P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com
Research Article https://doi.org/10.47278/journal.ijvs/2021.074

Safety Evaluation of β-mannanase Enzyme Extracted from the Mutant Pathogenic Fungal Strain *Penicillium citrinum* in Female Wistar Rats

Doaa S Foda[1*], Shaimaa A Nour[2], Siham A Ismail[2] and Amal M Hashem[2]

[1]Therapeutic Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, El Buhouth St., Dokki, Giza, Egypt.
[2]Chemistry of Natural and Microbial Products Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, El Buhouth St, Dokki, Giza, Egypt.
***Corresponding author:** E-mail: d.foda2018@gmail.com

Article History: 21-319 Received: 27-May-21 Revised: 19-Jun-21 Accepted: 21-Jun-21

ABSTRACT

External digestive exo-enzymes are enzymes used in simplifying animal food components and are now considered one of the most important top issues in the veterinary world. Introducing these exo-enzymes is believed to enhance increase in animals' body weights and help in reaching the maximum levels of digestion and absorption. The current research aimed to evaluate the safe use of the exo-enzyme β-mannanase extracted from the mutant fungal pathogen *Penicillium citrinum* Egy5 LC368457 before applying its use in animal feed. This was achieved by injecting daily different doses of the enzyme intraperitoneally to female rats and observing the change in body weights in addition to the determination of some hematological and biochemical parameters and the performance of histopathological examinations in rats' organs. The results showed significant retardation in body weight gain and significant changes in platelets and some immune cells count for different enzyme injected doses. An observed increase in serum ALT enzyme level was found in the high dose injected group compared to the control group. Histopathological examinations displayed the harmful impact of the high and the low doses of the injected enzyme on most of the body organs especially the liver, the kidneys, and the brain. Consequently, it can be concluded that using safe and non-pathogenic microorganisms in the extraction of exo-enzyme such as β-mannanase is an important aspect. Extraction of β-mannanase from the mutant *Penicillium citrinum* Egy5 LC368457 cannot be used for animal consumption but can be used for industrial purposes.

Key words: *Penicillium citrinum*, β-mannanase, Animal feed, Body weight gain, Biochemical analysis, Histopathological examinations

INTRODUCTION

Exo-enzymes are external natural enzymes that express high efficacy in animal feed conversion. This fact allows the farmers to use them as additives for animal diet so animals can benefit from most of the constituents of the diet (Daskiran et al. 2004; Sujani and Seresinhe 2015). Exo- enzymes are divided into many types according to their substrates and the bonds they break. Nowadays, many types of exo-enzymes are known such as fibrinolytic, proteolytic and amylolytic enzymes (Roque et al. 2017).

Recently, β-mannanases, which are considered as an example of fibrinolytic exo-enzymes, have been purified and extracted from various organisms such as bacteria, fungi, and plants for applying on animal feed conversion and for industrial uses (Chandra et al. 2011; Lv et al. 2013; Jana et al. 2018). These β-mannanases exo-enzymes express an influence on the bonds of carbohydrates and starch found in the animal diet. The enzyme cleaves the β-1,4- mannosidic linkages in mannans, galactomannans, glucomannans and galactoglucomannans and hydrolyzes mannan yielding mannotriose and mannobiose (Dawood and Ma 2020; Khattab et al. 2020).

β-mannanase showed good results when added to ruminant and non-ruminant diets due to its pH wide range tolerance and its resisting ability against temperature and gastric digestive enzymes in the animal 's stomach (Cai et al. 2011; Yang et al. 2015). It was found that β-mannanase exo-enzymes protect the animals from the harms resulted from the undigested β-mannans as they form high viscosity during animal feeding **(**Chauhan et al. 2012**)**. This problem

Cite This Article as: Foda DS, Nour SA, Ismail SA and Hashem AM, 2022. Safety evaluation of β-mannanase enzyme extracted from the mutant pathogenic fungal strain *Penicillium citrinum* in female Wistar rats. International Journal of Veterinary Science 11(1): 74-81. https://doi.org/10.47278/journal.ijvs/2021.074

can exist also in case of non-ruminant species such as swine and poultry **(**Rainbird et al.1984; Jackson et al.1999; Saenphoom et al. 2013). This viscosity leads to digestion and absorption depression in the gut in addition to diminishing the activity of the pancreatic enzymes in the intestine.

β-mannans also initiate an over stimulation of the immune responses in the intestinal mucosal cells and the probability of increasing different immune cells type in the animal's blood (Isaksson et al. 1982; Ikegami et al. 1990; Jackson et al. 2004; Alagawany and Attia 2015; Alagawany et al. 2017). Accordingly, the production of β-mannanase exo-enzyme is of a great importance for animal feed and health.

The production of the enzyme is preferred from fugal strains for their higher yields and higher enzymatic activity. Penicillium species represent one of the candidate fungi that are used as a source of microbial β-mannanase (Blibech et al. 2011; Wang et al. 2012; Khan et al. 2017). A new research was performed for the extraction of β-mannanase exo-enzyme from the pathogenic fungal strain *Penicillium citrinum* Egy5 LC368457. In this research the fungal strain spores were subjected to gamma rays for a maximum yield and stability of the exo-enzyme (Abosereh et al. 2019; Khattab et al. 2020). This newly extracted β-mannanase enzyme was evaluated in the current study for testing its safe and direct use to animals. So, the aim of this study is to identify the real effect of the enzyme extracted from the mutant pathogen; *Penicillium citrinum* Egy5 LC368457 on the animal's general body health before applying it as an additive for animal diet. This can be achieved by introducing the enzyme intraperitoneally with different doses to female rats for a period of a month and observing and determining the change in body weight, blood cell count, serum biochemical parameters and performing histological examinations in the various body organs.

MATERIALS AND METHODS

Chemicals and Kits

All chemicals used in the experiments were of analytical grade. Kits preformed for the quantitative determination of different parameters were purchased from Greiner Diagnostic Gmbh-Germany and Bio-Diagnostic Company, Egypt.

