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Hepatoprotection by 3-bromo-6-(4-chlorophenyl)-4--methylthio-2*H*-pyran-2-one against experimentally induced liver injury in rats

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Hepatoprotective activity of 3-bromo-6-(4-chlorophenyl)--4-methylthio-2*H*-pyran-2-one, an isostere of dimethyl ricinine, was evaluated in adult male albino rats intoxicated with carbon tetrachloride, paracetamol or thioacetamide. The test compound showed significant hepatoprotection at 6.0 mg kg^{-1} body mass daily dose, given to the rats for seven consecutive days. The carbon tetrachloride, paracetamol and thioacetamide were given, respectively, on days 3, 5, and 7, on day 6 and on day 6 post treatment with the test compound. The protective effect was evident in a battery of serum and liver biochemical parameters related to hepatotoxicity.

Keywords: 3-bromo-6-(4-chlorophenyl)-4-methylthio-2*H*--pyran-2-one, carbon tetrachloride, paracetamol, thioacetamide, hepatoprotection, hepatotoxicity

Leaves of *Ricinus communis* (*Euphorbiaceae*) are used in traditional medicine without any known side effects. In modern medicine, caster oil extracted from seeds of *R. communis* has been highly valued as a non-irritant purgative; a drop is sometimes used to allay eye irritation. Certain studies have shown that compounds isolated from this plant are good insecticidal (1), nematocidal (2–3) and hepatoprotective agents (4). *R. communis* leaves have also been shown to have a protective effect against galactosamine-induced hepatic damage (5). Its hepatoprotective activity has also been judged by its effect on hepatocytes isolated from paracetamol-treated rats and dose dependent choleretic and anticholestatic activities (6). Dimethyl ricinine isolated from *Ricinus communis* leaves though has been identified as a constituent responsible for hepatoprotective activity, showed undesired side effects (7–9). Thus, we undertook to design and prepare analogues of dimethyl ricinine in our laboratory. Among the various synthetic analogues prepared, 3-bromo-6-(4-chlorophenyl)-4-methylthio-2*H*-pyran-2-one, an isostere of dimethyl ricinine, showed significant hepatoprotective activity. The present report describes the hepatoprotective effect of this compound on carbon tetrachloride-, thioacetamide- and paracetamol-induced hepatotoxicity in rats.

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EXPERIMENTAL

Animals

Male Sprague-Dawley rats weighing 160 ± 20 g as obtained from the animal colony of Central Drug Research Institute, Lucknow (India), were kept in polypropylene cages and had free access to pellet diet (Lipton, India) and water. The following norms were followed for the animal room environment: temperature 22 ± 1 °C, humidity 50–60%, light 300 lux at floor level with a regular 12 h cycle, noise level 50 decibel, ventilation 10–15 air changes per hour.

Chemicals

Carbon tetrachloride (CCl_4) was procured from E. Merck (India), while thioacetamide was purchased from the Sigma Chemical Company (USA). Paracetamol was a product of U.P. Drug and Pharmaceuticals Ltd. (India). All other chemicals and reagents used were of highest purity grade.

Synthesis of the test compound

3-Bromo-6-(4-chlorophenyl)-4-methylthio-2*H*-pyran-2-one was prepared in our laboratories according to the method described earlier (10). Briefly, a mixture of 6-(4-chlorophenyl)-4-methylthio-2*H*-pyran-2-one (12.6 g) and *N*-bromosuccinimide (2.0 g) in carbon tetrachloride (100 mL) was refluxed for 20 hours. The solvent was removed and the residue was purified on silica gel column using hexane/chloroform (3:7) as eluent to give the title/test compound (10.1 g, m.p. 207 °C).

Preparation of solutions/suspensions of hepatotoxins/test compounds

Carbon tetrachloride as supplied by E. Merck (India) was injected into rats. However, 2.0 g paracetamol powder was suspended in 8.0 mL of normal saline, 400 mg thioacetamide was suspended in 8.0 mL of normal saline and 3.0 mg of test compound was suspended in 4.0 mL of normal saline before being administeried to rats.

