans and other animals [2]. Since ZEA is frequently present in foods and feeds such as corn and wheat, its contamination has turned into a global public health issue. These mycotoxin levels are low in grain that has been infected in the field, but they rise when it is stored with moisture levels of more than 30% to 40% [3]. The critical limit, or the amount regarded as unacceptable for ZEA is 0.5 ppm. Previous research has demonstrated that ZEA causes serious carcinogenic effects in humans and livestock, as well as severe reproductive toxicity, cytotoxicity, and immunotoxicity. In addition, the body is susceptible to oxidative stress and apoptosis due to ZEA [4,5]. In addition to eliminating endogenous and external wastes, including medications, the kidney also retains some of these compounds in the proximal tubular portion [6]. In the treatment of animals with ZEA, the kidneys excrete it via the hepatoenteral circulation [7]. Proteinuria, glomerular distortion, and renal tubular epithelial cell degeneration were all brought on by the accumulation of ZEA in the kidney. [8,9]. Jia et al. (2014) [10] mentioned that BUN and UA levels were significantly increased in the ZEA-treated groups, but the creatinine level was significantly decreased in all of the ZEA-treated groups.

Melittin (MEL) is a major component of honeybee (Apis mellifera) venom with various biological and pharmacological properties [11]. It has 26 amino acid residues and is a short, linear peptide. Melittin has antibacterial, antiviral, and anti-inflammatory characteristics in different cell types, and its biological and pharmacological actions have been the subject of several investigations [12]. Additionally, it has anti-rheumatoid arthritis and pain-relieving properties, anti-cancer cell proliferation effects, and aids immune modulatory activity [13,14]. Therefore, this study aimed to investigate the therapeutic effects of melittin to ameliorate the renal dysfunction induced by Zearalenone.

2. Materials and methods

2.1. Chemicals and Kits

Zearalenone (CAS No.17924-92-4) and melittin (CAS No.20449-79-0) were supplied by Sigma Aldrich, USA. Urea, creatinine, and uric acid were from Elabscience Biotechnology Co, Ltd, USA.

2.2. Zearalenone's Administration and Preparation

This study used a 2.7 mg/kg bodyweight chronic dosage of a ZEA solution that was dissolved in DMSO (dimethyl sulfoxide) and diluted to 1:100 in a sterile saline solution (0.9% NaCl) to create a working stock that was kept at -20 °C.

2.3. Animals and Experimental Design

The animal house of Sohag University in Sohag, Egypt, supplied 30 adult female Wistar rats (age 10–12 weeks, 180–200 g). All received a balanced pellet meal while being fed under regular laboratory settings, with unlimited access to water. Before the trial, animals were housed in typical home situations.

The rats were divided into three groups of ten at random, as follows:

Control group: Rats received weekly two doses of 2.7 mg/kg b.w. of 1% DMSO saline orally throughout two weeks.

ZEA group: During a period of two weeks, rats received 2.7 mg/kg b.w. of ZEA orally in two doses weekly, according to EL-Sawi et al., (2012) [15].

ZEA + MEL group: Rats received 2.7 mg/kg b.w. of ZEA orally twice a week for two weeks, followed by melittin 40 µg/kg b.w. orally three times per week for one month after intoxication, according to Abu-Zinadah et al., (2014) [16].

Rats were sacrificed and dissected after each time period, and blood samples from all groups were taken from the heart and centrifuged for 10 minutes at 4000 rpm to separate the serum. The serum was then divided into several aliquots and stored at -20°C until analysis was done. For weighting, renal samples were taken from the study groups.

2.4. Biochemical Assays

The concentration of blood urea nitrogen (BUN), creatinine (CRE), and uric acid (UA) in serum was determined according to Wang et al. [17]. Each measurement was based on the manufacturer's instructions.

2.5. Histological Studies

After the therapy, the abdomen was opened and dissected to take the right and left kidneys out for the histological preparations. For a light microscope examination, renal tissues were divided into small pieces and then fixed in 10% neutral buffered formalin for 24 hours. The slides were stained with hematoxylin and eosin.

