The Risk Assessment of Chlorantraniliprole and Imidacloprid on liver Enzymes and on Kidney Activity in Albino Mice

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ABSTRACT: The impact of chlorantraniliprole and imidacloprid as technical ingredients and formulated preparation on the enzyme levels in liver and on creatinine and uric acid in kidney of male albino mice (*Muss musculus)* were determined at certain concentrations (0.1, 0.01 and 0.001 of the LD_{50}). The obtained results of chlorantraniliprole showed that both technical ingredient (95%) and formulated preparation (Coragen® 20%) reduced the levels of GOT and GPT as liver enzymes and creatinine of kidney. On the other hand, both technical and formulated preparation increased uric acid levels except the formulated chlorantraniliprole at 0.01 of LD_{50} (2.8 mg/dL).The imidacloprid technical ingredient (95%) and formulated preparation (Admire® 20%) indicated that GOT and GPT liver enzymes were reduced except at 0.1 and 0.01of imidacloprid (Admire®).On the other hand, imidacloprid technical ingredient and formulated one increased creatinine and uric acid of kidney except 0.001 concentration. The data obtained for both tested compounds indicated that chlorantraniliprole showed less risk in the liver enzymes and kidney of the male albino mice than imidacloprid did.

Key words: Risk assessment, Chlorantraniliprole, Imidacloprid, Liver enzyme (GOT and GPT), Kidney (uric acid and creatinine)

INTRODUCTION

Food shortage is one of the most difficult problems around the world. The basic source for food supplying is agriculture which facing certain problems. The most important problem is the pests attacking cultivated crops. To manage these pests there are many choices. The chemical pesticides still one of these main choices for the farmers especially in the third world. The environmental pollution and pesticides residues in food are serious problems, because they represent a danger on human life. Many diseases were widespread on mammals because of the adverse effect of pesticides residue on food. One of the new pesticide groups is anthranilic diamine (chlorantraniliprole). Chlorantraniliprole was registered to be used against several insect-pests belonging to the order Lepidoptera and some to Coleoptera, Diptera and Isoptera species. Chlorantraniliprole belongs to a new class of selective insecticides (Ryanodine receptor), which regulates the release of intracellular, stored calcium critical for muscle contraction (Lahm *et al*., 2005 and 2007).

Chlorantraniliprole is of low use rates while it has a high biological activity against several insect-pests, with a very low mammalian toxicity and

selectivity to non-target arthropods (Bassi *et al*., 2007 and Lahm *et al*., 2005). The larvicidal and ovicidal activities were observed to some Lepidopteron pests (Bassi *et al*., 2009). Imidacloprid is a relatively new insecticide, first registered for use as a pesticide in (USEPA, 1994). Imidacloprid has a wide variety of uses against several insect-pests on cotton and vegetable crops, turf and ornamentals. Described the first kinetic determination of aspartate aminotransferase (glutamate oxalacetate transaminase) which belongs to the transaminases, and catalyze the inter conversion of amino acids and α-ketoacids by transfer of amino groups. Aspartate aminotransferase (GOT) is commonly found in human tissue. Although heart muscle is found to have the most activity of the enzyme. Significant activity has also been found in the brain, liver, gastric mucosa, adipose tissue, skeletal muscle, and kidneys. Wroblewski and Ladue (1956a) described the first kinetic determination of alanine aminotransferase (glutamate pyruvate transaminase) (GPT) which belongs to the transaminases, and catalyze the inter conversion of amino acids and α-ketoacids by transfer of amino groups.

In the muscle metabolism, creatinine is synthesized endogeneously from creatine and creatine phosphate. Under conditions of normal renal function, creatinine is excreted by glomerular filtration. Creatinine determinations are performed for the diagnosis and monitoring of acute and chronic renal disease as well as for the monitoring of renal dialysis. Uric acid is considered to be the final product of urine metabolism in the human organism. Uric acid measurements are used for the diagnosis and treatment of numerous renal and metabolic disorders, including renal failure, gout, leukemia, psoriasis, starvation or other wasting conditions, and of patients receiving cytotoxic drugs.

