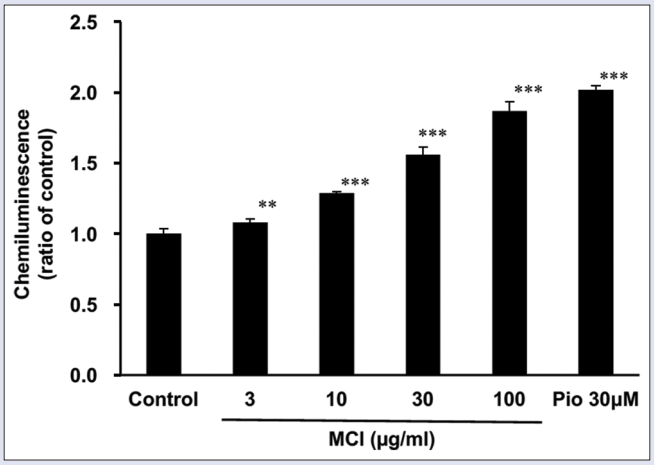

Figure 1: Peroxisome proliferator-activated receptor-γ agonistic activity of methanol extract of the capitulum of *Chrysanthemum indicum*. HEK293 cells were transiently transfected with pGL PPRE-luciferase reporter plasmid and pCMV-peroxisome proliferator-activated receptor-γ plasmid and incubated with the medium containing defatted fetal bovine serum for 24 h. methanol extract of the capitulum of *Chrysanthemum indicum* or pioglitazone (Pio) (30 μM) was dissolved in the media and further incubated for 24 h. Then, cells were lysed and the substrates for luciferase were added, and chemiluminescences were measured. Data are shown as the ratio of the control (without any treatment with methanol extract of the capitulum of *Chrysanthemum indicum* or pioglitazone) and expressed as mean ± standard error ($n = 4$). $^{**}P < 0.01$, $^{***}P < 0.001$ versus control group evaluated by Bonferroni–Dunnett's multiple *t*-test

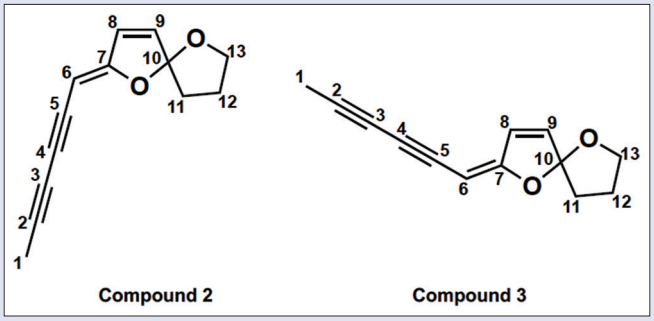

Figure 3: Chemical structures of compound 2 and 3

Analysis of F3 by HPLC revealed three distinct peaks. Peak 2 and Peak 3 were designated as compounds 2 and 3, respectively. On the basis of MS, NMR, optical rotation analysis, and comparison with previous studies,[14] compounds **2** and **3** were identified as (*Z*)-tonghaosu and (*E*)-tonghaosu, respectively [Figure 3]. The contents of (*Z*)- and (*E*)-tonghaosu in MCI were 0.063 ± 0.019 (w/w) % and 0.12 ± 0.04 (w/w) %, respectively. (*E*)-tonghaosu showed a significant concentration-dependent PPAR-γ agonistic activity, but (*Z*)-tonghaosu did not at a concentration of 100 μg/ml [Figure 4].

DISCUSSION

PPAR-γ agonists have a wide spectrum of biological functions, including regulating metabolism, reducing inflammation, influencing the balance of immune cells, inhibiting apoptosis and oxidative stress, and improving endothelial function; moreover, they are widely prescribed to type II diabetes mellitus in Japan.[15]