Animals

Healthy female albino Wistar rats of 100-130g body weight (bwt) were obtained from animal house of the National Research Center, Giza, Egypt. Animals were maintained under standard environmental conditions, i.e., ambient temperature of (25+2˚C), at 45-55% relative humidity, 12h light/dark cycle and were fed a standard pellet diet and water *ad-libitum*. The experiment was performed under the ethical approval of the medical ethical committee of the National Research Centre for the year 2020- 2021.

Fungal Isolate and Enzyme Preparation

The fungal strain *Penicillium citrinum* Egy5 LC368457 was obtained from the Pharoesmomes of Ancient Egyptian Musium, Cairo, Egypt. The culture was maintained on potato dextrose agar and incubated at 30°C for 7 days before storage at 4°C with monthly sub-culturing. Mutagenesis to the spore suspension of *Penicillium citrinum* was carried out using gamma rays produced from Cobalt-60 (Co60) as a source of gamma radiation (by Egyptian Atomic Energy Authority). The enzyme was produced according to the previously described method showed by Abosereh et al. (2019). The prepared medium consisted of (g/l):KH_2PO_4, 8.5; Nitrogen complex (peptone+ammoniumsulfate+Urea), 1.8% and coffee waste (10g/flask). The pH of the medium was adjusted at 5 before autoclaving.

Freshly prepared culture of the mutant *Penicillium citrinum* β-mannanase was performed. Each 250ml Erlenmeyer flask contained 50ml of the medium and was autoclaved for16min at 121°C. An inoculum culture was obtained by culturing the mutant fungal strain in the above medium at 30°C for 48h with shaking at 120rpm. The culture flasks were inoculated by 8% of the inoculums and incubated at 30°C in a shaking incubator at 120rpm for 12days. After the end of the fermentation, the culture was centrifuged, and the supernatant was used as the source of the enzyme.

β-Mannanase Assay

An assay was performed by incubating 0.5ml of appropriately diluted culture filtrate with 1 ml of 1% (w/v) locust bean gum in sodium citrate buffer (50mM) at pH 5.5 for 10min at 50°C (Hashem et al. 2001). The reducing sugars produced were determined using the Nelson–Somogyi technique (Smogyi et al. 1952). One unit of enzyme activity was defined as the amount of enzyme that released 1mmol of mannose/ml/min.

Protein Determination and Partial Purification of the Mutant *Penicillium citrinum* β-mannanase

The protein contents were determined according to Lowry et al. (1951) with bovine serum albumin as a standard. All the partial purification steps were carried out at 4˚C. The crude enzyme solution of mutant *Penicillium citrinum*(100ml) was partially-purified by fractional precipitations with ammonium sulfate (60–70%, w/v). The precipitated protein was obtained by centrifugation (5000rpm for twenty minutes) and was re-suspended in a minimum volume of distilled H_2O. The fraction of ammonium sulfate was dialyzed. The enzyme activity and the protein content were determined.

Experimental Design

Acute Toxicity Studies

Healthy female Wister rats (n=12) ranging about 80g bwt were divided into four groups (3 rats per each); representing one control group and three treatment groups. Each treated group was injected intraperitoneally with different single dose of β-mannanase enzyme; 80, 40 and 20Units/rat. All the rats were observed for two weeks (Sathya et al. 2012). The general observations were recorded daily for body weights as well as behavioral responses. They were continuously observed for spontaneous activity, irritability, corneal reflex, urination, and salivation. Any mortality during the experimental period of 14days was also recorded. At the end of the two weeks, the rats were sacrificed, and blood samples were collected for serum preparation and some biochemical parameters were detected.

Studies on Repeated Daily Doses

Another 25 healthy female Wistar rats ranging about 130g bwt were divided into three groups. Two groups represented the treated groups and were injected with 1/10 (8Uints) of the highest dose (80Units) and one tenth (2Units) of the lowest dose (20Units) that were used in the acute toxicity studies (Baliga et al. 2004).

In groups 1 and 2, 10 rats in each group were intraperitoneally injected with 8 and 2Units of β-mannanase enzyme/rat daily for a month, respectively. In group 3, five rats represented the negative control group. During the duration of the experiment, the body weights were recorded every week. At the end of treatment period, rats were sacrificed by decapitation and blood samples were drawn from the animals via puncturing the retro-orbital venous plexus with a thin sterilized capillary tube. Blood specimens were centrifuged at 4000rpm for 10min for serum preparation and was stored at -20°C for biochemical analysis. Body organs were immediately removed and fixed in 10% neutral formalin solution for histological examination. Blood samples for hematological analyses were collected in tubes containing ethylene diamine tetra acetic acid (EDTA). Red blood cell count, hemoglobin, platelet count and white blood cell count were determined using a multi-item automated hematology analyzer Medonic, Stockholm, Sweden.

Biochemical Analysis

Determination of Serum Parameters

All kits used in this study were purchased from Biodiagnostic Company, Egypt and Greiner Diagnostic GmbH, Germany. Glucose was determined calorimetrically (kit no.GL1320 Egypt) according to Trinder (1969). Total cholesterol and triglycerides were estimated as a quantitative enzymatic, colorimetric determination (Kits no.118 001, Germany) in serum according to Rifai et al. (1998). ALT was determined calorimetrically (kit no. AT 1034, Egypt) according to Reitman and Frankel (1957). Total protein, creatinine and urea were determined calorimetrically in serum according to Gornall et al. (1949), Bartels et al. (1972) and Fawcett and Soctt (1960) respectively (Kits no. TP2020, Egypt; CR 1251 Egypt, 147402, Germany).

Histological Examinations

Organs obtained from rats were fixed in 10% formalin and were prepared for sectioning and staining with hematoxylin and eosin as described by Afifi (1986). Light microscope was used in observing the stained sections.

