http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 6 (5): 448-451, 2003
ISSN 1028-880

Hepatoprotective Effect of Thymol on Chemical-induced Hepatotoxicity in Rodents

K.H. Janbaz, S.A. Saeed and [1]A.H. Gilani
Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan
[1]Department of Biological and Biomedical Sciences,
The Aga Khan University, Stadium Road, Karachi-47800, Pakistan

Abstract: The hepatoprotective activity of thymol, a terpenoid from essential oils of plant origin was investigated against paracetamol and CCl_4-induced hepatic damage. The results showed that paracetamol produced 100% mortality at the dose of 1 g kg^{-1} in mice while pre-treatment of animals with thymol (150 mg kg^{-1}) reduced the death rate to 30%. Oral administration of paracetamol (640 mg kg^{-1}) produced liver damage in rats as manifested by the rise in serum enzyme levels of alkaline phosphatase (ALP) and transaminases (AST and ALT). Pre-treatment of rats with thymol (150 mg kg^{-1}) prevented the paracetamol-induced rise in serum enzymes. The hepatotoxic dose of CCl_4 (1.5 ml kg^{-1}; orally) also raised the serum ALP, AST and ALT levels. The same dose of thymol (150 mg kg^{-1}) was able to prevent the CCl_4-induced rise in serum enzymes. The results indicated that thymol also prevented the CCl_4-induced prolongation in pentobarbital sleeping time confirming hepatoprotectivity. It was concluded that thymol possesses anti-hepatotoxic activity.

Key words: Thymol, essential oils, anti-hepatotoxic, paracetamol, CCl_4

Introduction

Thymol is one of the terpenoids present as essential oil in plants (Ali-Shtayeh *et al.*, 1997). The compound exhibited multiple biological activities including anti-bacterial (Didry *et al.*, 1994), anti-fungal (Mahmoud, 1994), anti-inflammatory (Azuma *et al.*, 1986), hypo-cholesteraemic (Case *et al.*, 1995), cytotoxic (He *et al.*, 1997), immunomodulating (Suzuki and Furuta, 1988) and also molluscidal (Singh *et al.*, 1999) activities.

The toxin-induced hepatic damages are known to be mediated through involvement of oxidized reactive intermediates (Aldridge, 1981) and compounds exhibiting anti-oxidant (Attri *et al.*, 2000), free radical scavenging (Sadanobu *et al.*, 1999) and anti-lipid peroxidant (Lim *et al.*, 2000) properties are reported to exhibit anti-hepatotoxic activities. Thymol possesses anti-oxidant (Aeschbach *et al.*, 1994), free radical scavenging (Fujisawa and Kadoma, 1992) and anti-lipid per-oxidation (Beach and Giroux, 1992) properties and is likely to curtail the sequence of events leading towards hepatocellular damage. The present study was under taken in an attempt to assess the possible anti-hepatotoxic potential of thymol against paracetamol and CCl_4-induced hepatotoxicity.

Materials and Methods

This study was conducted at Department of Biological and Biomedical Sciences, The Aga Khan University, Karachi during July-August, 2000.

Animals: Swiss male mice (20-25 g) and male albino wistar rats (200-250 g) were obtained from the Animal House of The Aga Khan University. The animals were housed in plastic cages (47x34x8 cm^3), mice (10 $cage^{-1}$) and rats (5 $cage^{-1}$), lined with sawdust renewed every 48 h, in air-conditioned quarters and had free access to tap water and food.

Pharmacological materials: Paracetamol, CCl_4, thymol, ketamine hydrochloride and methyl cellulose were obtained from Sigma Chemicals Company, St Louis, MO USA and olive oil (P. Sasso e Figili, Oneglia, Italy) was purchased from local market. Paracetamol and CCl_4 were suspended in 1% methyl cellulose (50 mg ml^{-1}) and olive oil (20% v/v), respectively.

Lethality study in mice: Preliminary experiments were performed on mice to estimate the protective effect of thymol against a lethal dose of paracetamol (1g kg^{-1}). Animals were divided into two groups of 10 animals each. One group was treated orally with thymol (150 mg kg^{-1}) followed after 1 h by oral administration of paracetamol. The 2nd group served as control and received the same treatment except that normal saline (0.9% NaCl) was administered instead of thymol. The mortality was observed for 24 h post-administration of paracetamol.

