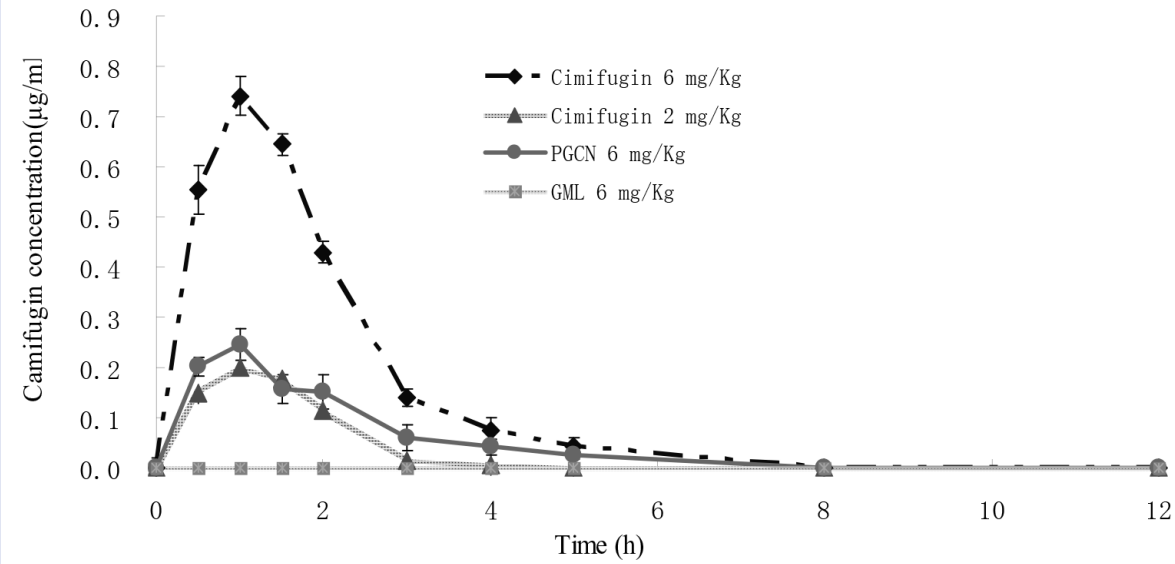

Figure 3: The concentration–time curve of cimifugin in rat plasma after oral administration of cimifugin, prime-O-glucosylcimifugin (PGCN), or 4′-O-β-D-glucosyl-5-O-methylvisamminol (GML). The rats were orally administered with cimifugin (2 or 6 mg/kg), PGCN (6 mg/kg), and GML (6 mg/kg), respectively. Blood samples from rats were then collected 0.5, 1, 1.5, 2, 3, 5, 8, and 12 h following the drug delivery for HPLC analysis.

Table 3: Effect of cimifugin, PGCN, and GML on xylene-induced ear edema in mice

Treatment	Dose (mg/kg)	Increase in ear edema weight (mg)	% Inhibition of edema weight (%)
Saline	0	7.01 ± 3.31	–
Cimifugin	1	4.57 ± 0.33**	35
	2	3.70 ± 0.29**	48
	4	2.16 ± 0.36**	70
PGCN	1	5.83 ± 0.23*	16
	2	5.45 ± 0.33**	22
	4	4.32 ± 0.42**	38
GML	1	6.64 ± 0.18	5
	2	6.42 ± 0.23	9
	4	6.13 ± 0.25	12

Table 4: Pharmacokinetic parameters of cimifugin in rat after p.o. administration of cimifugin, PGCN, and GML

Treatment	Dose (mg/kg)	Cmax (μg/mL)	Tmax (h)	AUC0-8 h (μg/mL·h)
Cimifugin	2	0.200	1.0	0.4023
	6	0.741	1.0	1.5852
PGCN	6	0.246	1.0	0.5405
GML	6	0.0000	–	0

cimifugin in SIF and a small proportion (1.99%) of transformation in SGF. As for GML, cimifugin metabolite was found neither in SGF nor in SIF. HPLC analysis showed that ~33.37% of GML degraded into a new compound, except for a small quantity of the drugs transformed into PGCN in SGF (3.09%) and SIF (0.38%). By HPLC-MS analysis, this new compound is confirmed to be the deglucosed derivative of GML, that is, MVL [Figure 4]. Stability studies in Figure 5 found that <20% cimifugin was degraded in the liver tissue homogenate and hardly any transformation occurred in plasma. In contrast, up to 44.9 and 36.0% MVL rapidly disappeared within a short span of 15 min co-incubation with *in vitro* hepatic system and plasma, respectively, suggestive of bio-inspired instability properties of this metabolite.

