Acute toxicity study and \textit{LD50} determination of MTC fraction from \textit{Aspergillus terreus Koji}

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Abstract

The acute oral toxicity of \textit{Aspergillus terreus koji} extracted by MTC and oral median lethal dose (\textit{LD50}) determination by Reed-Muench Method was evaluated in mice. Varying doses of the extract were administered orally for 14 days of treatment (2 g/kgbw, 4 g/kgbw, 8 g/kgbw and 16 g/kgbw) and produced six mortality in male mice and seven mortality in female mice, no acute signs of toxicity and abnormalities in gross findings were observed throughout the 14 days period of treatment. The daily body and organ weight showed no significant differences between the control and the mice treated with the extract. Oral \textit{LD50} by Reed-Muench Method in male mice had the same values as in female mice which is 16 g/kg body weight. These findings suggest that the MTC fraction of Koji \textit{Aspergillus terreus} could be relatively safe when administered orally in mice.

Keywords: Acute oral toxicity, A. terreus koji, \textit{LD50}, MTC fraction, Reed-Muench Method

Introduction

\textit{Aspergillus terreus} is a cosmopolitan fungus which is primarily isolated from compost, plant material, and from soil. It is more common in tropical or sub-tropical areas. \textit{Aspergillus terreus} is an especially prolific producer of secondary metabolites has biological activities, such as: terre cyclicol as antibiotic and antifungal (Almassi et al., 1996), lovastain as anti cholesterol (Schimel et al., 1998), asterriquinone as anti neoplastich (Kaji et al., 1994) and aspulvinone and kodaistatin as inhibitor of hepatic glucose-6 phosphatase system (Vertesy et al., 2000). Our previous study on MTC fraction of ethyl acetate extract of Koji \textit{A. terreus} Koji shows significant activity as inhibitory of \textit{α-glucosidase} (IC50<10 µg ML$^{-1}$) (Triana et al., 2006).

Diabetes mellitus and cardiovascular diseases are two of degenerative diseases which are placed in high rank in worldwide. It is a chronic metabolic disorder affecting approximately 4% population worldwide and is expected to increase by 5.4% in 2025 (Babu et al., 2006). It is characterized by abnormalities in carbohydrates, lipid and lipoprotein metabolism, which not only lead to hyperglycemia but also cause many complications, such as hyperlipidemia, hyperinsulinemia, hypertension and atherosclerosis (Chait and Brunzell, 1996). The association of hyperlipidemia and altering of lipid parameters present a major risk of cardiovascular diseases in diabetic patients (Ramesh and Pugalendi, 2005).

In diabetic person, the utilization of impaired carbohydrate leads to accelerate lipolysis resulted in hyperlipidemia and failed to cardiovascular diseases. The World Health Organization (WHO) estimates that every year 12 million people worldwide die from cardiovascular diseases, with most of them being from the developing world (Kniestowicz, 2002). The underlying primary cause is believed to be atherosclerosis, progressive multifactorial diseases of the arterial wall (Navah et al., 2002). Previous study showed that Koji has many biological activities therefore by studying the MTC fraction as the new candidate of antidiabetic and hypolipidemic substance. The present study was under taken to investigate acute toxicity in mice and determination of \textit{LD50} of MTC fraction from Koji \textit{Aspergillus terreus}.

Materials and Methods

Preparation of Extract

Koji (5 kg) was macerated with EtOAc then evaporated. The dry extract of KEE (90) was dissolved in MeOH (1:4) followed by fractionations with n-hexane, CH$_2$Cl$_2$ and EtOAc. Activity guided isolation was conducted by analysis the inhibitor \textit{α-glucosidase} activity. Fractions show potential activity (IC50<10 µg mL$^{-1}$) were further purified by vacuum column chromatography.

Experimental Animals

Male and female DDY mice weighing 25-30 g and eight weeks old were obtained from our laboratory. All animal were maintained in a controlled environment condition of temperature (24±1°C) on alternative 12 h light/dark cycles, they were fed with standard diets with regular supply of water ad libitum.
Acute Toxicity Study

Briefly, mice were divided into five groups of six male and female. The treated group was orally given the Koji MTC fraction in a single dose with varied doses (2 g/kgbw, 4 g/kgbw, 8 g/kgbw and 16 g/kgbw), while the control group received only water vehicle. The animals were monitored for apparent signs of toxicity for 14 days. The animals that died within this period were subjected to necropsies. All mice were weighted and sacrificed on the 15th day after administration, and then the vital organs including heart, livers, lungs, kidneys, spleen, adrenal, gaster, and sex organs were grossly examined.

