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ACUTE TOXICITY STUDIES OF TWO POTENTIAL ANTICONVULSANT 3-HYDRAZONO-1*H*-2-INDOLINONE DERIVATIVES

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SUMMARY

In this study, acute toxicity studies of 3-[[3-ethyl/phenyl-4(3*H*)quinazolinone-2-yl-mercaptoacetyl]hydrazono]-1*H*-2-indolinone (**1** and **2**) whose anticonvulsant activity have previously been reported, are evaluated. Also, their LD_{50} values were determined. Test substances were administered to mice orally by gavage method or injected intraperitoneally. At the end of the experimental study, determination of LD_{50} was made by SPSS (Statistical Package for the Social Sciences) statistical method. In order to evaluate the possible toxic effects of substances a histopathological examination was made on the organs such as liver, kidney, lungs, brain, cerebellum and testis.

Consequently, estimated LD_{50} values and pathological findings reveal that safety of **1** and **2** are suitable for consideration as new drugs and hence these two 3-hydrazono-1*H*-2-indolinone derivatives can be employed as candidates for new drug development.

ÖZET

Bu çalışmada, antikonvülsan etkinlikleri gösterilmiş olan 3-[[3-etil/fenil-4(3*H*)kinazolinon-2-il-merkaptoasetil]hidrazono]-1*H*-2-indolinonun (**1** ve **2**) akut toksisite araştırması ve LD_{50} tayinleri yapılmıştır. Test maddeleri farelere gavaj yöntemi ile oral ya da intraperitonal olarak tatbik edilmiştir. Deneysel çalışmanın sonunda SPSS (Statistical Package for the Social Sciences) istatistiksel analiz metodu kullanılarak maddelerin LD_{50} tayinleri yapılmıştır. Ayrıca ilaçların muhtemel toksik etkilerini araştırmak amacıyla karaciğer, akciğer, böbrek, beyin, beyincik ve testislerde histopatolojik inceleme yapılmıştır.

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Sonuç olarak, saptanan LD_{50} değerleri ve yapılan histopatolojik incelemeler **1** ve **2** nin ilaç olabilmek için gerekli ileri araştırmalar konusunda güvenliklerinin yüksek olduğunu göstermiştir. Bu iki 3-hidrazono-1*H*-2-indolinon türevinin yeni ilaç geliştirme çalışmaları kapsamında araştırılması uygun olacaktır.

Key words: LD_{50}, acute toxicity, 3-hydrazono-1*H*-2-indolinone derivatives

INTRODUCTION

New drugs' pharmacodynamic, pharmacokinetic and toxicologic properties need to be evaluated and *in vivo* animal studies should be done before the trials in humans (1). Among them toxicity studies, that belong to preclinical studies, are so important for determining the safety of a new drug (2). Toxicity studies are done for monitoring the effects of chemicals that are taken into the body. The most common acute toxicity test is the determination of lethal dose (LD_{50}) (3). LD_{50} is a statistically derived single dose of a substance that can be expected to cause death in 50 percent of animals when administered by the oral route.

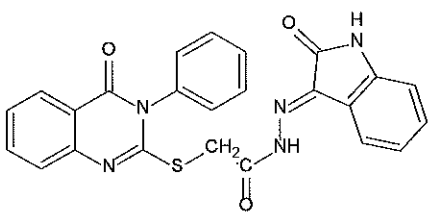
The aim of this work was to evaluate acute toxicity of two 3-hydrazono-1*H*-2-indolinone derivatives (**1** and **2**) whose anticonvulsant activity has been reported previously (4).

RESULTS AND DISCUSSION

Indole derivatives are endogenous compounds that possess a wide range of actions such as CNS-MAO inhibiting, anticonvulsant and anxiogenic activities (5-8). Furthermore, 3-imino/hydrazono-1*H*-2-indolinone derivatives have already been found to be effective on the central nervous system (9-11). The screening results of anticonvulsant activity against pentylenetetrazole induced seizures of some 3-aryloxy/arylthioxyacetylhydrazono-1*H*-2-indolinones have been reported. It has been found that all the compounds tested at 100 mg/kg showed varying degrees of activity (4).

The investigation of the toxicity of an anticonvulsant drug is very important since they are used chronically. Most of the anticonvulsant drugs are known to have serious side effects such as alopecia, bone marrow depression and fatigue (12). The toxicological investigations of new drugs on experimental animals are important prequisities for the clinical trials on humans (13). In the assesment and evaluation of the toxic characteristics of a substance the determination of acute oral toxicity is usually an initial step (14). As it is mentioned before, the most common acute toxicity test is the determination of LD_{50}. It is known that there are many parameters that affect the LD_{50} value such as gender, species, race, diet and method of drug administration.

This study was undertaken to give further contribution to the knowledge on the oral and intraperitoneal acute toxic effects of 3-[[3-ethyl-4(3*H*)quinazolinone-2-yl-mercaptoacetyl]hydrazono]-1*H*-2-indolinone **1** and 3-[[3-phenyl-4(3*H*)quinazolinone-2-yl-mercaptoacetyl]hydrazono]-1*H*-2-indolinone **2** in mice whose anticonvulsant activity was previously reported (4) (Figure 1).