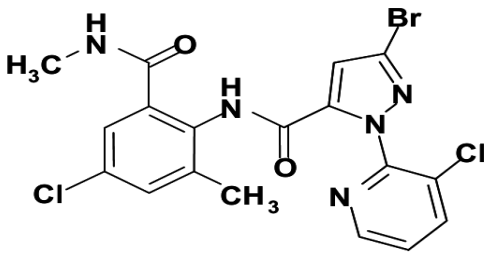
Therefore, the main target of the current investigation is to evaluate the risk and adverse effect of chlorantraniliprole and imidacloprid on liver and kidney of the male albino mice.

MATERIALS AND METHODS

Chemicals

A) Anthranilic diamide.
- Technical grade (Chlorantraniliprole 95%)
- Formulated preparation (Coragen® 20% SC)

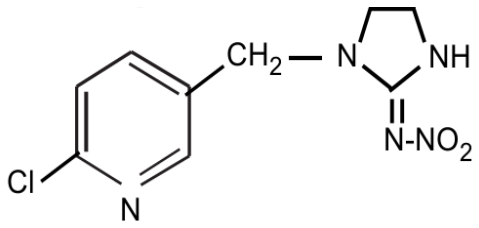
Chemical Structure



IUPAC name: 3-bromo-4′-chloro-1-(3-chloro-2-pyridyl)-2′-methyl-6′-(methylcarbamoyl) pyrazole-5-carboxanilide.

B) Neonicotinoid
- Technical grade (Imidacloprid 95%)
- Formulated preparation (Admire® 20% SC)

Chemical Structure



IUPAC name: *N*-{1-[(6-Chloro-3-pyridyl) methyl]-4, 5-dihydroimidazol-2-yl} nitramide

Evaluated indicators of risk:

A) Liver enzymes: (AST/GOT and ALT/GPT)

The International Federation of Clinical Chemistry (IFCC) recommended the standardized procedure for AST /GOT determination, including optimization of substrate concentrations, employment of TRIS* buffers, preincubation of combined buffer and serum to allow side reactions with NADH to occur (Bergmeyer *et al*., 1985). The IFCC confirmed their recommendation for ALT/GPT and extended it to 37^{o}C (Schumann *et al*., 2002). The used method is derived from the formulation recommended by the IFCC and was optimized for the performance and stability.

B) Kidney: (creatinine and uric acid)

Creatinine concentrations in urine can be used as reference values for the excretion of certain analytes (albumin, α-amylase). The creatinine determination method is based on the Jaffe reaction described by Seelig and Wust (1969) which was modified by Bartels *et al.* (1972). This modified version has a higher sensitivity and better precision. The assay of uric acid was carried colorimetric method that described by Town *et al*. (1985).

The acute toxicity determination

The acute toxicity of chlorantraniliprole and imidacloprid was carried for liver enzymes and kidney of the male of albino mice (*Mus musculus*). The used individuals of mice in this investigation were homogenous in age (4 weeks) and weight (15±1 g). There were 12 treatments in addition to untreated check, each

treatment replicated three times and each replicate contains 5 individuals of the mice. Samples of blood of the albino mice were collected after 45 day post-oral treatment. The evaluated concentrations of the tested forms of both compounds were 0.1, 0.01 and 0.001 of LD_{50} of the International Tax. Data published in the Manual of Pesticides. The mice were treated orally.

Statistical analysis

Data were subjected to the analysis of variance test (ANOVA) as Complete Randomized Design (CRD) and the least significant differences (LSD) at the 5% level were determined according to computer program Costat software (version 6.400).

RESULTS AND DISCUSSION

The effects of chlorantraniliprole (95%) and Coragen® (20%) were evaluated on GOT, GPT, creatinine and uric acid of the albino mice. The obtained data in Table (1) indicated that the levels of GOT enzyme of all treatments were decreased when they were compared with that of the untreated check. Chlorantraniliprole decreased the GOT levels to 218,260 and 262U/L with the tested concentration of 0.001, 0.1 and 0.01 of LD_{50}, respectively. Meanwhile, the formulated preparation Coragen® showed the same trend toward GOT levels. The recorded levels of GOT were 334,372 and 310 U/L with 0.1, 0.01 and 0.001concentrations of LD_{50}, respectively. In respect to GPT, the same trend was obtained by chlorantraniliprole showing the least levels of GPT that recorded 60, 63 and 64 U/L with 0.01, 0.1 and 0.001 of LD_{50}, respectively. On the other hand, Coragen® indicated the values of 81, 72 and 65 U/L with 0.01, 0.1 and 0.001 concentrations of LD_{50}, respectively. Kidney activity indicators creatinine and uric acid have been assessed for their levels regarding the efficacy of both chlorantraniliprole and Coragen® in the body of the mice.

