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Sensitivity in rats and *Artemia salina* to biogenic amines formed in sardine *Sardina pilchardus*

*Sensibilité des rats et d'*Artemia salina *aux amines biogènes formées dans la sardine* Sardina pilchardus

Mohamed Elamin Afilal
Université Mohamed I[er], Laboratoire de microbiologie appliquée,
Faculté des sciences, département de biologie, BP 764,
60000 Oujda, Maroc

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ABSTRACT

Afilal M.E., 1998 - Sensitivity in rats and Artemia salina *to biogenic amines formed in sardine* Sardina pilchardus. Mar. Life, ***8*** *(1-2): 45-50.*

In this work, the effect of biogenic amines on rats and Artemia salina *was studied. Amines were added to a mixture of fresh sardines in quantities similar to those normally found in altered sardine. Intraperitoneal injection of this preparation caused symptoms of histamine intoxication in white rats. When tested on* Artemia salina, CL_{50} *for histamine, tryptamine, tyramine, cadaverine, putrescine and spermidine, were respectively: 85 µg/5ml, 44.7 µg/5ml, 25.8 µg/5ml, 21.3 µg/5ml, 15 µg/5 ml and 7.5 µg/5 ml. The effects of different amines on histamine toxicity is discussed.*

RÉSUMÉ

*Afilal M.E., 1998 - [Sensibilité des rats et d'*Artemia salina *aux amines biogènes formées dans la sardine* Sardina pilchardus*].* Mar. Life, ***8*** *(1-2) : 45-50.*

Dans ce travail, nous étudions la toxicité des amines biogènes chez Artemia salina *et chez les rats blancs. Les amines étaient ajoutées à de l'infusion de sardine dans des concentrations similaires à celles trouvées dans la sardine altérée. En injection intrapéritonéale, cette préparation provoque des symptômes de l'intoxication histaminique chez les rats blancs. Pour* Artemia salina, *les* CL_{50} *pour l'histamine, la tryptamine, la tyramine, la cadaverine, la putrescine et la spermidine, étaient respectivement : 85 µg/5ml, 44,7µg/5ml, 25,8 µg/5ml, 21,3 µg/5ml, 15 µg/5ml et 7,5 µg/5ml. L'effet des différentes amines sur la toxicité histaminique est également discuté.*

INTRODUCTION

Importers and public health authorities from the European Economic Community have expressed dissatisfaction regarding the wholesomeness of Moroccan canned sardine, because several outbreaks of food poisoning have occured in Europe, implicating sardine (Taylor, 1983; Ababouch, Afilal,1989). For example Murray *et al.* (1982), conducted an investigation in the United Kingdom on 26 incidents of scombrotoxin poisoning that involved 71 people, and found that the highest rate of incidents were observed after the consumption of Moroccan sardines.

Historically, it has been referred to as scombrotoxic fish poisoning because of its frequent association with the consumption of spoiled scombroid fish such as tuna and mackerel (Gilbert *et al.*, 1980). However, pelagic fish such as sardines, herring and pilchards, and some types of cheese have been involved in outbreaks of this illnes (Taylor, 1985).

This intoxication occurs with a variety of symptoms of a cutaneous, gastrointestinal, hemodynamic and neurological nature (Gilbert *et al.*, 1980; Murray *et al.*, 1982). The intoxication occurs within a few minutes to several hours following the consumption of food that contains unusually high levels of histamine (Taylor, 1983, 1985). Some

intoxications involved fish with low content of histamine (Murray *et al.*, 1982), indicating that other substances might be involved, possibly as histaminic toxicity potentiators (Bjeldanes *et al.*, 1978). Biogenic amines such as cadaverine, putrescine, tyramine, tryptamine, and spermidine, are synthesized during the spoilage of Moroccan sardines (*Sardina pilchardus*) by bacteria such as *Proteus* (Afilal *et al.*, 1995, 1997; Ababouch, Afilal, 1989; Ababouch *et al.*, 1991 b).

Data on the role of biogenic amines in food, especially in sardines, are needed to improve our understanding of the mechanism of histamine toxicity, and to provide a scientific basis for sound regulatory actions. This study reports on the toxicity of different biogenic amines that were found in sardines. We discuss the role of different amines in histamine poisoning.

METHODS

Solutions of the monohydrochloride of tyramine and tryptamine, dihydrochloride of cadaverine, histamine, putrescine and trihydrochloride of spermidine (Sigma) were prepared at different concentrations, for intraperitonial injection to groups of ten male white rats weighing 200-300 g. Then we added sterile fresh sardine infusion that was prepared according to the method described by Eitenmiller *et al.* (1981) and Ababouch *et al.* (1991 a). Fresh sardines were mixed with twice their weight in distilled water and homogenized in an ultraturax mixer. The mixture was then boiled for 1 hour and filtered.