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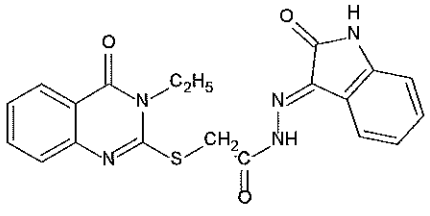

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Figure 1

A single dose of **1** and **2** were administered to 20-25 g male albino mice starved for 12 hours preceding the procedure. Test substances were administered to mice orally by gavage or injected intraperitoneally. Logarithmic increasing doses were chosen. During the studies compounds couldn't be sucessfully administered at some doses because of their wetting problem. All animals were observed individually during experiments. Animals that died during the tests were necropsied and at the conclusion of the the surviving animals were sacrificed and necropsied. Mortality ratios of treated animals, demonstrated that death of mice that received two drugs, started at the dose of 100 mg/kg. Both **1** and **2** were administered at 100 mg/kg, 300 mg/kg, 1 g/kg, 3 g/kg, 10 g/kg doses in order to estimate their LD_{50} values. All pathological changes were recorded. LD_{50} value of ethyl derivative **1** was determined to be as 881 mg/kg whereas the phenyl derivative **2** as 1153 mg/kg. The determination of LD_{50} value was made by using SPSS statistical method (Tables 1 and 2, Figure 2).

Table 1. Number of mortalities (n/6) induced by oral and intraperitoneal (i.p.)injection of **1** and **2**

Dosage	**100 mg/kg**	**300 mg/kg**	**1 g/kg**	**3 g/kg**	**10 g/kg**	**10 g/kg (vehicle)**
Compound1						
oral	0	0	0	*	*	
i.p.	0	0	4	6	6	0
Compound2						
oral	0	0	0	*	*	
i.p.	0	0	4	4	*	0

* The compound could not be administered at this dose.

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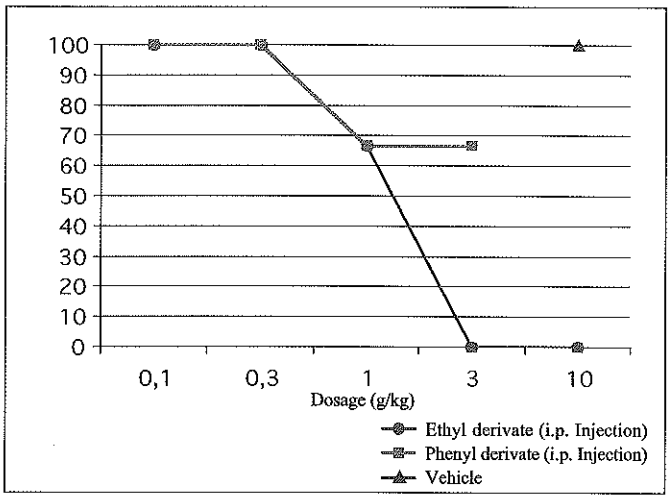
Table 2. Symptoms induced by intraperitoneal injection of **1** and **2**

Compound	Dosage	Symptoms
1	100 mg/kg	No serious symptom
	300 mg/kg	Slow motion, sedation
	1 g/kg	Immobility, lethargy, death
	3 g/kg	Immobility, death
	10 g/kg	-
2	100 mg/kg	No symptoms
	300 mg/kg	Sedation
	1 g/kg	Slow motion, death
	3 g/kg	Immobility, death
	10 g/kg	-

Figure 2. Comparison of survived test animals after intraperitoneal injection



The results demonstrated that similar chemical compounds which have different substituents, have different LD_{50} values. When LD_{50} and ED_{50} values of **1** and **2** are compared, it was found that LD_{50} value is higher than ED_{50} value. It is known that anticonvulsant drugs have so limited therapeutic index, their LD_{50} value is generally so close to their ED_{50} value. So, this study showed that these two molecules can be considered as safe compounds.

On the other hand, the results of the pathological evaluation on the organs demonstrated that compounds have toxic effects on liver and kidney only at the doses higher than their LD_{50} values. No histological alterations were observed on lungs, brain, cerebellum and testis at any dose and no significiant differences of the body and organ weights compared to controls were recorded.

Consequently, estimated LD_{50} values and pathologic findings reveal that safety of these two new molecules are convenient to be considered as new drug candidates. These two new molecules can be employed for new drug development .

EXPERIMENTAL

Chemistry

Synthesis of 3-[[3-ethyl/phenyl-4(3H)quinazolinone-2-yl-mercaptoacetyl] hydrazono]-1H-2-indolinone (1 and *2)*

A mixture of 0.05 mol isatin and 0.005 mol 3-ethyl/phenyl-4(3*H*)-quinazolinone-2-yl-mercaptoacetylhydrazide in 25 ml ethanol containing a few drops of concentrated sulphuric acid was heated under reflux for three hours, cooled and the separated solid was filtered and recrystallized from ethanol to give test compounds **1** and **2**.

Pharmacology

Methyl cellulose solution (3% c) was used as a vehicle. This vehicle was determined to have no toxic effects and did not alter the chemical as well as toxicological properties of the test substance (15).

Test Procedure: Healthy animals were kept at laboratory conditions for at least five days prior to the test. For each dose level six male albino mice (20-25 g) were used. They were starved twelve hours preceeding the procedure. The temparature of the experimental animal room was 22°C (±3°C) and relative humidity was 30-70 %. Lighting was artificial, the sequence was 12 hours light, 12 hours dark. The weight variation in animals did not exceed ±20 percent of the mean weight. Test substances were administered to mice orally by gavage and injected intraperitoneally. Doses were increased in a logarithmic manner. First administration method was chosen as gavage because it was the route that was intended to be used in human. Both of the new substances were administered at 100 mg/kg, 300 mg/kg, 1 g/kg, 3 g/kg, 10 g/kg doses in order to estimate their LD_{50} values. All animals were observed individually during the experiments. At the end of the test animals that died during the tests were necropsied whereas the surviving animals were sacrificied and then necropsied. Samples of organs were fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned. Sections were deparaffinized and stained with haemotoxylin and eosin for histological evaluation. All pathological changes were recorded. The determination of LD_{50} value was made by using SPSS statistical method.

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