ORIGINAL ARTICLE

*Momordica Foetida* (Cucurbitaceae) Extract Alleviates Parastar (Insecticide) -Induced Toxicity on Pancreatic and Duodenal α-amylase Activity in Male Rats

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**KEYWORDS**

α-amylase; *Momordica foetida*; Parastar; Protective effect; Toxicity

**ABSTRACT:** Parastar is a pesticide formulation made up of two insecticides lambda-Cyhalothrin and Imidacloprid. Parastar is one of the frequently used agrochemicals in the North West Region of Cameroon to protect crops. However, exposures to pesticides result in health alteration as well as therapeutic effects of medicinal plants have been largely acknowledged. One of such plants is *Momordica foetida*, which exhibits antidiabetic and antioxidant properties, suggesting its possible efficiency in toxicity alleviation. This study was designed to evaluate the effect of Parastar on pancreatic and duodenal α-amylase activity, and assess the protective effects of the methanol extract of *M. foetida* in albino male rats. Groups of 8 rats each were orally intubated with either distilled water (5 mL/kg), Parastar (6.23 mg/kg) alone or combination of Parastar and *M. foetida* (50 - 200 mg/kg) daily for 64 days. Once the follow-up period was over, animals were sacrificed, the pancreas and duodenum excised out and weighed. The pancreatic and duodenal homogenates were prepared and used for assessment of α-amylase activity. Results revealed increased pancreas weight in animals treated with Parastar. However, the latter variation was prevented when the animals were co-administered with the methanol extract of *M. foetida*. Parastar decreased pancreatic and duodenal α-amylase activity, which was also prevented by co-treatment of the animals with the methanol extract of *M. foetida*. These findings highlight the toxicity of Parastar on pancreatic and duodenal functions and support the use of *M. foetida* in protecting against alteration of the digestive system by the pesticide.

**INTRODUCTION**

The use of pesticides to control pests and increase food production is a common practice that adversely affects the environment and poses a great danger to many non-target species including humans [1]. Pesticides are commonly used in Cameroon, especially in Santa sub-division in the North West Region, to protect crops against diseases [2].

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One of the most commonly used pesticides is Parastar composed of active components Imidaclorpid and lambda-Cyhalothrin. Parastar is used to control pests that affect crops like tomatoes, potatoes, carrots, vegetable and spices [2, 3]. The use of pesticides has been shown to be associated with health hazards such as hypertension, skin rashes, asthma, and cancer, breathing difficulty, dizziness, vision problems, cough and gastrointestinal tract disorders. Other associated disorders related to pesticides exposure include alteration of the immune, hormonal, reproductive, and neurological and digestive systems [3-5]. Exposure to pesticides may occur through multiple routes including dermal, inhalation, and oral. Indirect exposures to pesticides occur following contact with surfaces sprayed with the chemicals or ingestion of contaminated foods and water contaminated with the pesticide residues [3, 5]. The digestive system represents one of the prevalent exposure routes to the pesticides in human. The human digestive system comprises different degradative and absorption areas from the mouth to anus. The nutrient degradative activity of the digestive system is mainly carried out by hydrolytic enzymes including α-amylase [6, 7]. α-Amylase (EC 3.2.1.1) catalyzes the initial step of starch hydrolysis to glucose and is thus a key enzyme in energy acquisition [8]. Pancreatic α-amylase is synthesized by pancreatic acinar cells and secreted into the duodenum as a major component of pancreatic fluid. In the duodenum, α-amylase digests starch into maltose or maltooligosaccharides, which are subsequently hydrolyzed by brush-border membrane enzymes [8, 9]. α-amylase has been a target molecule in the treatment of type 2 diabetes. The relationship between α-amylase inhibitors and diabetes has been extensively investigated [10, 11].