Statistical Analysis

Data were analyzed by with SPSS (Statistical Package for the Social Sciences) version 7.5 software. All values were expressed as the mean±SD. Significant differences between the groups were statistically analyzed by one way analysis of variance (ANOVA). A statistical difference of $P<0.05$ was considered significant.

RESULTS

Enzyme Production

The results at the end of fermentation showed that the mutant *Penicillium citrinum* could produce 351IU/ml with specific activity 21.57U/mg protein for each ml. The fraction obtained with 70% ammonium sulfate gave 172.44IU/mg protein, which reached about 7.99 fold purification.

Acute Toxicity Studies

Table 1 showed the effect of injected different single doses of β-mannanase enzyme on body weight and some serum parameters in female rats. The table cleared that after 2weeks from the intraperitoneal injection of single different doses (80, 40 and 20Units) of the β-mannanase enzyme, no mortality or any toxicity signs appeared during the study. The data showed that the group injected with 80Units of the enzyme displayed significant changes in some of the detected parameters (glucose and cholesterol) compared to the control group. Significant increase in body weight was also observed. This group also showed non-significant decreasing levels of triglycerides and normal levels of ALT and creatinine.

The groups injected with 40 and 20Units of the enzyme expressed non-significant results in all the detected serum parameters compared to the control group except only significant decrease in glucose level was detected in case of rats injected with 40Units of the enzyme. Non-significant changes in body weight were found in both groups (Table 1).

Studies on Repeated Daily Doses

Table 2 displayed the change in body weights after one month from the intraperitoneal injection of 8 and 2Units of β-mannanase enzyme to rats**.** There was a similar increase in body weights in both groups. Though this increase was significantly retarded by the action of the enzyme when compared to the increasing rate of the control group. This retardation rate was represented by a percent change equal –16% in weight.

Table 3 cleared the effect of the daily injection of different doses of β- mannanase enzyme on blood cell count and general blood picture. Normal red blood cells count, and hemoglobin content were found in both groups (8 and 2Units) compared to the control group. There were slightly significant changes in blood picture in case of the two injected groups (8 and 2Units) compared to the control group. These changes can be observed in the platelets count and some kinds of the white blood cells such as granulocytes and MID cells which represent rare cells correlating to monocytes, eosinophils, basophils, blasts and other precursor white cells.

Determining the effect of daily injection of different doses of β-mannanase enzyme on some serum biochemical parameters was shown in Table 4. Slightly significant changes in most of the serum biochemical parameters were observed due to injection with 8Units compared to the control group. Though these changes can be considered within the normal ranges (as found in glucose, cholesterol and total protein levels). High significant increase was observed in ALT enzyme levels which reflected the disruption of hepatocytes and expressed the toxicity of the 8Units injected dose. Also, there was a non-significant increase in the triglycerides level which may represent another risk factor on the animal general health on the short run. Normal urea and creatinine levels were recorded in the group injected with

Table 1: Effect of injected different single doses of β-mannanase enzyme on body weight and some serum parameters in female rats

Parameters	Units	Doses (Units/Rat)			
		80	40	20	Control
Initial average weight	G	82.22± 10.92	82.22±10.92	82.22±10.92	82.22 ±10.92
Final average weight	G	140±0.01*	132±2.51	130±9.01	123±15.27
Glucose	mg/dL	52.37±4.76*	51.15±1.15*	59.5±0.01	62.69±7.27
Cholesterol	mg/dL	76.79±3.70*	70.63±0.40	60.45±2.11	64.6±6.80
Triglycerides	mg/dL	44.35±7.72	49.37±1.76	59.7±20.88	69.83±23.80
Alanine aminotransferase (ALT)	U/L	11.84±0.015	12.90±1.58	11.63±0.99	11.45±1.27
Creatinine	mg/dL	0.66±0.09	0.70±0.06	0.66±0.13	0.9±0.14

Data represented as mean±SD. Asterisks indicate significant (P≤0.05) difference compared to control group in a row.

Table 2: Effect of daily injected different doses of β-mannanase enzyme on body weight of female rats

Parameters	Units	Doses (Units/Rat)		
		8	2	Control
Initial average weight	g	130.20±11.17	126.00±11.97	133.60±5.76
Final average weight	g	152.00±8.74 *	152.10±10.91*	181.20±6.83
% Change compared to control group.	%	-16.11	-16.05	----------
% Change compared to corresponding initial weight	%	+14.34	+17.15	+26.26

Data represented as mean±SD. Asterisks indicate significant (P≤0.05) difference compared to control group in a row.

Table 3: Effect of the daily injected different doses of β-mannanase enzyme on blood cell count and general blood picture.

Parameters	Units	Doses (Units/Rat)		
		8	2	Control
RBCs x10 ^{12}	cells/L	6.10±0.33	6.07±0.47	6.11±0.21
HB	g/dl	11.46±0.30	10.93±0.85	11.45±0.95
Platelets x10^{9}	cells/L	571.0±16.52*	523.66±37.54	484.0±81.00
WBCs x 10 ^{9}	cells/L	8.63±2.33	8.53±1.60	9.90±1.20
Lymphocytes	%	65.16±3.93	67.33±2.10	61.55±2.95
Granulocytes	%	23.20±1.34*	15.93±0.60*	30.70±2.50
MID	%	12.36±3.68*	14.20±0.70*	7.75±0.45

Data represented as mean±SD. Asterisks indicate significant (P≤0.05) difference compared to control group in a row.

Table 4: Effect of daily injected different doses of β-mannanase enzyme on some serum biochemical parameters.