Administration of test substances and induction of hepatic damage

Three separate experiments were performed to evaluate the hepatoprotective effect of the test compound against hepatotoxicity caused by carbon tetrachloride, paracetamol or thioacetamide in albino rats. Each experiment involved eighteen rats, which were further divided into three groups consisting of six rats each – group I, II and III. Animals of group I received normal saline for seven consecutive days whereas the animals of group II received normal saline for seven days and hepatotoxin on the desired day(s) (0.7 mL kg^{-1} body mass of carbon tetrachloride intraperitoneally on days 3, 5 and 7, 2.0 g kg^{-1} body mass of paracetamol orally on day 6 at and 400 mg kg^{-1} body mass of thioacetamide subcutaneously on day 6. The rats of group III received also the test compound at 6.0 mg kg^{-1} body mass orally for seven consecutive days.

In experiments with CCl_4 normal saline was given to rats of group I and group II. The amount equivalent to CCl_4, whereas an additional amount equivalent to the test substance was given orally to animals of group II. In experiments with thioacetamide normal saline was given to rats of group I and II subcutaneously in the amount equivalent to thioacetamide, whereas an additional amount equivalent to the test substance suspension was given orally to animals of group II. In experiments with paracetamol normal saline was given to rats of group I and II only orally: the amount equivalent to paracetamol suspension was given to animals of group I and II, whereas an additional amount equivalent to the test substance suspension was given to animals of group II.

On day 8, blood of each animal (after 16 h starvation) was collected from the retro-orbital plexus and then rats were sacrificed by decapitation and the liver was quickly excised and kept on ice till processed for macromolecular content and enzyme assays. Serum was separated after keeping the blood for two hours at room temperature by centrifugation at $1000 \times g$ for 10 min. Liver tissue was homogenized in 150 mmol L^{-1} KCl (10%, *m/V*) and taken as such for the estimation of macromolecular contents whereas the clear supernatant after centrifugation of the homogenate at $1000 \times g$ was used for enzymatic activity determination.

Macromolecular content and enzyme activity determinations

The macromolecular contents, *i.e.*, DNA (11), RNA (12), total protein (13), cholesterol (14) and glycogen (15), in crude liver homogenates and activities of glutamate oxaloacetate transaminase (GOT) (16), glutamate pyruvate transaminase (GPT) (16), and alkaline phosphatase (ALP) (17), sorbitol dehydrogenase (SBDH) (19) and levels of bilirubin (18) and cholesterol (14) in serum were estimated according to the reference given in brackets. The activities of aniline hydroxylase (20), aminopyrine-*N*-demethylase (21) and glucose-6-phosphate dehydrogenase (22) in liver homogenates were estimated according to the cited references.

RESULTS AND DISCUSSION

Tables I–III depict the serum GOT, GPT, ALP, and SBDH activity profiles, levels of bilirubin, cholesterol and the hepatic levels of DNA, RNA, total protein and hepatic activity profiles of aniline hydroxylase, aminopyrine-*N*-demethylase and glucose-6-phosphate dehydrogenase of the control (group I), hepatotoxicant treated group (group II) and hepatotoxicant plus test substance-treated group (group III).

It is evident from Table I that CCl_4 caused significant ($p < 0.001$) elevation in the serum GOT (104%), GPT (244%), ALP (59%), SBDH (589%) activites and in bilirubin (96%) and cholesterol (60%) levels. Hepatic glucose-6-phosphate dehydrogenase activity and cholesterol levels were increased by 58 and 57%, respectively, whereas aniline hydroxylase and aminopyrine-*N*-demethylase declined to nearly 80 and 87%, respectively. The percent decrease/increase in the above said parameters was significantly reduced in the presence of the test compound 3-bromo-6-(4-chlorophenyl)-4-methylthio-2*H*-pyran-2-one (group III). The increase in serum activities of GOT, GPT, ALP and SBDH were

found to be 53, 155, 32 and 417%, respectively, in the CCl_4 plus test compound-treated group. The increase in bilirubin and cholesterol levels was 55% and 40%, respectively. The decline in the level hepatic DNA, RNA and total protein was found to be 13, 11 and 11%, respectively, whereas the decrease in the level of aniline hydroxylase, aminopyrine--*N*-demethylase and glucose-6-phosphate dehydrogenase was found to be 60 and 76%.