DISCUSSION

To the best of our knowledge, this study is the first report on antipyretic, analgesic, and anti-inflammatory activities and pharmacokinetics of GML. The present investigation showed that this compound was not absorbed into blood and does not possess antipyretic, analgesic, and anti-inflammatory effects when orally administered, although it has long been used for quality control for Radix Saposhnikoviae in many editions

Table 5: Metabolic study of PGCN and GML in SGF and SIF

	Content(nmol/mL)					Transformation rate (%)
	Drugs	Cimifugin	PGCN	GML	MVL	
SGF	PGCN	1.99	126.12	–	–	1.55
	GML	–	3.09	141.94	–	2.13
SIF	PGCN	133.69	0.38	–	–	99.64
	GML	–	–	119.82	59.99	33.37

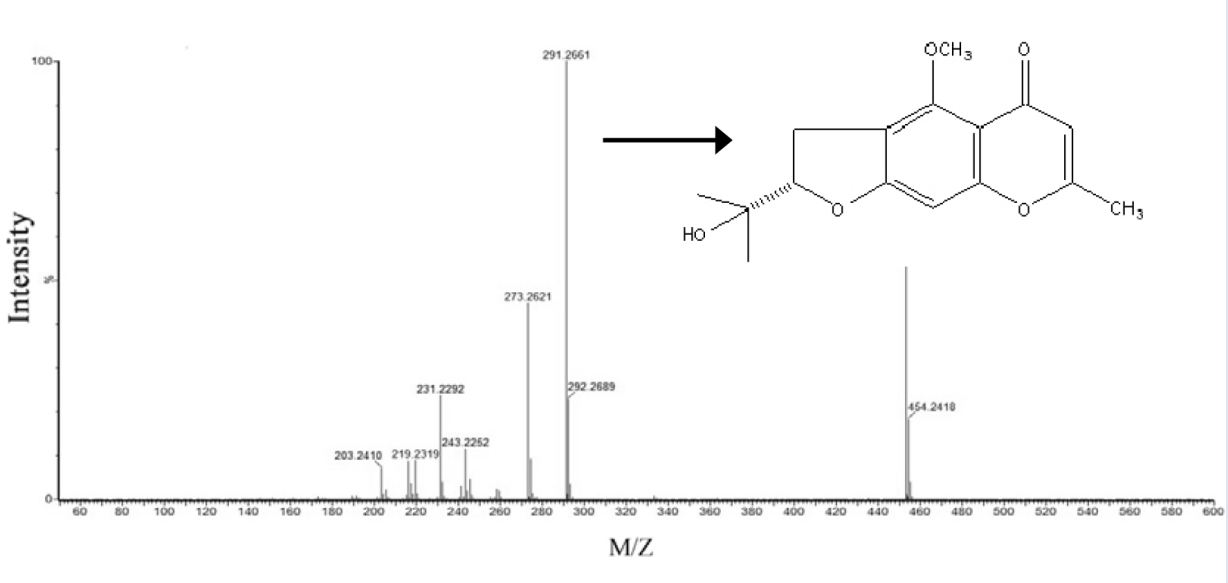

Figure 4: Mass spectrum analysis of the new metabolite of 4′-O-β-D-glucosyl-5-O-methylvisamminol (GML) in simulated intestinal fluid (SIF). The related substance was identified as 5-O-methyvisammiol (MVL)