The different solutions were injected according to the method reported by Billon (1978).

For the *Artemia salina* test, the different concentrations were prepared in a physiological solution (8,5 $g.l^{-1}$ NaCl). Eggs were incubated in the dark at 22°C for 24 h, in the physiological solution for eclosion (Meyer *et al.*, 1982). The tests were then conducted in 5 ml of the same solution.

RESULTS

Determination of CL_{50} in *Artemia salina*

Figures 1, 2, 3, 4, 5, and 6 show the mortality of *Artemia salina* larvae, with different concentrations of respectively: histamine, tryptamine, tyramine, cadaverine, putrescine and spermidine.

The curves were studied statistically with the probits method (Chevalier, Pairain, 1968), for the determination of CL_{50} (the concentration to kill 50% of the population). The results for histamine, tryptamine, tyramine, cadaverine, putrescine and spermidine, were respectively: 85 ± 7 µg/5ml, 44.7 ± 6 µg/5ml, 25.8 ± 5 µg/5ml, 21.3 ± 8 µg/5ml, 15 ± 7 µg/5ml and 7.5 ± 2 µg/5ml.

The sensitivity in rats

Results show that the injection of the different biogenic amines caused variable symptoms including immobilisation and respiration difficulties for 2 or 3 hours. The simultaneous injection of antihistaminic solution (2 ml of phenergan = promethazine at 0.1% of ste-

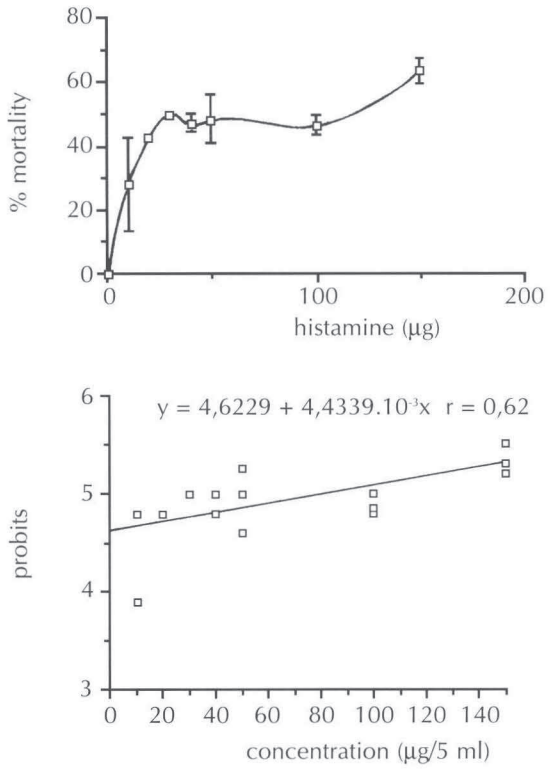

Figure 1 - Histamine effect in *Artemia salina.* / *Effet de l'histamine sur* Artemia salina.

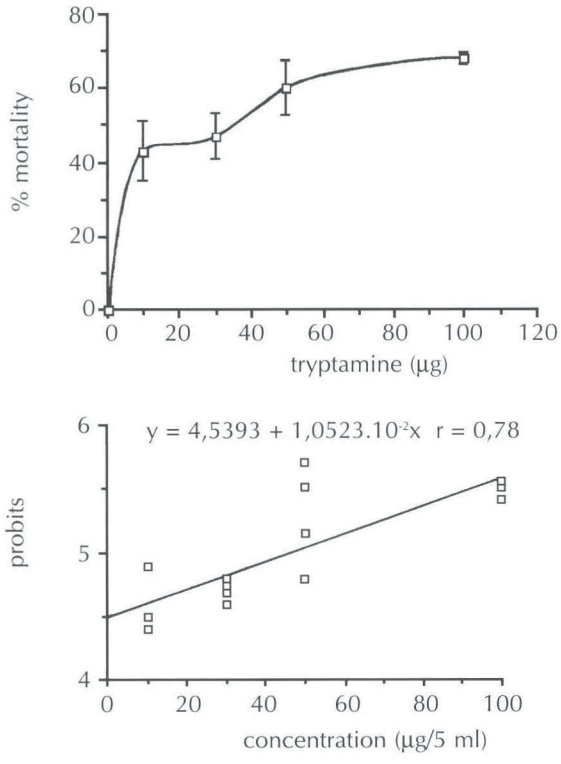

Figure 2 - Tryptamine effect in *Artemia salina.* / *Effet de la tryptamine sur* Artemia salina.