*Table I. Hepatoprotective effect of 3-bromo-6-(4-chlorophenyl)-4-methylthio-2*H*-pyran-2-one against carbon tetrachloride-induced hepatotoxicity*[a,b,c]

Biochemical parameter	Control (group I)	CCl_4 (group II)	Test compound + CCl_4 (group III)	Protection (%)
Serum				
GOT (IU L^{-1})	71.63 ± 5.57	146.28 ± 9.86[d] (104)	109.63 ± 6.60[d,g] (53)	49
GPT (IU L^{-1})	38.75 ± 2.22	133.75 ± 7.71[d] (244)	98.86 ± 10.4[d,g] (155)	37
ALP (IU L^{-1})	59.57 ± 1.78	94.51 ± 3.38[d] (59)	78.88 ± 5.36[d,g] (32)	45
SBDH (IU L^{-1})	0.028 ± 0.005	0.193 ± 0.003[d] (589)	0.145 ± 0.007[d,g] (417)	30
Bilirubin (mg dL^{-1})	0.905 ± 0.045	1.775 ± 0.030[d] (96)	1.40 ± 0.17[d,g] (55)	41
Cholesterol (mg dL^{-1})	102.00 ± 18.69	162.68 ± 14.88[d] (60)	143.56 ± 15.32[e] (40)	**32**
Liver				
Aniline hydroxylase (IU L^{-1})	0.136 ± 0.007	0.027 ± 0.002[d] (–80)	0.054 ± 0.002[d,g] (–60)	24
Aminopyrine-N demethylase (IU L^{-1})	0.157 ± 0.016	0.021 ± 0.002[d] (–87)	0.037 ± 0.007[d,g] (–76)	13
Glucose 6-phosphate dehydrogenase (IU L^{-1})	0.190 ± 0.009	0.301 ± 0.019[d] (58)	0.235 ± 0.021[e,g] (24)	59
DNA (mg g^{-1})	2.13 ± 0.19	1.66 ± 0.09[d] (–22)	1.86 ± 0.18[e,h] (–13)	42
RNA (mg g^{-1})	4.04 ± 0.35	2.92 ± 0.06[d] (–28)	3.59 ± 0.24[f,g] (–11)	60
Total protein (mg g^{-1})	145.44 ± 15.1	108.84 ± 9.47[d] (–25)	128.82 ± 14.1[,h] (–11)	31
Cholesterol (mg g^{-1})	7.16 ± 0.68	11.24 ± 0.99[d] (57)	9.95 ± 1.11[d] (39)	32
Glycogen (mg g^{-1})	41.63 ± 1.11	18.81 ± 1.31[d] (–54)	27.21 ± 1.29[d,g] (–35)	37

[a] Values are mean ± SE (n = 6). [b] Figures in parentheses indicate the % change compared to the control group. [c] Level of significance for groups II and III *vs.* group I: [d] $p < 0.001$, [e] $p < 0.01$, [f] $p < 0.05$; for group III *vs.* group II: [g] $p < 0.001$, [h] $p < 0.05$.

The test compound 3-bromo-6-(4-chlorophenyl)-4-methylthio-2*H*-pyran-2-one, therefore, caused significant protection in the increase in serum GOT (49%), GPT (37%), ALP (45%), SBDH (30%), bilirubin (41%) and cholesterol (32%) induced by CCl_4. The respective protection in all the other liver parameters studied, *i.e.*, aniline hydroxylase (24%), glucose-6-phosphate dehydrogenase (59%), DNA (42%), RNA (60%), total protein (31%) and glycogen (37%) were noted.

*Table II. Hepatoprotective effect of 3-bromo-6-(4-chlorophenyl)-4-methylthio-*2H*-pyran-2-one against paracetamol-induced hepatotoxicity*[a,b,c]