Parameters	Units	Doses (Units/Rat)		
		8	2	Control
Glucose	mg/dL	119±5.03 *	100±7.30	107±2.0
Cholesterol	mg/dL	56.75±4.57*	63.50±2.88*	77.00±10.16
Triglycerides	mg/dL	101.50±30.31	93.45±58.54	85.42±18.89
Total protein content	g/dL	7.11±0.46*	8.53±0.19	7.97±0.68
Alanine aminotrasferase (ALT)	U/L	56.02±7.26*	49.15±4.23	47.55±3.82
Creatinine	mg/dL	0.34±0.10	0.38±0.06	0.36±0.04
Urea	mg/dL	39.57±7.33	38.17±1.63	43.25±1.85

Data represented as mean±SD. Asterisks indicate significant (P≤0.05) difference compared to control group in a row.

8 Units. On the contrary, the rats injected with the 2Units dose showed mild and non-significant changes in serum parameters when compared to control rats as shown in Table 4.

Histological Examinations

Regarding to the histological examinations it was found that β-mannanase enzyme injection (8 and 2Units) to rats lead to many deteriorations on the level of the different organs compared to their corresponding controls. High doses of the enzyme represented by 8Units caused degenerations in the brain tissues and apoptosis of the neurons. The kidney tissue also showed degenerations in the epithelium lining the renal tubules and the presence of renal casts. The liver tissue was also affected expressing some mononuclear cells aggregation and dilation in the sinusoidal capillaries as shown in Fig. 1b, 2b and 3b.

Low doses of the enzyme represented by 2Units also caused pathological changes in the different tissues of rats. The brain tissue expressed scattered edemain virchow robin spaces. The liver showed congestion of portal blood vessels with proliferation of bile duct tubes and few mononuclear cells infiltration. Also, the kidney tissue showed mild degeneration changes in the renal tubular epithelium as shown in Fig. 1c, 2c and 3c.

DISCUSSION

Many studies have been performed on animals to evaluate the biological effects of β-mannanase enzyme addition to the animal diets. Kim et al. (2017) reported promising results from his experimental study on growing pigs fed low and high amounts of β-mannans in their diet with added β-mannanase enzyme. They approved a greater final body weights in animals and observed the high efficiency of β-mannanase enzyme on both diets. They also reported that adding the enzyme to diet had no effect on serum total cholesterol, triglycerides and urea. Also Tewoldebrhan et al. (2017) approved the safety of β-mannanase enzyme when it enters the animal body through the diet in a study performed on cows producing milk. Another experimental study on the enzyme's biological effect on pigs was performed by Huntley et al. (2018). The results of this study concluded that the enzyme had no effect on the immune status of the animals and had no effect in evoking cytokines and the leukocytes.

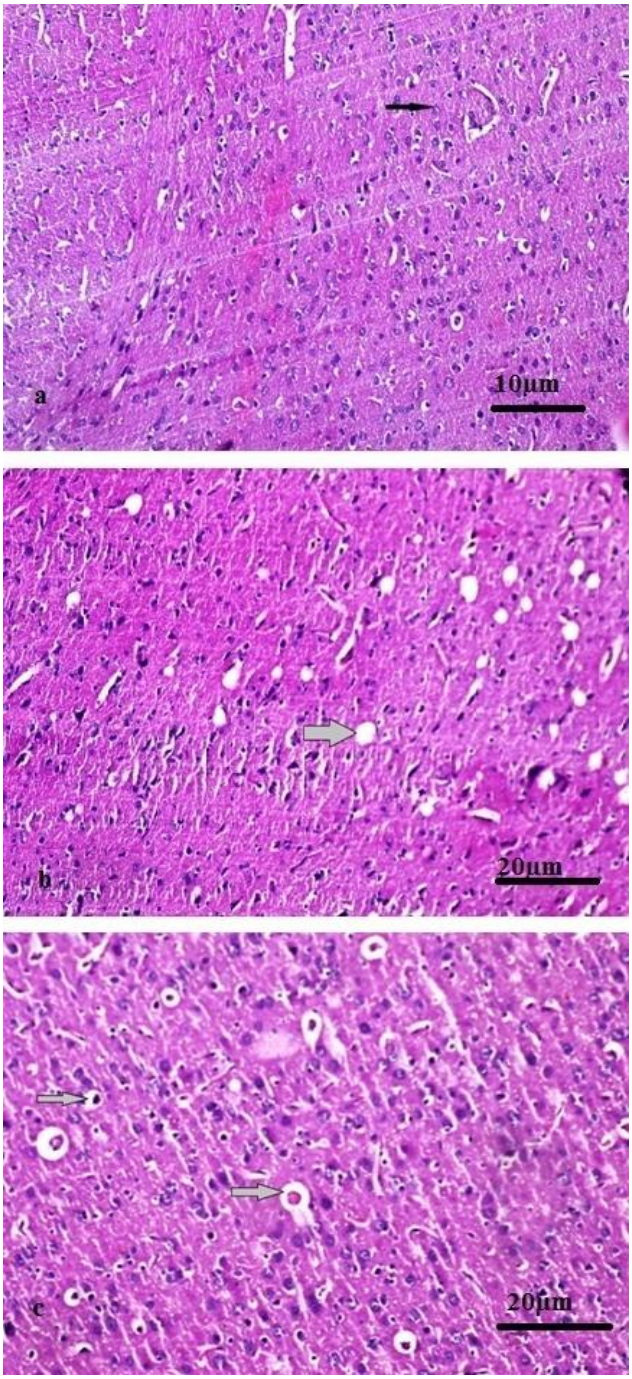

Fig. 1: Showing the brain tissue status in the different groups (a) Brain of control group disclosing normal structures, (b) Brain of rat injected with 8Uints showing spongy degeneration of brain tissue, and (c) Brain of rat injected with 2Units showing disclosing edema in virchow robin space and around astrocytes. H & E Stain.

Recently, Saeed et al. (2019) reported that β-mannanase enzyme is not only useful when added to ruminants' diet but to poultry diets also. They also reported that besides its great effect on mannan digestibility, the enzyme inhibited the growth of the harmful bacteria and enhanced the increase of the intestinal beneficial bacteria. They concluded that β-mannanase enzyme exhibited the digestion and absorption of important nutrient in addition to its reduction to the ammonia emission and so it can decrease the environmental pollution.