Hepatoprotective study: Hepatic injury in rats was induced separately by paracetamol (640 mg kg^{-1}) as well as CCl_4 (1.5 ml kg^{-1}), administered orally, whereas control animals

received an equal volume of respective vehicle (1% methyl cellulose or olive oil) as described previously (Janbaz and Gilani, 1995).

Rats were divided into 3 groups of 10 animals each. Group 1 served as vehicle control and received normal saline (10 ml kg^{-1}) and vehicle (1% methyl cellulose; 13 ml kg^{-1}, orally). Group 2 was given 4 doses of normal saline at 12 h intervals and paracetamol was administered orally 1 h post-treatment of the last dose. Group 3 was treated similarly to group 2, except that thymol (150 mg kg^{-1}, suspended in 10 ml saline) was administered instead of saline.

In a parallel study on 3 similar groups of rats (n = 10), normal saline (10 ml kg^{-1}) and vehicle (olive oil; 7.5 ml kg^{-1}) were administered orally to vehicle control group, whereas the remaining 2 groups were treated similarly to the study mentioned above except that paracetamol was replaced by Ccl_4.

Animals were anaesthetized with ketamine (100 mg kg^{-1}, i.m.) 24 h after the last treatment and blood (3 ml) was collected by cardiac puncture using sterile disposable syringes. Serum was separated by centrifugation (3000 rpm for 15 min) and serum alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) were estimated on the same day spectrophotometrically using Merck diagnostic kits.

Modification of CCl_4-induced prolongation in pentobarbital sleeping time: The effect of thymol on CCl_4-induced prolongation in pentobarbital sleeping time was studied in mice (Montilla *et al.*, 1990; Gilani *et al.*, 1998) (Table 2).

The animals were divided into three groups of 10 animals each. Group 1 animals received 4 doses of normal saline (10 ml kg^{-1}) orally at 12 h interval and vehicle (olive oil) was administered as bolus dose (7.5 ml kg^{-1}; orally) 1 h after the last dose of saline followed after 24 h by pentobarbital (75 mg kg^{-1}, i.p), while animals of group 2 were given the same treatment except vehicle was replaced by CCl_4 (1.5 ml kg^{-1}). Animals in group 3 were treated similar to group 2 except that thymol (150 mg kg^{-1}) was substituted for normal saline.

Statistical analysis: The statistical comparisons were made by means of the student's *t*-test and $P < 0.05$ was regarded as significant.

Results

Effect of thymol on paracetamol-induced lethality: Paracetamol at the dose of 1 g kg^{-1} killed all mice. In a group of animals pre-treated with thymol (150 mg kg^{-1}), the same dose of paracetamol killed only two out of ten resulting in 80% protection against the lethal effect of paracetamol (Table 1).

Effect of thymol on CCl_4-induced prolongation in pentobarbital sleep: Pentobarbital at a dose of 75 mg kg^{-1}, i.p., caused sleep in mice of control group for a period of 124 ± 11 min (mean " SEM; n = 10). Whereas treatment of

Table 1: Effect of thymol on paracetamol-induced lethality in mice

Groups	Treatment	Mortality (%)
1	Thymol + Paracetamol (150 mg kg^{-1} + 1g kg^{-1})	20
2	Saline + Paracetamol (10 ml kg^{-1} + 1g kg^{-1})	100

Table 2: Effect of thymol on CCl_4-induced prolongation in pentobarbital sleeping time in mice

Groups	Treatments	Sleeping time (min)
1	Saline + Vehicle + Pentobarbital (10 ml kg^{-1}+7.5 ml kg^{-1} + 75 mg kg^{-1})	124±11
2	Saline + CCl_4 + Pentobarbital (10 ml kg^{-1} + 1.5 ml kg^{-1} + 75 mg kg^{-1})	219±27*
3	Thymol + CCl_4 + Pentobarbital (150 mg kg^{-1} + 1.5 ml kg^{-1} + 75 mg kg^{-1})	132±15**

Values represent the mean ±SEM of 10 determinations. Saline/Thymol/CCl_4 was given orally, while pentobarbital was given intraperitoneally. *P<0.01; Compared to group 1 (control); **P<0.05; Compared to group 2.