Biochemical parameters	Control (group I)	Paracetamol (group II)	Test compound + paracetamol (group III)	Protection (%)
Serum				
GOT (IU L^{-1})	70.68 ± 5.72	136.61 ± 6.19[d] (93)	100.27 ± 14.37[d,g] (42)	55
GPT (IU L^{-1})	40.53 ± 5.12	107.23 ± 6.66[d] (164)	59.24 ± 6.64[d,g] (46)	72
ALP (IU L^{-1})	58.60 ± 5.90	140.60 ± 3.12[d] (140)	48.20 ± 7.10[f,g] (–17)	100
SBDH (IU L^{-1})	0.0264 ± 0.007	0.0476 ± 0.008[d] (80)	0.0403 ± 0.006[e] (52)	35
Bilirubin (mg dL^{-1})	0.915 ± 0.152	1.504 ± 0.0042[d] (64)	0.896 ± 0.064[g] (–2)	100
Cholesterol (mg dL^{-1})	123.00 ± 4.65	171.98 ± 3.86[d] (40)	134.63 ± 5.67[d,g] (9)	76
Liver				
Aniline hydroxylase (IU L^{-1})	0.136 ± 0.007	0.027 ± 0.002[d] (–80)	0.054 ± 0.002[d] (–60)	25
Aminopyrine-*N*-demethylase (IU L^{-1})	0.1845 ± 0.020	0.0527 ± 0.007[d] (–71)	0.0595 ± 0.006[d] (–67)	6
DNA (mg g^{-1})	1.97 ± 0.07	1.61 ± 0.05[d] (–18)	1.63 ± 0.091[d] (–17)	6
RNA (mg g^{-1})	3.82 ± 0.34	3.24 ± 0.16[e] (–15)	3.61 ± 0.033[g] (–5)	64
Total protein (mg g^{-1})	122.50 ± 4.91	107.98 ± 3.62[d] (–11)	115.76 ± 1.05[e,g] (–5)	54
Cholesterol (mg g^{-1})	7.23 ± 0.69	10.96 ± 1.73[d] (52)	9.25 ± 0.38[d,h] (28)	46
Glycogen (mg g^{-1})	45.30 ± 5.38	22.16 ± 2.32[d] (–51)	34.75 ± 4.01[e,g] (–23)	54

[a] Values are mean ± SE ($n = 6$). [b] Figures in parentheses indicate the % change compared to the control group. [c] Level of significance for groups II and III *vs.* group I: [d] $p < 0.001$, [e] $p < 0.01$, [f] $p < 0.05$; for group III *vs.* group II: [g] $p < 0.001$, [h] $p < 0.05$.

Table II/IIa shows the serum GOT, GPT, ALP and SBDH activity profiles; levels of bilirubin, cholesterol and the hepatic levels of DNA, RNA, total protein and hepatic activity profiles of aniline hydroxylase and aminopyrine-*N*-demethylase of the control (Group I), paracetamol alone (Group II) and paracetamol plus test substance treated groups (Group III). It is evident from Table II that paracetamol alone caused significant ($p < 0.001$) elevation in the serum GOT (93%), GPT(164%), ALP (140%) and SBDH (80%) activities and bilirubin (64%) and cholesterol (40%) levels. The aniline hydroxylase and aminopyrine-*N*-demethylase declined to nearly 80 and 71% respectively. Hepatic levels of DNA, RNA, total protein and glycogen were decreased by 18, 15, 11 and 51%, respectively in the paracetamol-treated group (group II). The percent change in the above parameters was significantly reduced in the paracetamol plus test compound-treated group (group III). The increase in the serum activities of GOT, GPT and SBDH were found to be 42 and 52%, whereas ALP activity decreased even bellow the control value. The bilirubin and cholesterol levels in group III were nearly equal to profiles obtained in sham treated control (group I). The decrease in the levels of hepatic DNA, RNA and total protein were only 17, 5 and 5%, respectively, compared to the sham treated control, whereas the decrease in the levels of aniline hydroxylase, aminopyrine-*N*-demethylase and glycogen were found to be 60, 67 and 23% respectively, in the test compund-treated group. Hepatic cholesterol concentration increased 28% only. The test compound 3-bromo-6-(4-chlorophenyl)-4-methylthio-2*H*-pyran-2-one, therefore showed significant protection against the rise in serum GOT (55%), GPT 872%), ALP (100%) activities and in the levels of bilirubin (100%) and cholesterol (76%) caused by paracetamol treatment. Hepatic glycogen and cholesterol levels were protected to around 54% and 46%, respectively. Hepatic RNA and the total protein content were protected by 64 and 54%, respectively.