Previous studies have illustrated ability of pesticides to interfere with the activity of hydrolytic enzymes of the gastrointestinal tract including α-amylase. For instance Cypermethrin, Chlorpyrifos, Diazinon and Deltamethrin decreased α-amylase activity in the insect Eurygaster integriceps [12]. One of the active ingredients of Parastar, Imidaclorpid, altered α-amylase activity in microorganisms found in tomato cultivated soils [13]. On the other hand plants have been used since time immemorial for management of human ailments. Momordica foetida (Cucurbitaceae) is a perennial climbing vine native to tropical Africa and traditionally used against various ailments including malaria, stomachache and diabetes [14,15]. In vitro and in vivo scientific studies have demonstrated the antioxidant properties and blood glucose lowering effect of M. foetida [16-18]. Though pesticides including Parastar used in agriculture are able to cause digestive intoxications with short and long terms consequences on farmers and consumers, very limited research studies have assessed such intoxication and possible implication of medicinal plants in the management of such ailments. This study therefore aimed at evaluating the effects of the methanol extract of M. foetida on pancreatic and duodenal α-amylase activity in male rats exposed to Parastar.

MATERIALS AND METHODS

Materials

Plant materials and preparation of the methanol extracts of M. foetida

Momordica foetida (Cucurbitaceae) fresh plant was harvested in Bambili (Mezam division, Cameroon) in February 2018, and identified at the Cameroonian National Herbarium (specimen No 33420 HNC). The plants were cleaned with water, dried at room temperature, chopped and finely grinded into powder. A quantity of 2.4 kg of the powder was macerated into 1 L of methanol for 48 hours, the mixture filtered using Whatman #1 filter paper, and the solvent evaporated using a rotary evaporator under reduced pressure at 40°C to obtain 15 g solid residue or extract.

Chemicals

Parastar 40WP containing imidaclorpid (20g/kg) and lambda-cyhalothrin (20 g/kg) was purchased from the local market of Santa. It was manufactured by Elanco Novartis, imported and distributed in Cameroon by FIMEX international SABP, Douala, Cameroon. Potassium diphosphate was obtained from Guandong Guanghua Sci-
Tech Co. Ltd (Guangdong, China), while iodine was purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). All other chemicals were of analytical grade.

**Experimental animals**

Forty male albino Wistar rats (average weight of 150 ± 10 g) raised in the animal house of the Department of Biochemistry (Faculty of Science, University of Bamenda) were used for the experiments. They were housed under standard conditions (room temperature of 25°C) and had free access to food and water. They were handled according to ethical guidelines of the Cameroon National Veterinary Laboratory as reference by the approval and health control No 001/17 CCS/MINEPIA/RD-NW/DD-ME/SSV.

**Methods**

**Experimental design**

Forty male rats were divided into 5 groups of 8 animals each. The 5 groups were treated orally with distilled water (5 mL/kg), Parastar pesticide (6.23 mg/kg) alone or Parastar plus 50, 100 and 200 mg/kg of the methanol extract of *M. foetida*, respectively. The animals were treated daily by gavage for 64 days and their body weight recorded once every three days. The dose of Parastar used in this study was based on toxicity of its constitutive ingredients, LD₅₀ values, as described previously [19] while those of the plant extract were defined from previous study on animal models [20,21]. After 64 days of treatment, the rats were fasted overnight, anaesthetized using diazepam, sacrificed and organs (pancreas and duodenum) dissected out and weighed. A 15% homogenate of each of the dissected organs was prepared in phosphate buffer (pH 7.1; 0.01M) and used for the estimation of α-amylase activity. The enzyme activity was corrected using protein levels (assessed as described by Gornall et al. [22]) in the homogenate.

**Determination of α-amylase activity**

The homogenate containing α-amylase was incubated with starch following by detection of residual substrate in the medium using iodine solution, as reported elsewhere [23]. Briefly, the blank or assay test tubes containing 2 mL of starch solution (10 mg/mL) were pre-incubated in the water bath at 25°C for 5 min and 2 mL of either pancreatic or duodenal homogenate were added. After homogenization, the mixture was incubated at 25°C for 5 min, and then 2 mL of 5 mM iodine solution were added. The absorbance of the incubation media was recorded against the blank at 590 nm, and the concentration of residual starch determined using standard curve obtained from different starch concentrations (0.5 – 10 mg/mL). The pancreatic and duodenal α-amylase activities were then calculated as mg of starch transformed/min. The enzyme activities were thereafter corrected using the concentration of proteins in different homogenates (expressed as mg of starch transformed/min/mg proteins).

**Statistical analysis**

Data were presented as mean ± standard deviation (Standard deviation). The data were analyzed using One Way analysis of variance (ANOVA) and any difference between groups assessed with the Student-Newman-Keuls test. All analysis was performed using MedCalc® software Version 8.0.01.