Creatinine levels indicated that all the tested treatments decreased these levels as compared with the untreated check (0.30mg/dL). On the contrary, the used treatments increased uric acid levels, except for Coragen® at 0.01 of LD_{50}which recorded a determination of 2.8mg/dL as compared with untreated check (3.9mg/dL). Since chlorantraniliprole belongs to selective Ryanodine receptor, which regulates the release of intra cellular stored calcium critical for muscle concentration, it is suggested that the release of calcium in the muscles could be responsible for the decrease of GOT and GPT levels (liver enzyme) and creatinine and uric acid (kidney). On the other hand, imidacloprid belongs to nicotinoids chemical class which is nicotinic acetyl choline receptor agonists and that coned mean that there is a strong evidence to prove that protein is responsible for insecticidal effect which may plays a role in increasing the concentration of the liver enzymes and kidney activity indicators (creatinine and uric acid).

The results are in agreement with those obtained by Shipra *et al.*(2010) who found that imidacloprid has adverse effects on GOT and GPT levels at high

doses (20mg/kg/day) in female rats. Toor *et al*.(2013) proved that imidacloprid at higher doses significantly increased liver enzymes levels (GOT and GPT) in treated rats.

Table (1). The effect of chlorantraniliprole (95%) and formulated preparation (Coragen® 20% SC) on the levels of liver enzymes and kidney activity indicators at certain suggested concentrations

Treatments		Liver enzymes		Kidney indicators	
		G.O.T (U/L)	G.P.T (U/L)	Creatinine (mg/dL)	Uric acid (mg/dL)
Chlorantraniliprole (Techn. 95%)	0.1*	260	63	0.27	6.1
	0.01	262	60	0.22	4.6
	0.001	218	64	0.18	4.7
Coragen® (20% SC)	0.1	334	72	0.17	4.6
	0.01	372	81	0.24	2.8
	0.001	310	65	0.14	4.0
Untreated check		377	94	0.30	3.9
LSD $_{0.05}$		13.133	5.064	0.073	1.864

*** Concentrations of LD_{50}**

Data presented in Table 2 show the effect of technical imidacloprid and formulated preparation (Admire®) at certain levels of LD_{50} on liver enzymes and kidney of the albino mice. The obtained results indicated that all tested treatments affected the levels of GOT and GPT as compared with the untreated check.

The highest recorded level of GOT was 492U/L has been obtained by the formulation (Admire®) at 0.01 of LD50. On the other hand, the least recorded level of GOT was 327U/L with (Admire®) at 0.001 of the LD_{50}. GPT level fluctuated with all tested treatments. Imidacloprid at 0.1 and (Admire®) at 0.01 of LD_{50} gave the highest level of GPT (117 and 114U/L, consecutively). Meanwhile, the least level of GPT was recorded for both Admire® and technical imidacloprid at 0.001 of LD_{50} with values of 72 and 76U/L, respectively.

The activity on the kidney activity indicators such as creatinine and uric acid were determined on mice after treatment. The obtained data showed that high levels of creatinine were recorded with imidacloprid at 0.1 of LD_{50} while imidacloprid at 0.001of LD_{50} gave the lowest value. In the case of uric acid, all treatments recorded remarkable increasing values compared with the untreated check. The highest level of uric acid was 11.9 mg/dL at 0.1 of LD_{50}. On the other hand, the lowest value was obtained by Admire® at the concentration 0.1 of the LD_{50}.