Table III displays the serum GOT, GPT, ALP and SBDH activity profiles, levels of bilirubin, cholesterol and the hepatic levels of DNA, RNA, total protein and hepatic activity profiles of aniline hydrxylase, aminopyrine-*N*-demethylase and glucose-6-phosphate dehydrogenase of control (Group I), thioacetamide plus test substance treated group (Group III). It is evident from Table III that thioacetamide alone caused significant ($p < 0.001$) elevation in the serum GOT (62%), GPT (114%), ALP (81%), SBDH (248%) activities, and bilirubin (227%) and cholesterol (22%) levels. Hepatic glucose-6-phosphate dehydrogenase activity and cholesterol levels were increased by 107 and 58%, respectively, whereas aniline hydroxlase and aminopyrine-*N*-demethylase declined to nearly 72 and 67% respectively. Hepatic levels of DNA, RNA, total protein and glycogen were also decreased by 29, 22, 8 and 77%, respectively. The percent change in the above parameters in thioacetamide plus test compound-treated group (group III) were significantly lowered. The increase in serum activities of GOT, GPT, ALP and SBDH were reduced to 40, 35, 69 and 145%, respectively. The increase in bilirubin and cholesterol levels was 164 and 10, respectively. The hepatic levels of DNA, total protein and cholesterol declined to 18, 5 and 9%, respectively, whereas the decrease in the levels of aniline hydroxylase, aminopyrine-*N*-demethylase and glucose-6-phosphate dehydrogenase were found to be 34%, 40% and 83%. Significant protection by the test compound 3-bromo--6-(4- chlorophenyl)-4-methylthio-2*H*-pyran-2-one was noted in the serum GOT (36%), GPT (69%), ALP (15%), SBDH (41%), bilirubin (28%) and cholesterol (56%) levels caused by thioacetamide. All the other liver parameters studied were protected by the test sub-

stance from 23 to 100%, namely aniline hydroxylase (52%), aminopyrine-*N*-demethylase (40%), glucose-6-phosphate dehydrogenase (23%), DNA (36%), RNA (93%), total protein (100%), cholesterol (85%) and glycogen (75%).

*Table III. Hepatoprotective effect of 3-bromo-6-(4-chlorophenyl)-4-methylthio-*2H*-pyran-2-one against thioacetamide-induced hepatotoxicity*[a,b,c]

Biochemical parameters	Control (group I)	Thioacetamide (group II)	Test compound + Thioacetamide (group III)	Protection (%)
Serum				
GOT (IU L^{-1})	62.82 ± 1.90	101.86 ± 1.90[d] (62)	87.89 ± 1.86[d,g] (40)	36
GPT (IU L^{-1})	37.25 ± 2.99	79.74 ± 6.56[d] (114)	50.45 ± 5.00[d,g] (35)	69
ALP (IU L^{-1})	61.19 ± 6.50	110.58 ± 10.14[d] (81)	103.40 ± 3.92[d] (69)	15
SBDH (IU L^{-1})	0.0231 ± 0.002	0.0804 ± 0.006[d] (248)	0.0567 ± 0.001[d,g] (145)	41
Bilirubin (mg dL^{-1})	0.58 ± 0.04	1.90 ± 0.15[d] (227)	1.53 ± 0.15[d,h] (164)	28
Cholesterol (mg dL^{-1})	112.63 ± 7.12	137.76 ± 0.37[d] (22)	123.56 ± 7.21[g] (10)	56
Liver				
Aniline hydroxylase (IU L^{-1})	0.1818 ± 0.015	0.0507 ± 0.004[d] (–72)	0.1186 ± 0.015[d,g] (–34)	52
Aminopyrine-N-demethylase (IU L^{-1})	0.117 ± 0.011	0.0386 ± 0.0052[d] (–67)	0.070 ± 0.0013[d,g] (–40)	40
Glucose-6-phosphate dehydrogenase (IU L^{-1})	0.213 ± 0.028	0.441 ± 0.008[d] (107)	0.389 ± 0.017[d,g] (83)	23
DNA (mg g^{-1})	2.14 ± 0.19	1.53 ± 0.15[d] (–29)	1.75 ± 0.16[f,h] (–18)	36
RNA (mg g^{-1})	4.45 ± 0.25	3.45 ± 0.068[d] (–22)	4.38 ± 0.05 (–2)	93
Total protein (mg g^{-1})	148.55 ± 6.25	136.69 ± 2.00[f] (–8)	155.92 ± 4.04[g] (5)	100
Cholesterol (mg g^{-1})	7.99 ± 0.18	12.61 ± 0.27[d] (58)	8.69 ± 0.23[d,g] (9)	85
Glycogen (mg g^{-1})	40.57 ± 4.68	9.42 ± 0.65[d] (–77)	32.78 ± 2.65[e,g] (–19)	75

[a] Values are mean ± SE ($n = 6$). [b] Figures in parentheses indicate the % change compared to the control group. [c] Level of significance for groups II and III *vs.* group I: [d] $p < 0.001$, [e] $p < 0.01$, [f] $p < 0.05$; for group III *vs.* group II: [g] $p < 0.001$, [h] $p < 0.05$.