**RESULTS**

**Animal body weight**

The body weight of animals exposed to Parastar and the methanol extract of *M. foetida* for 64 days is presented Figure 1. In general the animal body weight increased over time and there was no difference among groups.
Figure 1. Body weight of animals exposed to Parastar and the methanol extract of *M. foetida* for 64 days
Values are expressed as mean ± SD for 8 rats per treatment.

**Weight of Pancreas and duodenum**

The relative weight of pancreas and duodenum from rats exposed to Parastar alone or in co-treatment with the methanol of *M. foetida* are illustrated in Figure 2. Exposure to Parastar resulted into significant increase (P < 0.05) of the pancreas weight when compared to negative control receiving distilled water. However, administration of the methanol extract of *M. foetida* to the animals alleviated the effect of Parastar on the pancreas weight, with the doses 50 and 100 mg/kg the plant extract being the most effective. The duodenal weight was not significantly affected by the different treatments investigated.

Figure 2. Pancreas and duodenum weight of animals exposed to Parastar and the methanol extract of *M. foetida*
Values are expressed as mean ± SD for 8 rats per treatment. Different letters mean significant difference among treatments for the same organ, P <0.05, student Newman Keul’s test.
Pancreatic and duodenal α-amylase activity

The activity of α-amylase in pancreatic and duodenal homogenates in rats exposed to Parastar alone or co-treated with Parastar and the M. foetida extract is shown in Figure 3. In control animals, α-amylase activity is higher (P < 0.05) in duodenal homogenate than pancreatic one. As compared to the control animals receiving distilled water, Parastar exposed rats showed significant (P < 0.05) decrease of both pancreatic and duodenal α-amylase activity. The addition of the methanol extract of M. foetida to Parastar accentuated the decrease of the pancreatic α-amylase while all doses of the plant extract significantly alleviated (P < 0.05) the decreasing effect of the pesticide on duodenal α-amylase activity.

![Figure 3](image)

**Figure 3.** α-amylase activity of the pancreatic and duodenal homogenates of animals exposed to Parastar and the methanol extract of M. foetida. Values are expressed as mean ± SD for 8 rats per treatment. Different letters mean significant difference among treatments for the same organ homogenate, P <0.05, student Newman Keul’s test.

DISCUSSION

The enzymes α-amylases or α-1,4-glucan-4-glucanohydrolases are endoglycosidases that belong to the glycosyl hydrolase family 13. These enzymes catalyze the first step in the digestion of starch [24]. Starch is normally the main source of digestible carbohydrate in the human diet and as such, is the major source of glucose that appears at relatively high concentrations in the blood circulation following intestinal digestion of a starch-containing meal. α-amylase progressively brings about hydrolysis of the polysaccharide resulting in the production of maltose, maltotriose and limit dextrins as the main products [7,25]. Pancreatic α-amylase is the main enzyme for luminal digestion of carbohydrate in the small intestine following the initiative digestive work of the salivary amylase in the mouth [26]. However pancreatic dysfunctions such as pancreatitis together with alterations of α-amylases have been noted upon exposure to pesticides [27-29].

In the present study, the toxicity of Parastar, one of the commonly used insecticide formulations in market gardening crop protection in Cameroon was evaluated on pancreatic α-amylase activity in male rats. Also a potential protective effect of a traditional medicinal plant
*Momordica foetida* against Parastar- associated toxicity on pancreatic and duodenal parameters was investigated. Parastar did not show any substantial effect on animal body weight. This observation on the body weight is consistent with observations reported earlier on this pesticide effect on male rats [19]. Moreover the animal body weight was not altered by *M. foetida* extract, suggesting that the plant extract does not induce toxicity at the investigated doses.