Table (2). The effect of imidacloprid (95%) and formulated preparation Admire® (20% SC) on the liver enzymes and kidney activity indicators at certain suggested concentrations

Treatments		Liver enzymes		Kidney indicators	
		G.O.T (U/L)	G.P.T (U/L)	Creatinine (mg/dL)	Uric acid (mg/dL)
Imidacloprid (Techn.95%)	0.1*	468	117	0.56	11.9
	0.01	458	93	0.47	7.3
	0.001	385	76	0.33	6.4
Admire® (20% SC)	0.1	335	85	0.53	4.1
	0.01	492	114	0.37	7.7
	0.001	327	72	0.26	5.0
Untreated check		377	94	0.30	3.9
LSD $_{0.05}$		11.776	6.561	0.075	5.039

*** Concentrations of LD_{50}**

The collected data of the technical active ingredients of chlorantraniliprole and imidacloprid is presented in Figure1. It is obvious that the trend of the effect of chlorantraniliprole on the liver enzymes GOT and GPT going to decrease the levels, while the trend in the case of imidacloprid on the liver enzymes GOT was going to increase of these levels. Furthermore, in the case of GPT a decreasing result was obtained at 0.01 and 0.001 concentrations of the LD_{50}. The effect of chlorantraniliprole on kidney creatinine showed also a decrease of the value, while in the case of imidacloprid showed an increase in creatinine level determined at 0.1, 0.01 and 0.001 concentrations. In the case of the effect of both chlorantraniliprole and imidacloprid on uric acid, it was observed that both active ingredients showed an increase of uric acid as compared with the untreated check samples.