2.6. Data Analysis

Results were expressed as the mean ± SD (standard deviation), and data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 26. Using the Shapiro-Wilk test with a normality level of $p < 0.05$, the data were examined for normality. Statistical analysis was done using an analysis of variance (ANOVA), followed by Tukey's multiple comparison test to test the difference between groups. Renal weight group values have a normal distribution with the same variance. While for the non-parametric data, the Kruskal-Wallis one-way ANOVA by ranks and Mann-Whitney U multiple comparison tests were also used to confirm that the means of the groups were equal. The level of significance was set at $p<0.05$, $p<0.01$, and $p<0.001$.

3. Results:

3.1. Ameliorative effect of MEL on Renal weight

Table 1 indicates that the renal weight (mg) after administration of ZEA has a highly significant decrease (***P<0.001) in the mean value compared to the control group. While the treatment with MEL showed a highly significant increase in the mean value of renal weight compared to the ZEA group (###P<0.001).

Values are represented by mean ± S.D. Parametric data are tested by ANOVA Test for comparison between groups. ***P ≤ 0.001: Highly Significant vs. control, ###P < 0.001: Highly Significant vs. ZEA group, and ns: non-significant.

Table 1: Impact of ZEA (2.7 mg/kg b.w.) and MEL (40 µg/kg b.w) on renal weight of female rats.

Parameter/ Groups	Control group	ZEA group	ZEA + MEL group
Renal Weight (mg)	0.58 ± 0.04	0.19 ± 0.10***	0.49 ± 0.13 ns, ###

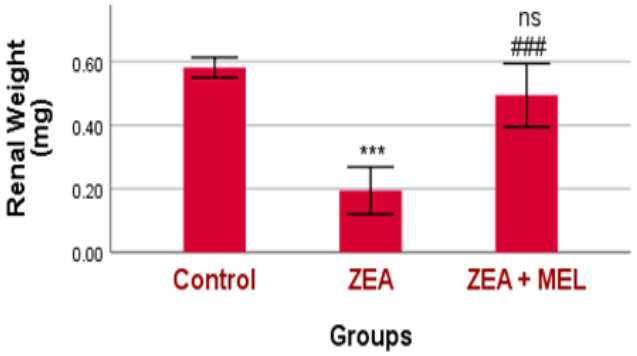

Fig .1. Impact of melittin (40 µg/kg b.w.) and zearalenone (2.7 mg/kg b.w.) on renal weight in the study groups.

3.2. Ameliorative effect of MEL on Renal Biomarkers

There was renal dysfunction and damage in the ZEA group, as indicated by the significant elevation in the mean value of serum renal biomarkers such as blood urea nitrogen (BUN), creatinine (CRE), and uric acid concentrations compared to the control group and the reduction in renal weight. while rats that received ZEA plus MEL showed a reduction in renal biomarkers and a restoration of all altered parameters, as shown in Table 2 and Figure 2, and improved renal weight (Table 1 and Figure 1). demonstrating that MEL had an adaptative ameliorative impact.

3.2.1. Blood Urea Nitrogen concentration (BUN)

The data included in Table 2 showed that the ZEA group's mean serum BUN concentration (mg/dL) –increased significantly (***P<01) as compared to the control group. In comparison to the ZEA group, the ZEA + MEL group showed an extremely significant decline and became non-significant to the control.

3.2.2. Creatinine concentration (CRE)

The mean serum CRE (mg/dL) levels in the ZEA group were significantly higher (***P<0.001) than those in the control group, according to Table 2. When compared to the ZEA group, the MEL therapy resulted in a highly significant drop (###P<0.001) in the mean value of serum CRE (mg/dL).

3.2.3. Uric acid concentration (UA)

Table 2 showed that the UA concentration (mg/dL) was highly significantly increased (***P<0.001) after administration of ZEA for two weeks compared with the control group, while the treatment with MEL showed a highly significant decrease (###P<0.001) in the mean value of UA concentration compared to the ZEA group. UA concentration did not return to the normal control value after MEL treatment

and there is still a significant difference between the control and ZEA+MEL group (0.01<p<0.05).