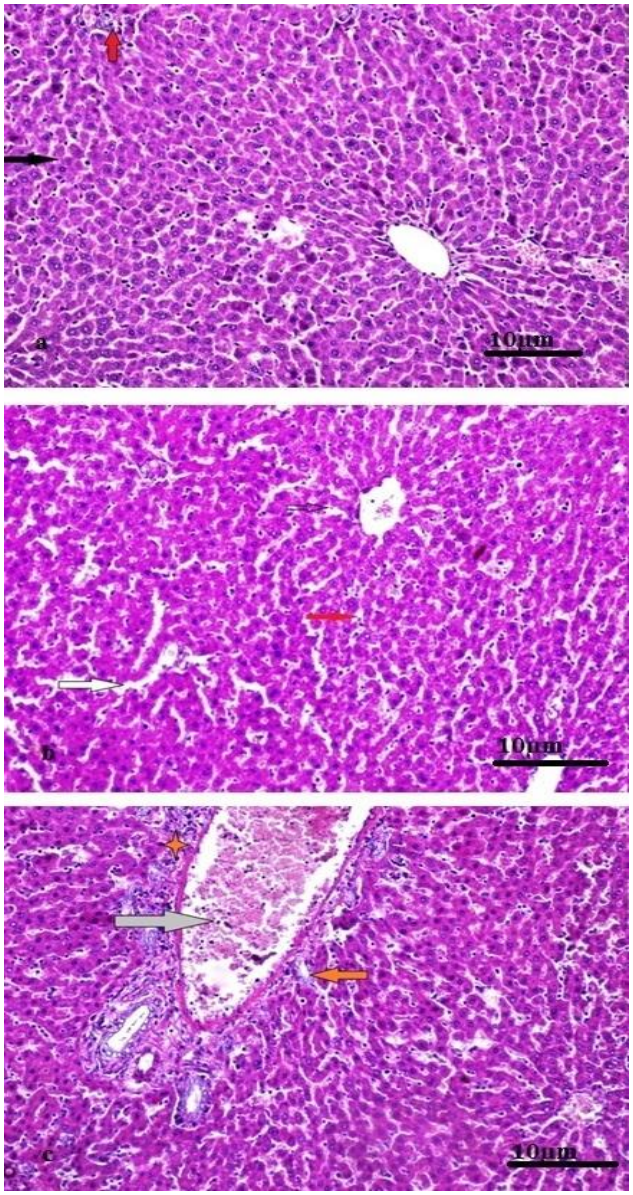

Fig. 2: Showing the liver tissue status in different groups. (a) Liver of control group showing normal histological structures of hepatic cells, (b) Liver of rat injected with 8Units showing mild dilatation of sinusoidal capillaries and few mononuclear cells aggregation and (c) Liver of rat injected with 2Units showing congestion of portal blood vessels with proliferation of bile duct tubes and few mononuclear cells infiltration. H & E Stain.

In the current study we will focus on the safety evaluation of β-mannanase enzyme extracted from a new micro-organism (*Penicillium citrinum* Egy5 LC368457) which was subjected to mutation and testing its biological effect on animals ' health. The intraperitoneal (i.p) injection of β-mannanase was chosen in the current study as a short and a direct way to demonstrate and clarify the effect of the enzyme on the animals' blood and body organs excluding the oral route to avoid any loss of the used enzyme doses. Another important reason for choosing the (i.p) injection is that the constituent of the diet of rats is simpler than that of ruminants and does not need any exo-enzymes. Accordingly, if it is added to the rats' diet it will have no complex substrates to work on which will lead to false results.

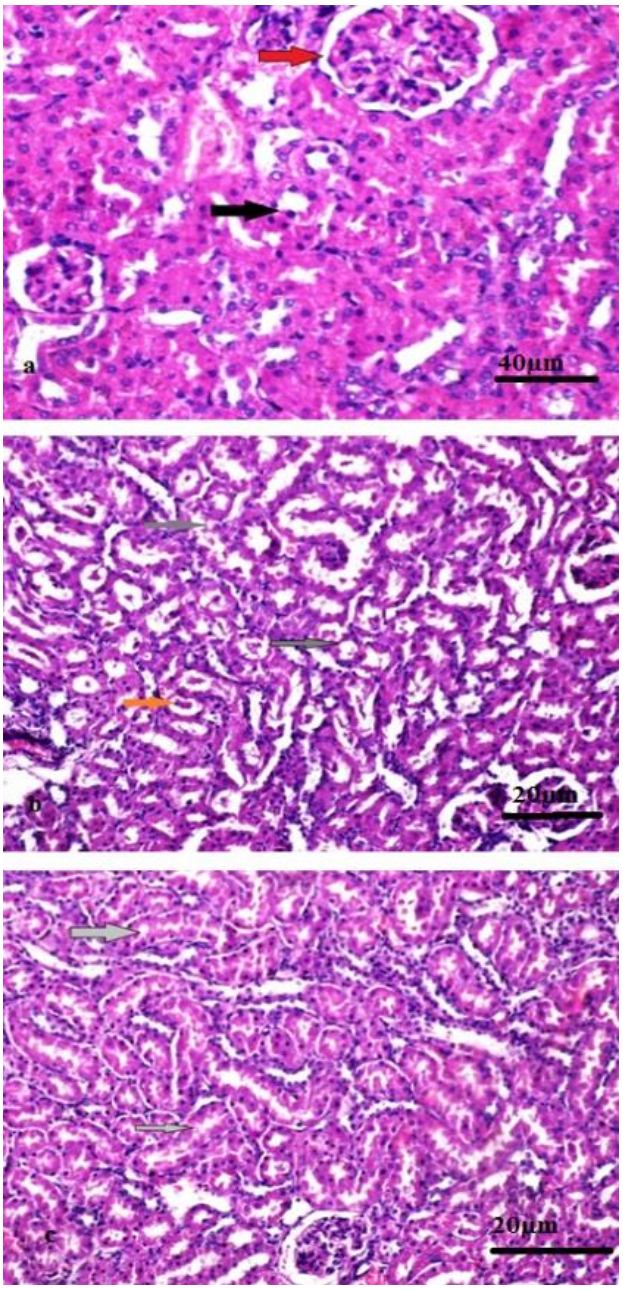

Fig. 3: Showing the kidney tissue status in different groups. (a) Kidney of control group showing normal histological structures of renal tubules and glomeruli, b) Kidney of rat injected with 8Units showing focal area of degenerative changes of lining renal tubular epithelium and renal casts and c) Kidney of rat injected with 2Units showing mild degeneration changes in the renal tubular epithelium. H & E Stain.