Carbon tetrachloride, paracetamol and thioacetamide have been extensively used in experimental studies to assess prospective hepatoprotective substances. In our study, these toxic substances caused extensive damage to the liver, reflected in the elevation of serum GOT, GPT, ALP, SBDH, followed by a concomitant rise in bilirubin and impairment of the activities of hepatic drug metabolising enzymes, *viz*. aniline hydroxylase and aminopyrine-N-demethylase. Most of the hepatotoxic effects caused by carbon tetrachloride, paracetamol and thioacetamide in serum and liver have already been reported by us (23–25). In normal rats, the test compound had no effect per se on liver and serum parameters (data not shown). However, pre-treatment of rats with the test compound 3-bromo-6-(4-chlorophenyl)-4-methylthio-2*H*-pyran-2-one provides significant ($p < 0.001$) protection against the carbon tetrachloride, paracetamol and thioacetamide induced elevation in the serum GOT, GPT, ALP, SBDH activities and bilirubin levels. Besides serum parameters, the test compound also provided significant ($p < 0.05$) protection to the altered activities of drug metabolising enzymes as well as glucose-6-phosphate dehydrogenase. Decreased activities of the drug metabolising enzymes will limit the ability of the animals to metabolise a variety of therapeutic drugs or environmental agents to which they may be exposed. The decreased glycogen content in liver tissue following intoxication is also due to impaired energy metabolism, which leads to a breakdown of glycogen, an impaired source of the production of ATP to meet the energy requirement (26). In all the three models, the test compound caused a significant ($p < 0.001$) reversal of the hepatic glycogen content. Protection of the cholesterol level in liver and serum shows hypolipidemic activity of compound 3-bromo-6-(4-chlorophenyl)-4--methylthio-2*H*-pyran-2-one. Decreased protein synthesis following intoxication by any of the above said hepatotoxins was evident in the decrease in hepatic DNA, RNA and total protein levels and was found restored to their normal levels in the test substance-treated groups. This shows that the test compound may have some role in protein biosynthesis. It is evident from the results that the levels of DNA, RNA and protein were higher in the test substance-treated group compared to the toxin alone-treated group.

CONCLUSIONS

The findings of this study suggest that the compound 3-bromo-6-(4-chlorophenyl)--4-methylthio-2*H*-pyran-2-one has hepatoprotective activity because it offers significant protection against hepatotoxicity caused by either carbon tetrachloride, paracetamol or thioacetamide in rats, as reflected in various serum and liver biochemical parameters. Though the exact mechanism of hepatoprotective action of this compound is not known at the moment, its hypolipidemic nature and membrane stabilizing effect may be either partially or fully responsible for it.

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S A Ž E T A K

Hepatoprotektivno djelovanje 3-brom-6-(4-klorfenil)-4-metiltio-2*H*-piran-2-ona kod eksperimentalno induciranih oštećenja jetre u štakora

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Ispitivano je hepatoprotektivno djelovanje 3-brom-6-(4-klorfenil)-4-metiltio-2*H*-piran-2-ona, izostera dimetil ricinina, na odraslim albino štakorima muškog spola prije izlaganja toksičnom djelovanju tetraklormetana, paracetamola, odnosno tioacetamida. 3-Brom-6-(4-klorofenil)-4-metiltio-2*H*-piran-2-on pokazao je značajno hepatoprotektivno djelovanje u dnevnim dozama 6,0 mg kg^{-1}. Tetraklormetan, paracetamol i tioacetamid su dani treći, peti i sedmi dan ili šesti dan nakon davanja testiranog spoja. Zaštitni učinak uočen je praćenjem biokemijskih parametara u serumu i jetri karakterističnih za hepatotoksičnost.

Ključne riječi: 3-brom-6-(4-klorfenil)-4-metiltio-2*H*-piran-2-on, tetraklormetan, paracetamol, tioacetamid, hepatoprotektivno djelovanje, hepatotoksičnost

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