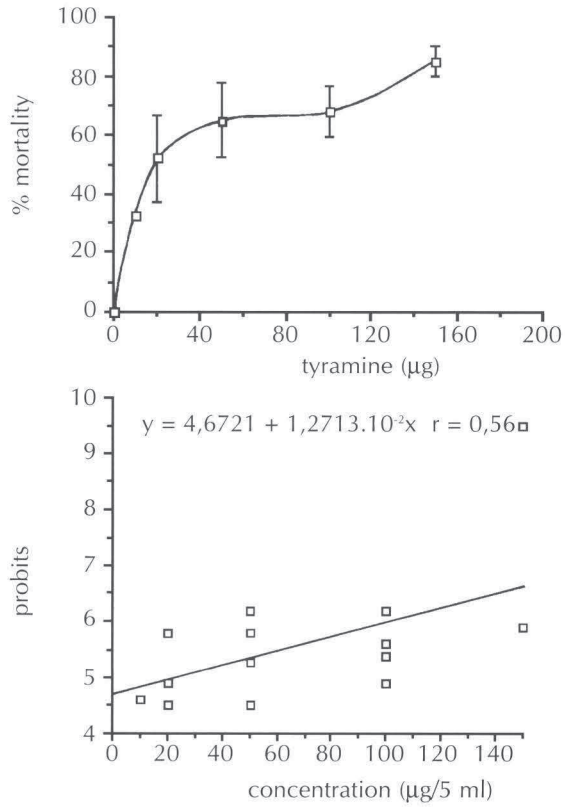

Figure 3 - Tyramine effect in *Artemia salina.* / *Effet de la tyramine sur* Artemia salina.

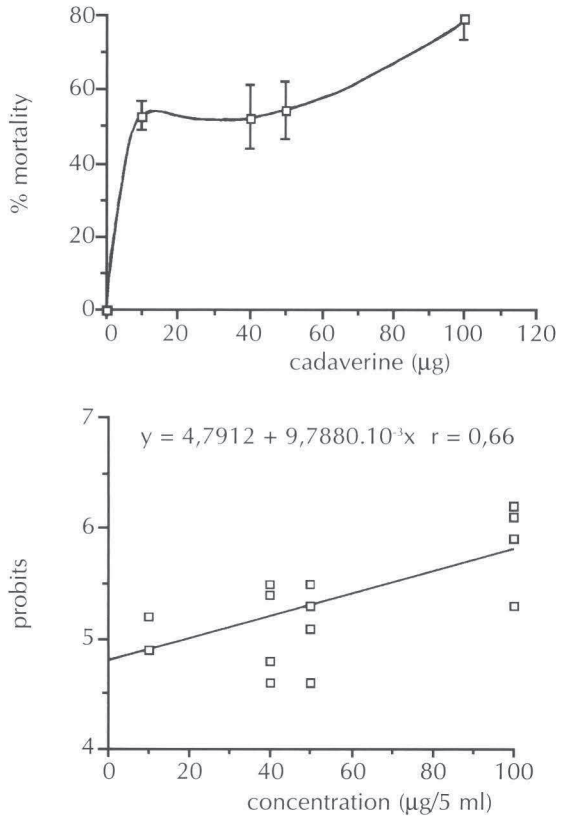

Figure 4 - Cadaverine effect in *Artemia salina.* / *Effet de la cadaverine sur* Artemia salina.

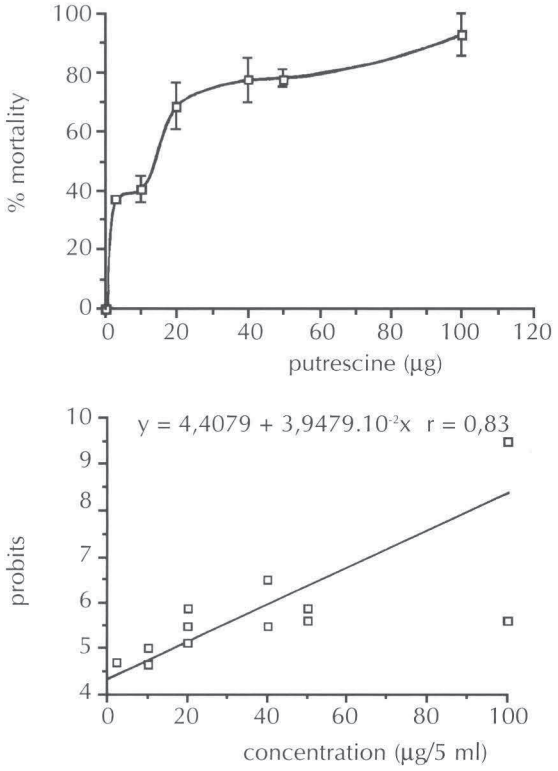

Figure 5 - Putrescine effect in *Artemia salina.* / *Effet de la putrescine sur* Artemia salina.