Table 3: Effect of thymol on paracetamol-induced rise in serum enzyme levels in rats

Groups	Treatments	ALP	AST	ALT
1	Saline + Vehicle (10 ml kg^{-1} + 13 ml kg^{-1})	207±15	97.0±13	48.0± 09
2.	Saline + Paracetamol (10 ml kg^{-1} + 640 mg kg^{-1})	309±28*	943.0±181*	438.0± 119*
3.	Thymol + Paracetamol (150 mg kg^{-1} + 640 mg kg^{-1})	208±19**	133.0± 37**	69.0± 21**

Values shown are mean ± SEM of 10 determinations expressed as IU. Group 3 animals received four doses of thymol (150 mg kg^{-1}) at 12 h interval before paracetamol (640 mg kg^{-1}) administration. *P < 0.01; Compared to group 1. **P < 0.01; Compared to group 2.
ALP, alkaline phosphatase; AST, aspartate transaminase; ALT, alanine transaminase

Table 4: Effect of thymol on CCl_4-induced rise in serum enzyme levels in rats

Groups	Treatments	ALP	AST	ALT
1	Saline + Vehicle (10 ml kg^{-1} + 7.5 ml kg^{-1})	205.0±17	92.0± 11	51.0±12
2	Saline + CCl_4 (10 ml kg^{-1} + 1.5 ml kg^{-1})	318.0±32*	781.0± 169*	449.0±114*
3	Thymol + CCl_4 (150 mg kg^{-1} + 1.5 ml kg^{-1})	211.0±21**	119.0± 29***	75.0±19***

Values shown are mean " SEM of 10 determinations expressed as IU. Group 3 animals received four doses of thymol (150 mg kg^{-1}) at 12 h interval before CCl_4 (1.5 ml kg^{-1}) administration. * P < 0.01; Compared to group 1 (control). **P < 0.05; ***P < 0.01; Compared to group 2.
ALP, alkaline phosphatase; AST, aspartate transaminase; ALT, alanine transaminase

animals with CCl_4, prolonged the pentobarbital sleeping time to 219 ± 27 min, the value that is significantly higher ($P < 0.01$) than that of control (Table 2). However, prior treatment of animals with thymol (150 mg kg^{-1}) returned this CCl_4-induced prolongation of pentobarbital sleeping time to 132 ± 15 min, which is significantly lower than group 2 animals ($P < 0.05$) and close to the control sleeping time ($P > 0.05$).

Effect of thymol on paracetamol-induced hepatotoxicity: Control (saline + vehicle) serum values of ALP, AST and ALT in rats were found to be 207 ± 15, 97 ± 13 and 48 ± 09 IU, respectively (Table 3), while toxic dose of paracetamol (640 mg kg^{-1}) raised significantly ($P < 0.01$) the respective serum enzyme values to 309±28, 943±181 and 438±119. Group 3 animals were pre-treated with thymol (150 mg kg^{-1}) to determine its effect on paracetamol-induced rise in serum enzymes. The serum values of enzymes in pre-treated group were found to be 208 ± 19 (ALP), 133 ± 37 (AST) and 69 ± 21 (ALT), which were significantly lower ($P < 0.01$) than the values of toxic control and similar to the control values ($P > 0.05$).

Effect of thymol on CCl_4-induced hepatotoxicity: The estimated values of serum alkaline phosphatase (ALP) and transaminases (AST and ALT) in control (saline + vehicle) group of rats were found to be 205±17, 92±11 and 51±12 IU, respectively (Table 4), which were raised significantly ($P < 0.01$) to the respective values of 318±32, 781 ± 169 and 449 ± 114 after administration of a toxic dose of CCl_4 (1.5 ml kg^{-1}). However, pretreatment of animals with thymol (150 mg kg^{-1}) returned the serum ALP, AST and ALT values to 211±21, 119±29 and 75±19 IU, respectively, which are significantly lower ($P < 0.0$ 5; 0.01; 0.01) than values of toxic control and were close to normal values ($P > 0.05$).

Discussion

Paracetamol and CCl_4-induced hepatic injuries are commonly used models for hepatoprotective drug screening (Plaa and Hewitt, 1982) and the extent of hepatic damage is assessed by the level of increased cytoplasmic enzymes (ALP, AST and ALT) in circulation (Sallie *et al.*, 1991). Thymol when administered prophylactically exhibited protection against paracetamol-induced lethality in mice suggesting hepatoprotective actions.

The treatment of mice with CCl_4 caused a damage to microsomal drug metabolizing enzymes in hepatocytes leading to a substantial decrease in hepatic drug metabolizing capacity, being reflected in prolongation of pentobarbital-induced sleeping time (Javatilaka *et al.*, 1990). Whereas, pretreatment of animals with thymol prevented the CCl_4-induced prolongation in pentobarbital-sleeping time, suggesting a protective effect of thymol against CCl_4-induced damage to hepatocytes.