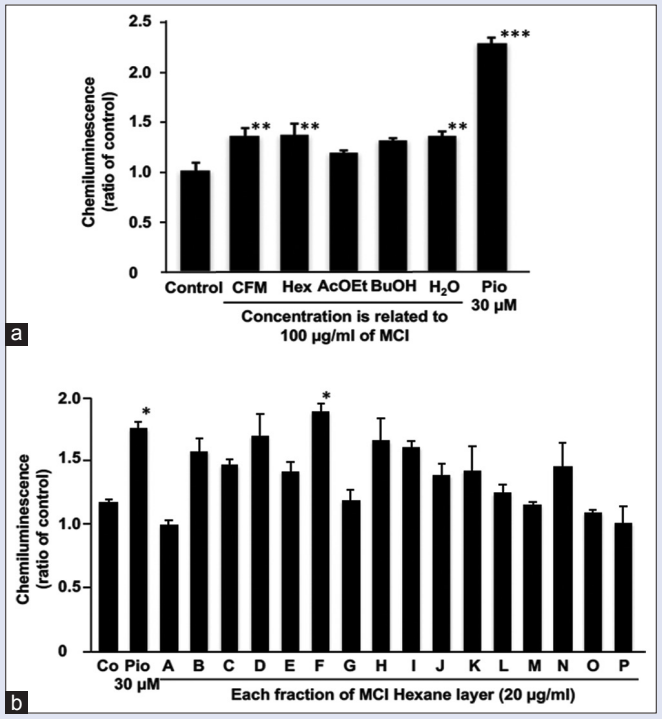

Figure 2: Peroxisome proliferator-activated receptor -γ agonistic activity of the fractions of methanol extract of the capitulum of *Chrysanthemum indicum*. (a) methanol extract of the capitulum of *Chrysanthemum indicum* was partitioned according to the descriptions of Materials and Methods, and the cells was treated with the medium containing each layer (Hex, hexane layer; AcOEt, ethyl acetate layer; BuOH, buthanol layer, H_2O, water layer; Pio, pioglitazone) at the concentration related to the original methanol extract of the capitulum of *Chrysanthemum indicum* (100 μg/ml). (b) Fractionated hexane layer of methanol extract of the capitulum of *Chrysanthemum indicum* shown as Fr. A– P. The cells were treated with the medium containing each fraction (20 μg/ml). Co, Control; Pio, pioglitazone. Data are shown as the ratio of the control and expressed as mean ± standard error ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus control group evaluated by Bonferroni–Dunnett's multiple *t*-test

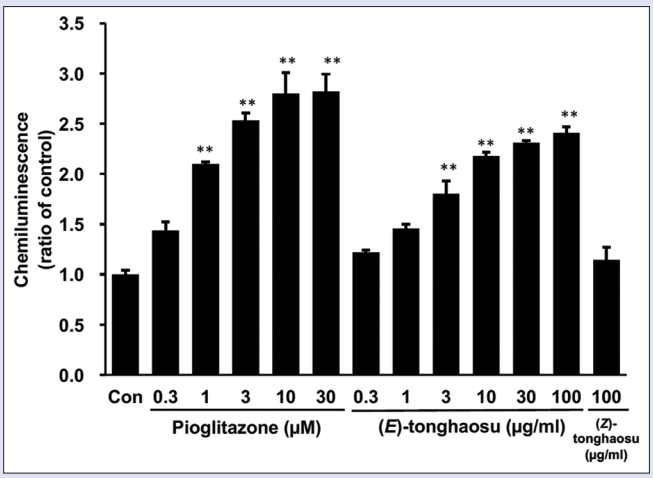

Figure 4: Peroxisome proliferator-activated receptor -γ agonistic activity of (*Z*)- and (*E*)-tonghaosu. (*Z*)-tonghaosu, (*E*)-tonghaosu, or pioglitazone was dissolved in the media and further incubated for 24 h. Then, cells were lysed and the substrates for luciferase were added, and chemiluminescence was measured. Data are shown as the ratio to the control and expressed as mean ± standard error ($n = 4$). $^{**}P < 0.01$ versus control group evaluated by Bonferroni–Dunnett's multiple *t*-test

In the present study, MCI at 100 μg/ml showed PPAR-γ agonistic activity approximately double of an experimental control. Previous study indicated that the hot water extract of *C. morifolium* edible flower (100 μg/ml) caused doubling of PPAR-γ mRNA expression in 3T3-L1 adipocyte cells.[16] Although *Chrysanthemum* species and the extracting methods are different from our study, the results are consistent with the previous report.[16]

We isolated the active constituents from CFM by activity-guided fractionation. Results show that PPAR-γ agonistic activities were distributed among several fractions of the extract, suggesting that Chrysanthemi Flos contained several active constituents. We isolated three peaks from the fraction exhibiting the highest activity and found that two of these (compounds **2** and **3**) had PPAR-γ agonistic activity. We further analyzed the chemical structures of compounds **2** and **3**. Both compounds were geometric isomers of tonghaosu, and only (*E*)-isomer had PPAR-γ agonistic activity. The polyacetylene motif binding to carbon 6 of tonghaosu with different geometry would affect the binding of tonghaosu to the target site of PPAR-γ. Although conjugated linoleic acids have different anti-inflammatory activities among geometric isomers,[17] the geometric character of tonghaosu could affect the PPAR-γ agonistic activity. (*E*)-tonghaosu showed significant PPAR-γ agonistic activity at 3 μg/ml = about 15 μM, suggesting that (*E*)-tonghaosu have about one-tenth activity of pioglitazone.

CONCLUSION

We identified (*E*)-tonghaosu as PPAR-γ agonistic constituents in the capitulum of *C. indicum*. Considerable chemical modulation would be needed to derive a new PPAR-γ agonist using (*E*)-tonghaosu as the seed.

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Conflicts of interest

There are no conflicts of interest.

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