The obtained results showed that no toxicity signs were observed due to (i.p) injection of high single doses of the enzyme to rats (80, 40 and 20units) during two weeks as shown in Table 1. On the other hand, applying the (i.p) injection of daily small doses of β-mannanase to rats caused some complications to the animals. Though these complications were significant and appeared more clearly in the group injected with the 8Units.

Beginning with the body weight, there was a retarded increase in weights in case of both of the injected doses; the 8Units and the 2Units compared to the control group as shown in Table 2. This represented the first toxicity sign attributed to the enzyme injection. The study also detected that there were some abnormalities related to the blood parameters especially in the platelets count and some immunological parameters in both groups as shown in Table 3. These changed blood markers may indicate the presence of inflammation or trauma and may be an indication for the growth of some tumor or undesired cells in the rats' bodies (Mahmoud et al. 2019).

The high and significant serum alanine aminotransferase enzyme (ALT) level detected in the rats' group injected with 8Units can be considered as a pathological case. This case reflected hepatocytes rupture which led to the release of ALT in serum (Simon-Giavarotti et al. 2002; Cotran et al. 2010; Arafa et al. 2019). These results are in accordance with the histological findings applied on the liver tissue as shown in Fig 2b. Also decreasing levels in serum total protein were detected in this group. This decrease in serum proteins indicated the low production of albumin or globulin or other serum proteins synthesized by the liver for hormonal transportation directed to body organs through blood (Foda and Shams 2021). The significant changes in the serum cholesterol and glucose level could be clear signs for approving the liver dysfunction in addition to the previous stated parameters.

It was noticed that (i.p) injection with 2Units dose to rats, caused a retardation in displaying the complications in the serum biochemical parameters which are expected to be apparent when consuming the dose daily for a long time. This can be shown in the determined serum biochemical parameters and in the histopathological examinations as well. It could be said that the pathological changes detected (due to the injection of the enzyme to rats) in the brain, the kidney in addition to the liver tissues clarified the toxicity of the enzyme.

The expressed toxicity of β-mannanase in this study can be attributed to the toxic mycotoxin secreted normally by the pathogenic fungal strain *Penicillium citrinum* which is represented in citrinin**.** This mycotoxin is proved to be secreted by the used fungal strain (He and Cox 2016; Gohar et al. 2020). Accordingly, citrinin seemed to be secreted in association to the β-mannanase during the enzyme release in the culture medium. The toxicity of citrinin was tested previously and the results approved that this mycotoxin had hepatotoxic and nephrotoxic effects (Speijers and Speijers 2004; Flajs and Peraica 2009; Imran et al. 2020).

On the other hand, there were few studies for extracting β-mannanases from penicillium species without evaluating its impact on animal health. Blibech et al. (2011) produced high yields of mannanase enzyme from *Penicillium occitanis*. Also, Wang et al. (2012) in their research were able to colon from *Penicillium freii* F63an alkali-tolerant endo-β-1,4-mannanase in addition to the over expression of the obtained enzyme. Their study approved the valuable use of the obtained enzyme in many industrial directions.

Conclusion

Applying extractions of exo-enzymes from pathogenic micro-organisms is not a safe way for extraction and the obtained exo-enzymes cannot be used in animal feed but it can be used for industrial purposes.

Author's Contribution

DSF performed the biochemical experimental design, the biochemical practical work, analyzed the data and wrote the manuscript. SAN performed the practical work of the enzyme preparation and extraction. SAI contributed in the enzyme production and revised the practical work. AMH performed the enzyme production experimental design, supervised and authorized the manuscript.

Acknowledgment

The National Research Centre (NRC) is gratefully acknowledged for providing the scientific aspects and tools to perform this study.

REFERENCES

Abosereh NA, Ismail SA, Khattab OmK H, Nour SA, Abo-Elnasr AA and Hashem AM, 2019. Genetic improvement of fungal β-mannanase and its molecular differentiation. Egyptian Pharmaceutical Journal 18: 403–410. http://doi.org/10.4103/epj.epj_32_19

Afifi A, 1986.Contrbution to the chemical carcinogensis in Xenopus. Ph. D. Thesis, Louvain La Neuve, Belgium, pp: 90- 91.

Alagawany M and Attia A, 2015. Effects of feeding sugar beet pulp and Avizyme supplementation on performance, egg quality, nutrient digestion and nitrogen balance of laying Japanese quail. Avian Biology Research 8: 79-88. https://doi.org/10.3184/175815515X14274754281188

Alagawany M, Attia AI, Ibrahim ZA, Mahmoud RA and El-Sayed SA, 2017. The effectiveness of dietary sunflower meal and exogenous enzyme on growth, digestive enzymes, carcass traits, and blood chemistry of broilers. Environmental Science and Pollution Research 24: 12319-12327. https://doi.org/10.1007/s11356-017-8934-4

Arafa AF, Foda DS, Mahmoud AH, Metwally NS and Farrag ARH, 2019. *Bombax ceiba* flowers extract ameliorates hepatosteatosis induced by ethanol and relatively moderate fat diet in rats. Toxicology Reports 6:401–408. https://doi.org/10.1016/j.toxrep.2019.04.008