Parastar increased the pancreas weight of animals without affecting the duodenum weight. The pancreas is an organ with both exocrine and endocrine functions. The exocrine function plays vital role in the digestion and general metabolism by secreting digestive enzymes, ions and water into the gastrointestinal tract [30]. The increase of the pancreas weight upon pesticide administration could be a sign of affection commonly known as pancreatitis. However, it was previously observed that one of Parastar active ingredients, Imidacloprid (0.06 – 6 mg/kg), did not affect pancreas weight in mice [31]. Alteration observed in the present study could thus be due to the other active component of the pesticide, lambda-Cyhalothrin, or to any synergetic effect of the two compounds. Moreover Parastar adjuvants or excipients in the insecticide formulation may also contribute to the effects of the pesticide. It is hoped that further studies could help to better assess the intrinsic effect of each active component of Parastar to the alteration of pancreas weight. It was also observed that the negative effect of Parastar was alleviated when the animals were co-administered *M. foetida* extract and the pesticides. This suggests a beneficial potential of the plant extract against Parastar-induced toxicity on the pancreas. Such protective effect could be related to the presence of antioxidant compounds in the *M. foetida* extract [16-18]. Besides, *M. foetida* is traditionally used in the management of diabetes and has shown interesting effects on the control of glucose levels in rats and humans [32, 33]. These beneficial effects of *M. foetida* together with its protective effect on pancreas weight reported herein, sustain a beneficial role of this plant extract on pancreatic function.

The exocrine secretions of the pancreas represent nearly 90% of the pancreatic functions and consist of salts and digestive enzymes including α-amylase [34]. Pancreatic α-amylase is synthesized by pancreatic acinar cells and secreted into the duodenum where it digests starch into maltose or maltooligosaccharides, which are subsequently hydrolyzed by brush-border membrane enzymes [8]. α-amylase like other pancreatic enzymes are produced and secreted into a network of pancreatic ductules and ducts in response to a meal [34]. The high levels/ activity of α-amylase are expected not in the production site which is the organ itself (pancreas) but in its milieu of action, that is the duodenum, where it is released to assist in digestion [8, 11]. This was evidenced by results of this study with higher enzyme activity in the duodenal homogenate as compared to the pancreatic homogenate. Exposure to the insecticide Parastar resulted in decreased α-amylase activity in both pancreatic and duodenal homogenates. This ‘anti’-amylase effect of Parastar is an additional toxicity point to the alteration of glucose metabolism (glucose levels, insulin) of its ingredient Imidacloprid [31]. Alteration of α-amylase activity by Parastar is consistent with altered pancreas weight following exposure to the pesticide formulation, and further illustrates the negative effect of this chemical on such vital organ as amylase is a major component of pancreatic fluid [8, 9]. Affection of the pancreas by Parastar could explain the signs and symptoms such as abdominal pain, nausea and vomiting usually experienced by farmers upon exposure to agropesticides [3, 5]. Though the negative effect of the Parastar is evident, the mechanism of such toxicity is still to be clarified as the chemical could act on the biosynthetic or activity levels to affect the hydrolytic capacity of the enzyme.

Co-administration of the methanol extract of *M. foetida* and Parastar showed differential effect on the enzyme by decreasing the activity of α-amylase in pancreas while alleviating the activity in duodenum. For α-amylase in the pancreatic homogenate, the plant was not able to prevent or reverse possible pancreatitis due to the pesticide. However in the duodenum which is the actual milieu of α-amylase action, *M. foetida* extract prevented the negative effect of the pesticide on the enzyme. This action of the plant extract indicates a certain specificity of the active compound(s) of
the plant extract which may be affected by the conditions of the medium. In fact *M. foetida* is rich in phytochemicals such as phenolic compounds that may be more available through the action of hydrolase enzymes present in intestinal lumen. Factors such as pH could also affect the composition/functionality of groups on bioactive compounds of the plant extract [18, 35]. Previous studies have shown the potential of Parastar to induce oxidative stress [19], and it can be speculated that *M. foetida* extract, which possesses antioxidant substances [18], attenuate the oxidative stress induced by Parastar. The antioxidants in *M. foetida* extract may inactive or prevent the negative effect of Parastar on α-amylase activity or enzyme molecular structure. Protective and ameliorative effects have been demonstrated with several plants and plant derived products. For example, garlic oil, vitamin E and C alleviate alteration of amylase activity and induction of oxidative stress in pancreatic cells upon exposure to furan and the pesticide Phosalone [36, 37].

Overall, results from this study suggest that Parastar induced toxic effects on pancreatic function by increasing organ weight, decreasing α-amylase activity in the pancreas and duodenum. The methanol extract of *M. foetida* showed protective property on Parastar toxicity by preventing the increase of pancreas weight and alleviating inhibition of the duodenal α-amylase activity. *M. foetida* could therefore be a promising plant to prevent toxicity of pesticide on the pancreatic function.

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**Conflict of interest**

The author(s) declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

**REFERENCES**