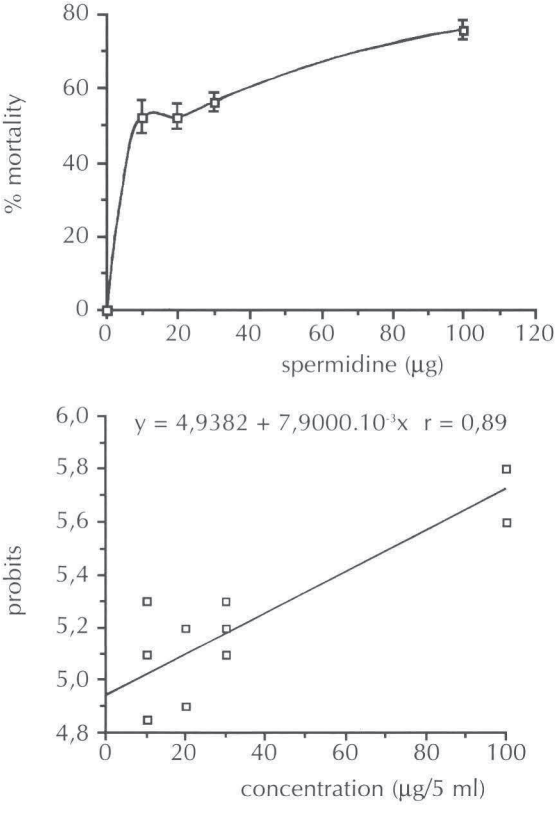

Figure 6 - Spermidine effect in *Artemia salina.* / *Effet de la spermidine sur* Artemia salina.

rile distilled water) relieves the symptoms of tyramine, but not those of spermidine. This may be due to the release of histamine by tyramine and not by spermidine.

Table I shows that intraperitonial injection of 250 mg histamine (1 $g.kg^{-1}$) does not cause the death of rats; these animals, therefore, are highly resistant, but death occurs 20 mn after the injection of 275 mg of tyramine (1.1 $g.kg^{-1}$), 100 mg of tryptamine (0.4 $g.kg^{-1}$), 250 mg of spermidine (0.66 $g.kg^{-1}$).

DISCUSSION

This work illustrates very well the complexity of the effect of biogenic amines and their role in histamine poisoning, when due to the ingestion of altered sardine. Three hypotheses could explain the mechanisms:

1st hypothesis: Taylor and Lieber (1979) reported that since oral histamine, when taken by itself, is not toxic even at high doses, a barrier to histamine absorption must be present in the gut. Biogenic amines, especially cadaverine and putrescine, affect the histamine absorption by inhibiting intestinal histamine metabolism, or by altering the physical barrier function of the small intestine. They allow the diffusion of greater quantities of histamine into the circulation, that results in an increase in histamine toxicity (Cheu-Hsia, Bjeldanes, 1982; Lyons *et al.*, 1983).

2nd hypothesis: The results reported in this work (with different animals) support the idea that all biogenic amines, found in sardine, and not histamine alone, causes different reactions, the symptoms depending on the sensitivity of individuals. The variability of the symptoms observed after ingestion of altered sardine, therefore, might be attributed, in part, to different biogenic amines, which accumulate at different rates in different kinds of food, and not to histamine alone. This hypothesis is supported by the fact that symptoms of such intoxication vary with individuals when the food is incriminated. Taylor *et al.* (1982) report that histamine in cheese does not cause the same symptoms as when eaten in fish.

Arnold and Brown (1978) reported that *Artemia salina* could be used for the biological dosage of histamine. However, our study shows that this method is not specific because death of larvae could be due to other amines, which are very toxic, even if they take place at rates far below that of histamine in food products such as sardines (table II).

Figure 7 shows the structure of biogenic amines, so the aliphatic forms are more toxic than the cyclic forms.

3rd hypothesis: This work shows also that some biogenic amines could provoke the release of physiological histamine stocked in mastocyte cells (Dumain, Pailler, 1987) and increase the doses of histamine in blood to become toxic. Tyramine can act indirectly in releasing histamine.

CONCLUSION

The relatively high frequence of scombroid toxicity by sardine urges the public health services to focus on quality control and set standards for the acceptance or the rejection of commercial marine products.