Baliga MS, Jagetia GC, Jagadish N. Ulloor JN, Baliga MP, Venkatesh P, Rosi Reddy R, Rao KVNM, Baliga BS, Devi S, Raju SK, Veeresh V, Reddy TK and Bairy KL, 2004. The evaluation of the acute toxicity and long term safety of hydro-alcoholic extract of Sapthaparna (Alstoniascholaris) in mice and rats. Toxicology Letters 15: 317–326. https://doi.org/10.1016/j.toxlet.2004.01.015

Bartels H, Böhmer M and Heierli C, 1972. Serum creatinine determination without protein precipitation. Clinica Chimica Acta 37: 193-197. https://doi.org/10.1016/0009-8981(72)90432-9

Blibech M, Ellouz Ghorbel R, Chaari F, Dammak I, Bhiri F, Neifar M and Chaabouni SE, 2011. Improved mannanase production from *Penicillium occitanis* by fed-batch fermentation using acacia seeds. International Scholarly Research Notices 2011: Article ID 9383471. https://doi.org/10.5402/2011/938347

Cai H, Shi P, Luo H, Bai Y, Huang H, Yang P and Yao B, 2011. Acidic β-mannanase from Penicillium pinophilum C1: Cloning, characterization and assessment of its potential for animal feed application. Journal of Bioscience and Bioengineering 112: 551-557. https://doi.org/10.1016/j.jbiosc.2011.08.018

Chandra MRS, Lee YS, Park IH, Zhou Y, Kim KK and Li CY, 2011. Isolation, purification and characterization of a thermostable β-mannanase from Paenibacillus sp. DZ3. Journal of Applied Biological Chemistry 54: 325–331. https://doi.org/10.3839/jksabc.2011.052

Chauhan PS, Puri N, Sharma P and Gupta N, 2012. Mannanases: microbial sources, production, properties and potential biotechnological applications. Applied Microbiology and Biotechnology 93: 1817–1830. https://doi.org/10.1007/s00253-012-3887-5

Cotran R, Kumar V and Robins S, 2010. Robin's pathological basis of disease. 4th Ed. W.B. Saunders Co. Harcourt, pp: 212-217.

Daskiran M, Teeter RG, Fodge D and Hsiao HY**,** 2004.An evaluation of endo-β-D-mannanase (Hemicell) effects on broiler performance and energy use in diets varying in β-Mannan content. Poultry Science 83: 662–668. https://doi.org/10.1093/ps/83.4.662

Dawood A and Ma K, 2020. Applications of microbial β-mannanases. Frontiers in Bioengineering and Biotechnology 8: 598-630. https://doi.org/10.3389/fbioe.2020.598630

Fawcett JK and Scott, 1960. A rapid and precise method for the determination of urea. Journal of Clinical Pathology 13: 156-159. https://doi.org /10.1136/jcp.13.2.156

Flajs D and Peraica M, 2009. Toxicological properties of citrinin. Archives of Industrial Hygiene and Toxicology 60: 457–464. https://doi.org/10.2478/10004-1254-60-2009-1992

Foda DS and Shams GS, 2021.A trail for improving thyroid gland dysfunction in rats by using a marine organism extract. Brazilian Journal of Biology 81: 592-600. https://doi.org/10.1590/1519-6984.226829

Gornall AC, Bardawill CJ and David MM, 1949.Determination of serum proteins by means of the biuret reaction. Journal of Biological Chemistry 177: 751-766.

Hashem AM, Ismail AMS, El-Refai MA and Abdel-Fattah AF, 2001. Production and properties of β-mannanase by free and immobilized cells of Aspergillusoryzae NRRL 3488.Cytobios 105: 115–130.

He Y and Cox RJ, 2016. The molecular steps of citrinin biosynthesis in fungi. Chemical Science 7: 2119–2127. https://doi.org/10.1039/c5sc0427 b

Huntley NF, Nyachoti CM and Patience JF, 2018. Lipopolysaccharide immune stimulation but not β-mannanase supplementation affects maintenance energy requirements in young, weaned pigs. Journal of Animal Science and Biotechnology 9: 1-16. https://doi.org/10.1186/s40104-018-0264-y

Ikegami S, Tsuchihashi F, Harada H, Tsuchihashi N, Nishide Eand Innami S, 1990. Effect of viscous indigestible polysaccharides on pacreatic-biliary secretion and digestive organs in rats. Journal of Nutrition 120: 353-360. https://doi.org/10.1093/jn/120.4.353

Imran M, Cao S, Wan SF, Chen Z, Saleemi MK, Wang N, Naseem MN and Munawar J, 2020. Mycotoxins - a global one health concern: A review. Agrobiological Records 2: 1-16. https://doi.org/10.47278/journal.abr/2020.008

Isaksson G, Lundquist I and Ihse I, 1982. In vitro inhibition of pancreatic enzyme activities by dietary fiber. Journal of Digestive Diseases 24: 54-59. https://doi.org/10.1159/000198775

Jackson ME, Fodge DW and Hsiao HY, 1999. Effects of ß-mannanase in corn-soybean meal diets on laying hen performance. Poultry Science 78: 1737-1741. https://doi.org/10.1093/ps/78.12.1737

Jackson ME, Geronian K, Knox A, McNab J and McCartney E, 2004. A dose response study with the feed enzyme β-mannanase in broilers provided with corn-soybean meal based diets in the absence of antibiotic growth promoters. Poultry Science 83: 1992-1996. https://doi.org/10.1093/ps/83.12.1992.