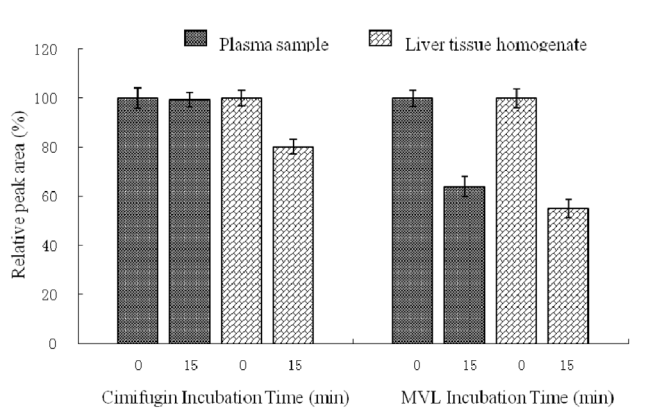

Figure 5: In vitro stability studies of 5-O-methyvisammiol (MVL) in rat hepatic system and plasma.One hundred microliters of simulated intestinal fluid (SIF) containing MVL or cimifuginwas first coincubated with 30% in vitro liver tissue homogenate or plasma sample of rats at 37°C in a shaking water bath.Fifteenminutes later, metabolic stability of these two chemicals was evaluated and compared by HPLCanalysis of parent drugs left usingrelative peak area, which was obtained by designating the actual peak area at 0 min as a value of 100%.

of Chinese Pharmacopoeia.

Regarding the pharmacodynamic effects of the three chromone derivatives in Radix Saposhnikoviae, only PGCN, as far as we know, was documented to possess antipyretic, analgesic, anti-inflammatory, anti-platelet aggregation, and other actions.[10,14,15] However, both previous and our present study showed that it was the aglycone, cimifugin, rather than the parent drug (PGCN) that represented the potential pharmacodynamic component.[4] This is compatible with a previous study which suggested that PGCN was of a moderately absorbed compound.[16] It is worthwhile to note that in this study only cimifugin, but not any trace of PGCN, was found in the plasma samples of PGCN orally administered rats, which is inconsistent with previous reports, which also found a certain amount of parent drug, although its content in blood is much lower than that of cimifugin.[5] We speculated that the discrepancy might be primarily due to the dose used for study. Indeed, Our used dose of PGCN was 2–6 mg/kg, which was much lower than those used in other studies (2–6 vs. 10 mg/kg).[5] In spite of that, the dose in our study might represent the real *in vivo* process of PGCN, becausethe dose of 2–6 mg/kg rat b.w. was designated based on the actual usage dosage and human–rat dose conversion.

As aforementioned, because except for cimifugin, no other components were found in the blood, the pharmacological effects of orally administered PGCN might totally arise from its dyglucosated metabolite cimifugin. Indeed, we herein found that cimifugin monomer solution took comparatively stronger antipyretic, analgesic, and anti-inflammatory effects,and PGCN have comparatively weaker pharmacological activities when it is orally administered. But when it comes to GML, no antipyretic, analgesic, and anti-inflammatory activities were found with this compound when it was orally used. Moreover, we reckon that GML might not produce any pharmacodynamic effects when orally administered, becausenot any form of this compound was traced in the blood for this delivery route.

It is rather curious that GML was not traced either in the blood or in the feces. As thus, we detected its biotransformation products in gastrointestinal using SGF and SIF. As a result, an extremely unstable metabolite MVL was obtained in the SIF. We thus hypothesizedthat GML might first was metabolized to unstable MVL, which then was absorbed into the blood and rapidly degraded by the enzymes in the blood and liver; the degradation process was perhaps exceedingly fast, and thereby we failed to detect MVL in the plasma samples. This hypothesis warrants further confirmation.

CONCLUSION

To conclude, no antipyretic, analgesic, and anti-inflammatory action was observed with GML. Meanwhile, this compound is hard to be absorbed into bloodstream. Given that Radix Saposhnikoviae extract is generally administered orally, we speculate that this compound might be a nonpharmacologically active agent in real usage. Thus, it might be

unscientific to evaluate the quality of Radix Saposhnikoviae based on the content of GML.

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Conflicts of interest

There are no conflicts of interest.

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