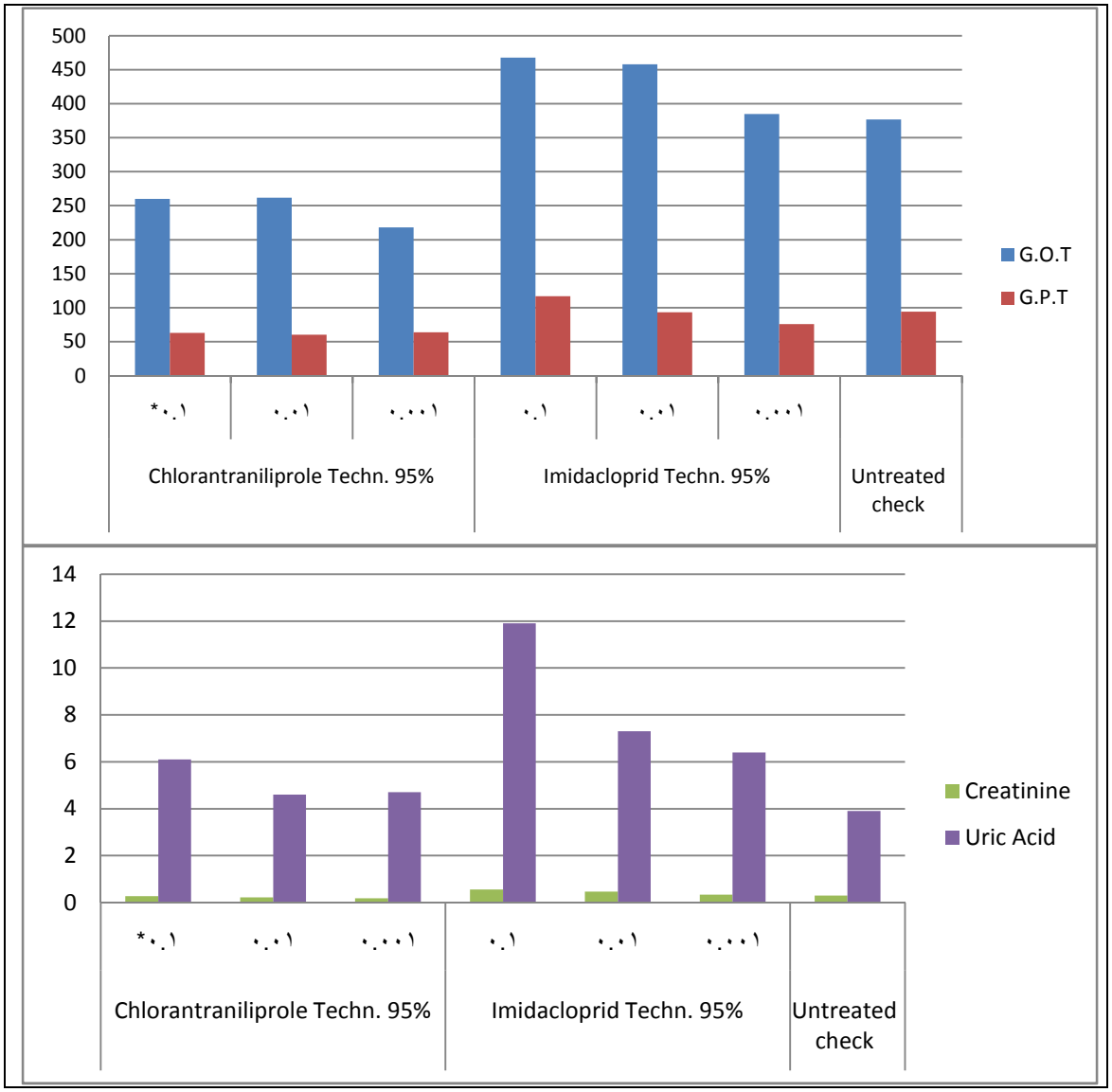
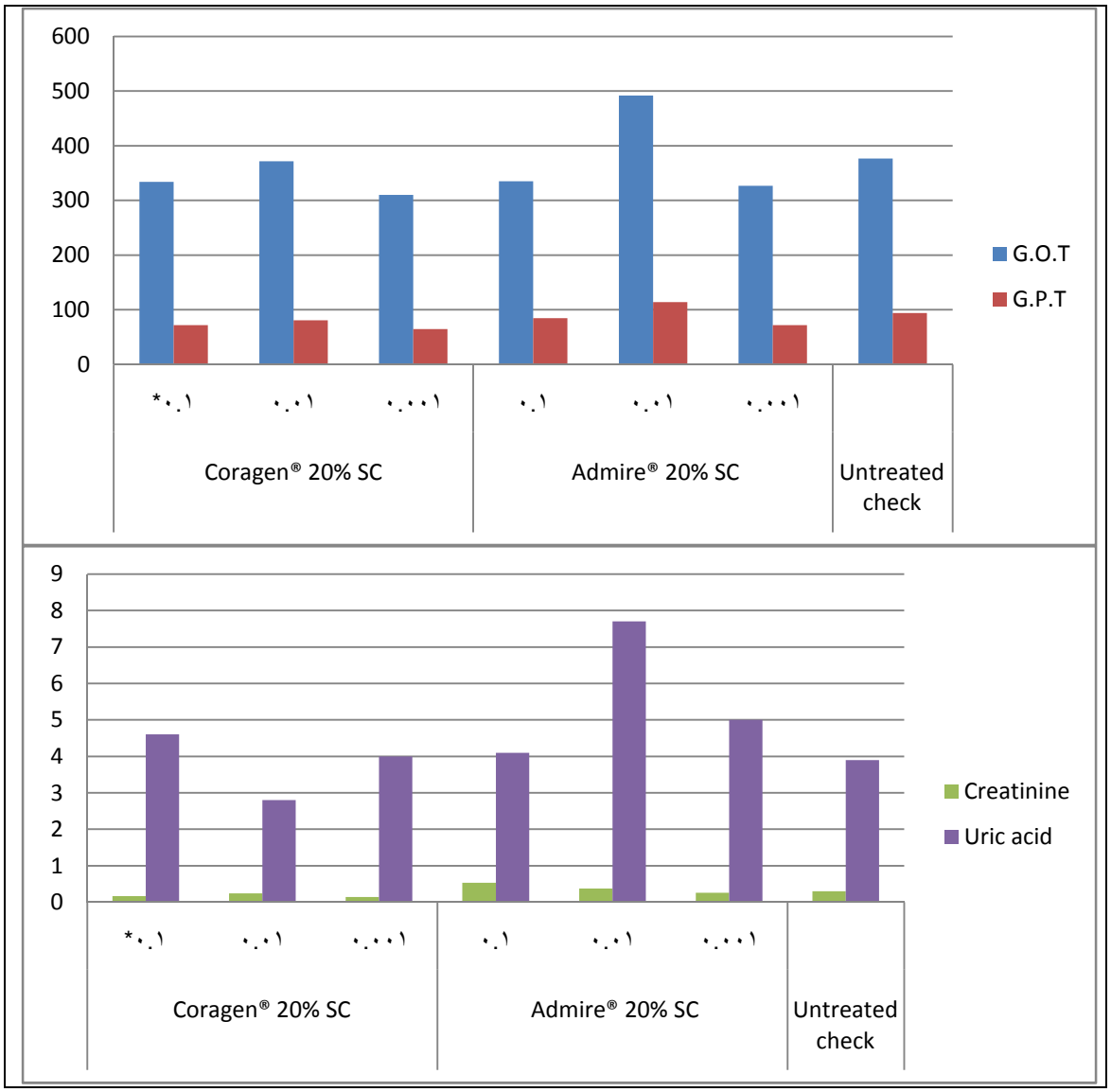