Only few countries have defined official standards and strict regulations. This work makes evident the fact that all biogenic amines, and not histamine alone, have to be taken into account in evaluating the hazard posed by canned sardines. The best approach consists in establishing an M.T.C. (Maximum Tolerated Concentration), for each amine, low enough to ensure efficient consumer protection, and take into account consumers who may be more sensitive to different amines.

Epidimiological studies are needed to determine the long term effects of the frequent consumption of such products.

Table I - Sensitivity in rats (a: sterile sardine infusion (100g/200 ml); b: amount found in altered sardines injected in 2.5 ml of sterile distilled water; c: amount which causes death at 100%). / *Sensibilité chez le rat (a : infusion stérile de sardine (100g/200ml) ; b : dose trouvée chez la sardine altérée, injectée dans 2,5 ml d'eau distillée ; c : dose léthale à 100%).*

Amines	amount injected	Observations
Fresh(*a*) sardine	3 ml	Nothing particular
Altered(*a*) sardine	3 ml	Intoxication symptoms after 20 min but no death
Histamine	250 mg(*b*)	Intoxication symptoms after 10 min but no death
Tyramine	18 mg(*b*)	Intoxication symptoms after 10 min but no death
	275 mg(*c*)	Intoxication symptoms and death after 20 min
Tryptamine	7 mg(*b*)	Intoxication symptoms after 10 min but no death
	100 mg(*c*)	Intoxication symptoms and death after 20 min
Putrescine	12.5 mg(*b*)	Intoxication symptoms after 10 min but no death
Spermidine	6 mg(*b*)	Intoxication symptoms after 10 min but no death
	66 mg(*c*)	Intoxication symptoms and death after 20 min
Cadaverine	50 mg(*b*)	Intoxication symptoms after 10 min but no death

Table II - Biogenic amines rates found in sardines (*Sardina pilchardus*) (Ababouch *et al.*,1991 b; Afilal *et al.*, 1995). / *Taux d'amines biogènes trouvés chez la sardine (*Sardina pilchardus*) (Ababouch* et al., *1991 b ; Afilal* et al., *1995).*

Amines	In fresh sardine ($mg.g^{-1}$)	after 24h of alteration at 20°C ($mg.g^{-1}$)
histamine	0.12	246
cadaverine	0.07	50
tyramine	0.07	19
putrescine	0.59	12
tryptamine	0.15	0.7
spermidine	0.26	0.4
spermine	0.47	0.5

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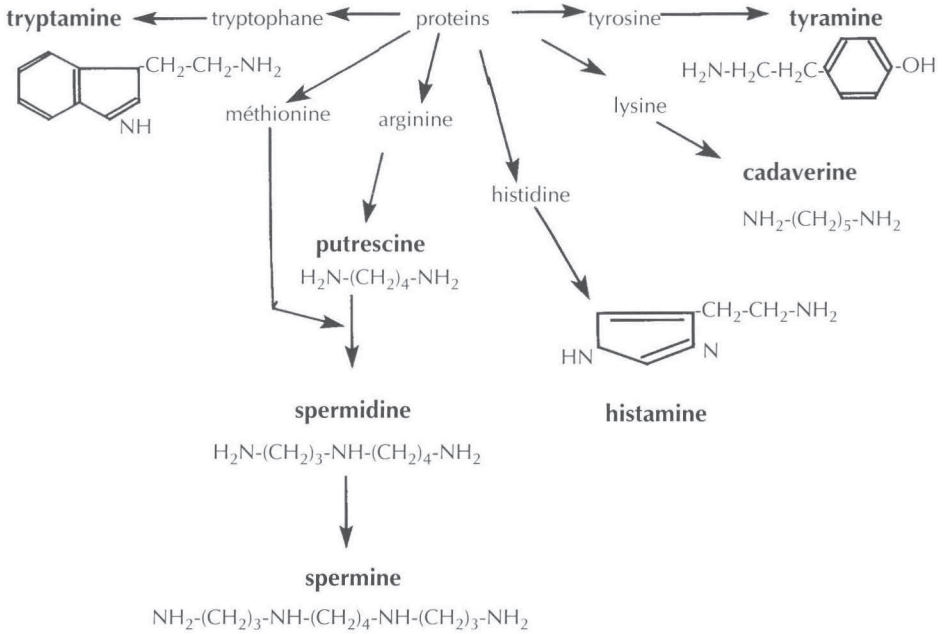

Figure 7 - Biochemical pathway of biogenic amines formation in sardine (*Sardina pilchardus*). *Voies biochimiques de formation des amines biogènes chez la sardine* (Sardina pilchardus).

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