Paracetamol is converted to a toxic reactive intermediate called N-acetyl-p-bezoquinone imine (NAPQI) following metabolism by a number of isozymes of cytochrome P-450 (CYPs), i.e., CYP 2E1 (Tanaka *et al.*, 2000), CYP 1A2 (Venkatakrishnan *et al.*, 1998), CYP 2A6 (Chen *et al.*, 1998), CYP 3A4 and CYP2D6 (Dong *et al.*, 2000), whereas CCl_4 is activated to halogenated free radicals (HFR) by CYP 2E1 (Jeong and Park, 1998). The massive production of reactive species may lead to depletion of protective physiological moieties (glutathione and "-tocopherol, etc.), ensuing widespread propagation of the alkylation as well as peroxidation, causing damage to the macromolecules in vital biomembranes (Aldridge, 1981). The reactive species mediated hepatotoxicity can be effectively managed upon administration of such agents possessing anti-oxidants (Attri *et al.*, 2000), free radical scavengers (Sadanobu *et al.*, 1999) and anti-lipid peroxidation (Lim *et al.*, 2000) activities. Thymol treatment was able to ameliorate the paracetamol and CCl_4-induced hepato-cellular damage as evidenced by prevention of any increase in serum enzymes (ALP, AST and ALT) levels subsequent to toxin exposure and the reported anti-oxidant (Aeschbach *et al.*, 1994), free radical scavenging (Fujisawa and Kadoma, 1992) and anti-lipid peroxidation (Beach and Giroux, 1992) properties might be the contributed factor towards the observed hepatoprotection.

Inflammation plays a central role during drug-induced acute hepatitis and products of arachidonic acid metabolism have been extensively involved in inflammatory processes (Perez-Alvarez *et al.*, 1993). Similarly, the reported anti-inflammatory (Azuma *et al.*, 1986) and cyclooxygenase inhibitory (Anamura *et al.*, 1988) activities of thymol may also be partly involved in the protective effect against paracetamol and CCl_4-induced hepatotoxicity observed in this study.

In conclusion thymol exhibited protection against paracetamol and CCl_4-induced liver injuries as manifested by the reduction in toxins-mediated rise in serum enzymes in rats, protection against lethal dose of paracetamol in mice and prevention of CCl_4-induce increase in pentobrbital sleeping time possibly through multiple pathways.

References

Aeschbach, R., J. Loliger, B.C. Scott, A. Murica, J. Butler, B. Halliwell and O.I. Aruoma, 1994. Anti-oxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. Food. Chem. Toxicol., 32: 31-36.

Aldridge, W.N., 1981. Mechanism of toxicity: New concepts are required in toxicology. Trends Pharmacol. Sci., 2: 228-231.

Ali-Shtayeh, M.S., M.A. Al-Nuri, R.M. Yaghmour and Y.R. Faidi, 1997. Antimicrobial activity of *Micromeria nervosa* from the Palestinian area. J. Ethnopharmacol., 58: 143-147.

Anamura, S., T. Dohi, M. Shirakawa, H. Okamoto and A. Tsujimoto, 1988. Effect of phenolic dental medicaments on prostaglandin synthesis by microsomes of bovine tooth pulp and rabbit kidney medulla. Arch. Oral Biol., 33: 555-560.

Attri, S., S.V. Rana, K. Vaiphei, C.P. Sodhi, R. Katyal, R.C. Goel, C.K. Nain and K. Singh, 2000. Isoniazid- and rifampicin-induced oxidative hepatic injury--protection by N-acetylcysteine. Hum. Exp. Toxicol., 19: 517-522.

Azuma, Y., N. Ozasa, Y. Ueda and N. Takagi, 1986. Pharmacological studies on the anti-inflammatory action of phenolic compounds. J. Dental. Res., 65: 53-56.

Beach, D.C. and E. Giroux, 1992. Inhibition of lipid peroxidation promoted by iron (III) and ascorbate. Arch. Biochem. Biophys., 297: 258-264.

Case, G.L., L. He, H. Mo and C.E. Elson, 1995. Induction of geranyl pyrophosphate pyrophosphatase activity by cholesterol-supressive isoprenoids. Lipids, 30: 357-359.

Chen, W., L.L. Koenig, S.J. Thompson, R.M. Peter, A.E. Rettie, W.F. Trager and S.D. Nelson, 1998. Oxidation of acetaminophen to its toxic quinone imine and nontoxic catechol metabolites by baculovirus-expressed and purified human cytochrome p450 2E1 and 2A6. Chem. Res. Toxicol., 11: 295-301.