Figure (1). The impact of chlorantraniliprole and imidacloprid technical's on GOT, GPT, Creatinine and Uric acid.

Also, the data obtained regarding the impact of Coragen® and Admire formulated preparation are presented in Figure2. The same trend was observed in the case of Coragen® the formulated preparation of chlorantraniliprole regarding the impact on GOT and GPT (liver enzymes), while it was flacuated in the case of Admire® the formulated preparation of imidacloprid. The same trend was recorded as decreasing values in the case of the impact of Coragen® on the creatinine (kidney) while an increasing value were obtained in the case of Admire®. Flacuated results were recorded regarding Coragen® in the case of uric acid (kidney). While Admire® increased the values of uric acid at the different tested concentrations as compared by the untreated check.

Summarizing the data obtained, it is obvious that both technical chlorantraniliprole and formulated preparation are less risky on the male albino

mice liver enzymes and kidney than imidacloprid and the formulated preparation.



Figuer (2). The impact of Coragen® and Admire® formulated preparation on GOT, GPT, Creatinine and Uric Acid on mice.

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الملخص العربى

تقييم أضرار مركبي كلورانترانيليبول وإيميداكلوبريد علي إنزيمات الكبد وعلى نشاط الكلي في الفأر الأبيض

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المعمل المركزي للمبيدات مركز البحوث الزراعية – الصبحية – الإسكندرية

تم تقدير أضرار كل من مبيدي كلورانترانيليبول وإيميداكلوبريد في صورتيهما النقية والمجهزة علي مستويات الإنزيمات في الكبد والكيريتتين وحمض اليوريك في كُلي ذكر الفأر الأبيض وذلك بإستعمال هذه المركبات بمعدل قيم ٠.1 ، ٠.01 ، ٠.٠٠١ من الجرعة المقدرة اللازمة لقتل ٥٠% من الأفراد المعرضة من ذكور الفأر الأبيض. وقد أظهرت النتائج المتحصل عليها أن المعاملة بمركب كلورانترانيليبول في صورة المادة النقية الفعالة منه (٩٥%) وكذلك التجهيزة المصنعة للتسويق التجاري (كوراجين®٢٠%) سببت أو أدت الى إنخفاض مستوى إنزيمي GOT و GPT الدالة علي نشاط ووظائف الكبد وكذلك نقص مستوى الكيريتتين في الكُلي. وعلي الجانب الأخر فإن كلا من المادة النقية والمجهزة أدت إلي زيادة مستويات حمض اليوريك فيما عدا التركيز ٠.٠١ (٢.٨ مجم/دالتون). كذلك سببت كل من المادة النقية الفعالة (٩٥%) وكذلك التجهيزة المصنعة للتسويق التجاري (أدمير®٢٠%) لمركب إيميداكلوبريد إيضاً إنخفاضاً في مستويات إنزيمات الكبد GOT و GPT فيما عدا التركيزات ٠.١ ، ٠.٠١ وفي نفس الوقت زيادة الكيريتتين و حمض اليوريك في الكُلي فيما عدا التركيز ٠.٠١ من الجرعة المقدرة اللازمة لقتل ٥٠% من الأفراد المعرضة من ذكور الفأر الأبيض. كذلك امكن من النتائج المتحصل عليها التوصل الى أن مركب كلورانترانيليبول يعتبر أقل ضرراً

وخطورة عن مركب إيميداكلوبريد وذلك عند مقارنة تأثيرهما علي مستويات الإنزيمات المقدرة في كبد الفار الأبيض وكذلك مستويات كل من الكيريتنين و حمض اليوريك في الكُلي عند تناول الفئران لجرعة فمية واحدة من المركبات المختبرة بالتركيزات المقترحة.