Table 2: Results of Kruskal–Wallis test and the post hoc Mann–Whitney U multiple comparisons test, showing the significance of differences in renal biomarker characters in studied groups. Explanation of symbols: 0, 1, 2, code of experimental groups (0= Control, 1= ZEA, 2= ZEA + MEL); H, H test value; ns, non-significant; p, significance level; * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001. * vs control and # vs ZEA group.

Characters	Mean ± SD	Kruskal – Wallis test		Mann – Whitney U test		
				0-1	0-2	1-2
		H	P	P	P	P
Serum Uric acid (mg/dL)	4.73 ± 2.59	21.68	0.000	0.000 ***	0.023 *	0.000 ***,###
Serum Creatinine (mg/dL)	1.83 ± 1.64	20.84	0.000	0.000 ***	0.070 ns	0.000 ***,###
Serum Urea (mg/dL)	7.83 ± 3.75	19.46	0.000	0.000 ***	0.650 ns	0.000 ***,###

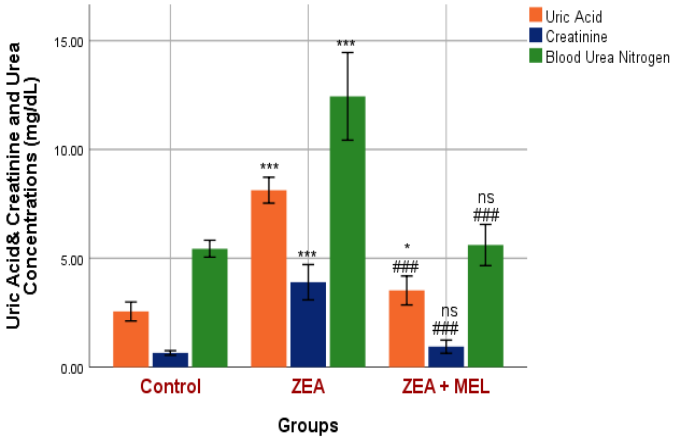

Fig .2. Impact of zearalenone (2.7 mg/kg b.w.) and melittin (40 µg/kg b.w) on renal biomarkers in study groups.

3.3. Histological result

Control group:

Paraffin sections of rat renal stained with hematoxylin and eosin, showed a rat kidney's typical anatomy. As shown in Fig. **3A**, it was composed of the well-known outer cortex, which contained the renal corpuscles and tubules, and an inner medulla, which contained the remaining renal tubules.

ZEA group:

Zearalenone (2.7 mg/kg b.w. for two weeks) treatment of rats resulted in severe alterations to the rat kidney capsule and tubules. In Fig. **3B**, endothelial rupture in the capsule, damaged glomeruli, dilated tubules, and loss of cellular border are seen.

ZEA + MEL group:

The rat kidney capsule and tubules showed a significant improvement after two weeks of zearalenone (2.7 mg/kg b.w.), which was followed by melittin (40 g/kg b.w. for one month). This improvement was equivalent to that of the control group, as shown in Fig. **3C**.

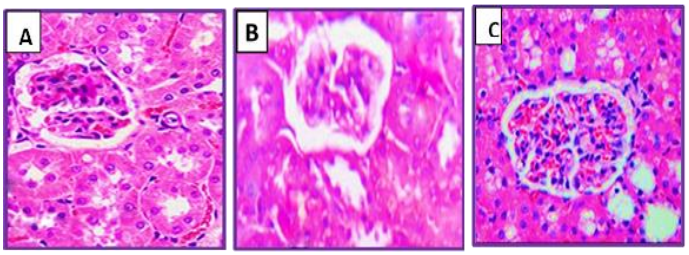

Fig .3. (A) Section of control rat kidney showing normal renal corpuscles, and tubules. **(B)** The section in the rat kidney of the ZEA group shows endothelial rupture in the capsule, damaged glomeruli, dilated tubules, and loss of cellular border. **(C)** Section from rat kidney of ZEA + MEL group showing equivalent improvement to that of the control group in kidney corpuscles, tubules, and cellular border.