Didry, N., L. Dubrevil and M. Pinkas, 1994. Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. Pharm. Acta. Helv., 69: 25-28.

Dong, H., R.L. Haining, K.E. Thummel, A.E. Rettie and S.D. Nelson, 2000. Involvement of human cytochrome p-450 2D6 in the bioactivation of acetaminophen. Drug Metab. Dispos., 28: 1397-1400.

Fujisawa, S. and Y. Kadoma, 1992. Effect of phenolic compounds on the polymerization of methyl methacrylate. Dent. Mater., 8: 324-326.

Gilani, A.H., K.H. Janbaz, B.H. Shah, 1998. Esculetin prevents liver damage induced by paracetamol and CCl_4. Pharmacol. Res., 37: 31-35.

He, L., H. Mo, S. Hadisusilo, A.A. Qureshi and C.E. Elson, 1997. Isoprenoids suppress the growth of murine B16 melanomas in *vitro* and *in vivo*. J. Nutri., 127: 668-674.

Janbaz, K.H. and A.H. Gilani, 1995. Evaluation of protective potential of *Artemisia maritima* extract on acetaminophen and CCl_4-induced liver damage. J. Ethnopharmacol., 47: 43-47.

Javatilaka, K.A.P.W., M.I. Thabrew and D.J.B. Perera, 1990. Effect of *Madder aspatana* on carbon tertachloride-induced changes in rat hepatic microsomal drug metabolizing enzyme activity. J. Ethnopharmacol., 30: 97-105.

Jeong, H.G. and H.Y. Park, 1998. The prevention of carbon tetrachloride-induced hepatotoxicity in mice by "-hederin: inhibition of cytochrome p-450 2E1 expression. Biochem. Mol. Biol., 45: 163-170.

Lim, H. K., H.S. Kim, H.S. Choi, S. Oh, C.G. Jang, J. Choi, S.H. Kim and M.J. Chang, 2000. Effects of acetylbergenin against D-galactosamine-induced hepatotoxicity in rats. Pharmacol. Res., 42: 471-474.

Mahmoud, A.L., 1994. Antifungal action and anti-aflatoxigenic properties of some essential oil constituents. Lett. Appl. Microbiol., 19: 110-113.

Montilla, M.P., J. Cabo, M.C. Navarro, S. Risco, R.S. Jimmenez and J. Aneiros, 1990. The protective and curative action of *Withania frutescens* leaf extract against CCl_4-induced hepatotoxicity. Phytother. Res., 4: 212-215.

Perez-Alvarez, V., R.A. Bobadilla-Lugo, P. Muriel, L. Favari, C. Villanueva-Lopez, 1993. Effects of leukotriene synthesis inhibition on acute liver damage induced by carbon tetrachloride. Pharmacol., 47: 330-336.

Plaa, G.L. and W.R. Hewitt, 1982. Quantitative evaluation of indices of hepatotoxicity. In: Toxicology of The Liver. Raven Press, New York, pp: 103-120.

Sadanobu, S., M. Watanabe, C. Nakamura and M. Tezuka, 1999. *In vitro* tests of 1,3-dithia-2-thioxo-cyclopent-4-ene to evaluate the mechanisms of its hepatoprotective action. J. Toxicol. Sci., 24: 375-381.

Sallie, R., J.M. Tredger and R. William, 1991. Drugs and the liver. Biopharmaceut. Drug Dispos., 12: 251-259.

Singh, V.K., S. Singh and D.K. Singh, 1999. Effect of active molluscicidal component of spices on different enzyme activities and biogenic amine levels in the nervous tissue of Lymnaea acuminata. Phytother. Res., 13: 649-654.

Suzuki, Y. and H. Furuta, 1988. Stimulation of guinea pig neutrophil superoxide anion-producing system with thymol. Inflammation, 12: 575-584.

Tanaka, E., M. Terada and S. Misawa, 2000. Cytochrome p-450 2E1: its clinical and toxicological role. J. Clin. Ther., 25: 165-175.

Venkatakrishnan, K., L.L. von Moltke and D.J. Greenblatt, 1988. Human cytochrome p-450 mediating phenecetin-O-deethylation *in vitro*: validation of the high affinity component as an index of CYP 1A2 activity. J. Pharm. Sci., 87: 1502-1507.