LD50 Determination

Sixty mice were randomly divided into five groups (6 in each group) male and female. The experimental group received the MTC fraction of \textit{A.terreus} koji through a gastric tube at several dose levels (2 g/kgbw, 4 g/kgbw, 8 g/kgbw and 16 g/kgbw) while the control group received only water vehicle. The animals were observed continuously during the 14 days of treatment. The analysis of LD50 was evaluated using the Reed and Muench Method (Reed and Muench, 1958).

Statistical Analysis

Results were expressed as mean ±standard error of mean (S.E.M.). Statistical significance was determined by one-way analysis of variance (ANOVA) and least significance difference (LSD) test. \( P \) values less than 0.05 were considered significant.

Results and Discussion

Oral administration of MTC fraction from \textit{A.terreus} koji in doses from 2 g/kgbw to 16 g/kgbw did not produce significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses and gastrointestinal effects in male and female mice (data not shown) these effect are observed during the experimental period (2 h after oral route). During the 14 days of treatment there were thirteen mortalities in both male and female groups and at the highest dose (16 g/kgbw) there was fifty percent of mortality. The alteration of body weight gain and organ weights from the control would reflect the toxicity of the substance (Carol, 1995) significant difference in organ weights between treated and untreated (control) animals may occur in the absence of any morphological changes (Bailey et al., 2004). Internal organ weights both in male and female mice were recorded as shown in table 1, while body weight in mice were illustrated in figure 1 for female group and figure 2 for male group, respectively. Neither body weight nor internal organs weight of treated mice was significantly changed relative to that of the control group. Gross examination of the internal organs revealed no pathological abnormality relative to the control (data not shown).

The median lethal dose (LD50) obtained from the Reed Muench Method (Reed and Muench, 1958) is 16 g/kgbw for male and female mice. The result showed that MTC fraction from \textit{A.terreus} koji is practically non toxic for oral administration.

| Table 1 Organ weight of mice in the acute toxicity study of the MTC fraction from Koji Aspergillus terreus |
|-----------------------------------------------|--------------|----------------|--------------|--------------|
| Koji MTC                                     | Control      | 2 g/kgbw       | 4 g/kgbw     | 8 g/kgbw     |
|                                              | Female       |                |              |              |
| Lung                                         | 291.62±20.33 | 248.5±28.30    | 243.05±8.77  | 220.08±20.75 |
| Heart                                        | 143.53±5.22  | 138.8±19.10    | 148.67±7.74  | 137.74±4.94  |
| Liver                                        | 1840±54.31   | 1710.9±232.42  | 1734.58±102.15| 1644.08±115.83|
| Spleen                                       | 103.42±4.47  | 148±32.7       | 148.3±14.82  | 164.0±290.67 |
| Adrenal                                      | 285.93±42.99 | 251.8±35.15    | 284.15±38.46 | 266.8±33.95  |
| Kidney                                       | 463.95±20.81 | 414±56.5       | 440.33±23.22 | 397.7±17.32  |
| Ovary                                        | 46.22±11.20  | 61.3±11.96     | 39.65±8.08   | 19.14±3.00   |
| Male                                         |              |                |              |              |
| Lung                                         | 231.65±15.99 | 236.4±11.06    | 249.87±8.89  | 238.34±16.61 |
| Heart                                        | 169.2±12.12  | 164.6±35.32    | 201.67±32.38 | 127.58±8.10  |
| Liver                                        | 1822±51.41   | 1899.3±71.55   | 1949.4±94.24 | 1652.3±105.69|
| Spleen                                       | 82.55±6.84   | 84.1±5.45      | 94.2±11.08   | 70.08±7.65   |
| Adrenal                                      | 419.65±7.69  | 256.2±22.25    | 726.4±36.71  | 408.18±47.41 |
| Kidney                                       | 479.35±24.78 | 464.6±77.04    | 505.62±28.21 | 468.94±49.76 |
| Testis                                       | 257.95±2.09  | 271.4±10.98    | 279.47±9.78  | 266.5±11.19  |

677
Figure 1 Body weights of female mice in the acute toxicity study of MTC fraction from A. terreus koji

Figure 2 Body weights of male mice in the acute toxicity study of MTC fraction from A. terreus koji

Conclusions

A single oral dose of 2 g/kgbw, 4 g/kgbw, 8 g/kgbw and 16 g/kgbw of MTC fraction from A. terreus koji are unable to induce toxic effects although there were thirteen deaths occurred during the experimental period. LD50 from Reed Muench Method is 16 g/kgbw for male and female mice. In summary, the data on the acute toxicity study indicates that MTC fraction from A. terreus koji is practically non toxic for oral administration.

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References