4. Discussion:

The kidney is a vital organ for the body's metabolism and is involved in the breakdown of medications and poisons. ZEA can harm the kidneys [6]. Therefore, we believe it is essential to research the kidney damage brought on by ZEA and discover a potent medicine to treat it.

In our study, a reduction in renal weight was seen in the ZEA group. The decrease in kidney weight can be regarded as evidence of tissue damage brought on by ZEA. There have been reports of reduced body and absolute kidney weight in rats fed ZEA [18]. While the treatment with MEL revealed a highly significant increase in renal weight, our findings indicated that MEL restored the reduction in kidney weight induced by ZEA.

A by-product of muscular action called creatinine circulates in the blood. Because it can only be removed by the kidneys, creatinine levels and renal health are related. The amount of creatinine that is freely filtered in renal glomeruli and the minimal amount that is filtered by the tubular component during kidney elimination serves as an effective measure of renal-glomerular function [19]. In this study, the CRE level in serum was highly increased in the ZEA group compared with the control (P<0.005). A similar conclusion was obtained by Virk et al. (2020) [20], who reported that administration of ZEA (2 mg/kg b.W.) significantly increased blood levels of CRE (p<0.05) showing that kidney function was affected. Abnormal levels of creatinine in the rats treated with ZEA that were reported in the current study could be a sign of kidney failure, as described by Braun et al. (2008) [21]. However, endotoxin-induced renal dysfunction and structural damage were greatly reduced by the injection of melittin.

BUN is the amount of nitrogen that circulates in the form of urea through the bloodstream. In healthy animals, the renal glomerulus removes urea from plasma. Although renal tubules allow it to return to circulation, the majority of it is eliminated through urination. Various physiological conditions, such as consuming a lot of protein, intestinal bleeding, infection, fever, dehydration, medication, burns, and poisoning, cause the BUN levels to rise. However, if the kidney is not functioning properly, enough urea cannot be eliminated from the plasma. [22]. In the present study, the BUN level in serum was significantly increased after administration of ZEA for two weeks. In agreement with Liang et al. (2010) [23], who found that a single dose of ZEA increased blood urea nitrogen levels

in the serum, our findings suggested that an increase in BUN levels brought on by ZEA might signify renal failure. In this investigation, the administration of MEL resulted in a highly significant drop in the amount of BUN. Our research showed that MEL inhibits ZEA's harmful effects. Similarly, An et al. (2016) [24] reported that melittin may be an effective therapeutic drug for preventing fibrosis that underlies the development of chronic kidney disease.

In the current investigation, UA levels rose after ZEA was administered for two weeks. Although serum uric acid is frequently increased in people with chronic kidney disease (CKD), this issue has traditionally received little attention. A putative contributory risk factor in the onset and progression of CKD has recently been revived: uric acid. The majority of research found that a higher-than-normal blood uric acid level can independently predict the onset of CKD. Arteriolosclerosis, glomerular damage, and tubulointerstitial fibrosis are signs of glomerular hypertension and renal illness, which can be brought on by raising the uric acid level in rats [25]. A similar finding was observed by Zhang et al. (2020) [6] who discovered that feeding male mice ZEA caused the amount of serum UA to rise. According to our findings, renal disease may be indicated by a high level of UA. In contrast, treatment with MEL dramatically decreased the amount of UA in the serum, demonstrating the ameliorative effects of MEL.

The histological analysis of this study's group, ZEA + MEL revealed a noticeable amelioration of the different alterations induced by ZEA. Improvements in kidney corpuscles, tubules, and cellular borders were comparable to those in the control group. These findings confirmed those of earlier researchers who indicated that melittin has an ameliorative effect on Kidney Injury [26].

4. Conclusion

Current findings confirmed the ameliorative effects of MEL against renal dysfunction that was induced by the oral administration of ZEA (2.7 mg/kg bw), where ZEA altered renal weight and biomarkers; therefore, our results supported the therapeutic potential use of MEL to ameliorate renal failure.

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