Jana UK, Suryawanshi RK, Prajapati BP, Soni H and Kango N, 2018. Production optimization and characterization of mannooligosaccharide generating β-mannanase from *Aspergillus oryzae*. Bioresource Technology 26: 308–314. https://doi.org/10.1016/j.biortech.2018.07.143

Khan A, Aalim MM, Khan MZ, Saleemi MK, He C, Khatoon A and Gul ST, 2017. Amelioration of immunosuppressive effects of aflatoxin and ochratoxin a in White Leghorn layers with distillery yeast sludge. Toxin Reviews 36: 275-281. http://doi.org/10.1080/15569543.2017.1303781

Khattab Om KH, Ismail SA, Abosereh NA, Abo-Elnasr AA, Nour SA and Hashem AM, 2020. Optimization and comparative studies on activities of β-mannanase from newly isolated fungal and its mutant. Egyptian Pharmaceutical Journal 19: 29-46. https://doi.org/10.4103/ epj.epj_48_19

Kim JS, Ingale SL, Hosseindoust AR, Lee SH, Lee JH and Chae BJ, 2017. Effects of mannan level and β-mannanase supplementation on growth performance, apparent total tract digestibility and blood metabolites of growing pigs. Animal 11: 202–208. https://doi.org/10.1017/S1751731116001385

Lowry OH, Rosebrough NH, Farr AL and Randall R, 1951. Protein measurement with the Folinphenol reagent. Journal of Biological Chemistry 193:265–275. https://doi.org/ 10.1016/S0021-9258(19)52451-6

Lv JN, Chen YQ, GuoXJ, PiaoXS, Cao YH and Dong B, 2013. Effects of supplementation of β-mannanase in corn-soybean meal diets on performance and nutrient digestibility in growing pigs. Asian-Australasian Journal of Animal Sciences 26: 579-587. https://doi.org/10.5713/ajas.2012.12612

Mahmoud AH, Foda DS, Ibrahim NE, El-Hagrassi AM and Yousef AMH, 2019.Secondary metabolites and biological activities of *Allium porrum* L. in attacking Ehrlich ascites carcinoma in mice. Egyptian Journal of Chemistry 62:211-227. https://doi.org/10.21608/ejchem.2019.20452.2228

Rainbird AL, Low AG and Zebrowska T, 1984. Effect of guar gum on glucose and water absorption from isolated loops of jejunum in conscious growing pigs. British Journal of Nutrition 52: 489-498. https://doi.org/10.1079/BJN19840116

Reitman S and Frankel S, 1957. A colorimeteric method for the determination of serum glutamic oxaloacetic, glutamic pyruvic transaminases. American Journal of Clinical Pathology 28: 56-63. https://doi.org/10.1093/ajcp/28.1.56

Rifai N, Bachorik PS and Albers JJ, 1998. Lipids. lipoproteins and apolipoproteins. In: Burtis CA and Ashwood ER (eds). Tietz Textbook of Clinical Chemistry, 3rd Ed, WB Saunders, Philadelphia, PA, USA, pp: 809-861.

Roque BM, Appuhamy JADRN and Kebreab E, 2017. Role of exogenous enzyme supplementation to improve nutrition and health of ruminants. Broadening Horizons 41: 1-10.

Saeed M, Ayaşan T, Alagawany M, El-Hack MEA, Abdel-Latif MA and Patra AK, 2019. The role of ß-mannanase (Hemicell) in improving poultry productivity, health and environment. Brazilian Journal of Poultry Science 21: 1-8. https://doi.org/10.1590/1806-9061-2019-1001

Saenphoom, P, Liang JB, Ho YW, Loh TC and Rosfarizan M, 2013. Effects of enzyme treated palm kernel expeller on metabolizable energy, growth performance, villus height and digesta viscosity in broiler chickens. Asian-Australasian Journal of Animal Sciences 26: 537-544. https://doi.org/10.5713/ajas.2012.12463

Sathya M, Kokilavani R and Ananta Teepa KS, 2012. Acute and subacute toxicity studies of ethanolic extract of *Acalypha indica* Linn in male Wistar albino rats. Asian Journal of Pharmaceutical and Clinical Research 5: 97-100.

Simon-Giavarotti KA, Giavarotti L, Gomes LF, Lima AF, Veridiano AM, Garcia EA, Mora OA, Fernandez V, Videla LA and Junqueira VB, 2002. Enhancement of lindane-induced liver oxidative stress and hepatotoxicity by thyroid hormone is reduced by gadolinium chloride. Free Radical Research 36: 1033-1039. https://doi.org/10.1080/10715760 21000028280

Smogyi M, 1952. Notes on sugar determination. Journal of Biological Chemistry 195: 19–23.

Speijers GJA and Speijers MHM, 2004. Combined toxic effects of mycotoxins. Toxicology Letters 153: 91–98. https://doi.org/ 10.1016/j.toxlet.2004.04.046

Sujani S and Seresinhe RT, 2015. Exogenous enzymes in ruminant nutrition: A review. Asian Journal of Animal Sciences 9: 85-99. https://doi.org/10.3923/ajas.2015.85.99

Tewoldebrhan TA, Appuhamy JADRN, Lee JJ, Niu M, Seo S, Jeong S and Kebreab E, 2017. Exogenous β-mannanase improves feed conversion efficiency and reduces somatic cell count in dairy cattle. Journal of Dairy Science 100: 244-252. https://doi.org/10.3168/jds.2016-11017

Trinder P, 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of Clinical Biochemistry 6: 24.

Wang Y, Shi P, Luo H, Bai Y, Huang H, Yang P, Xiong H and Yao B, 2012. Cloning, over-expression and characterization of an alkali-tolerant endo-*β*-1,4-mannanase from *Penicillium freii* F63.Journal of Bioscience and Bioengineering 113: 710–714. https://doi.org/10.1016/j. jbiosc.2012.02.005

Yang H, Shi P, Lu H, Wang H, Luo H, Huang H, Yang P and Yao B, 2015. A thermophilic β-mannanase from *Neosartorya fischeri* P1 with broad pH stability and significant hydrolysis ability of various mannan polymers. Food Chemistry 173: 283–289. https://doi.org/10.1016/j. foodchem.2014.10.022