Genistein: is the multifarious botanical a natural anthelmintic too?

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Abstract Genistein (4',5,7-trihydroxyisoflavone) is naturally present in plants of the soy family and is known to have various pharmacological activities, such as anti-cancer, anti-diabetic, anti-oxidant, etc. The phytoestrogen is one of the major isoflavones found in some medicinal plants having anthelmintic properties. This review describes the putative role of genistein as an anthelmintic, which has been tested on some helminth parasites in vitro. Genistein has been shown to cause paralysis and alterations in the tegument and tegumental enzymes (acid phosphatase, alkaline phosphatase, adenosine triphosphatase, and 5'-nucleotidase) of helminth parasites. Alterations in the activities of several enzymes associated with the coordination system (specifically non-specific esterases, acetylcholine esterase, and nitric oxide synthase), and changes in the concentration of nitric oxide, cGMP, free amino acid pool, and tissue ammonia are observed in helminth parasites treated with genistein. The phytoestrogen also affects the carbohydrate metabolism by altering the activities of key enzymes involved in glycogen- and glucose-metabolism of a cestode parasite. Considering the significance of phosphoenolpyruvate carboxykinase (PEPCK) in glycolysis of the cestode parasite, *K_i* of the phytoestrogen for PEPCK in the parasite has been determined, and molecular docking of genistein into the active site of the enzyme has also been described. The potential beneficial role of genistein as a natural alternative in

management of helminth parasites needs to be further explored, particularly considering its in vivo efficacy and pharmacokinetics.

Keywords Medicinal plants · Genistein · Anthelmintic · Helminths

Introduction

Since ancient times, traditional plant remedies have been the main source against several diseases. Plants or their products have potential to provide an alternative to current practices involving chemotherapy against helminths (Didier et al. 1988; Robinson et al. 1990; Tandon et al. 2011). Drug resistance against several diseases has surged in development of new topical and systemic medicines. Therefore, in view of the urgent need of developing new treatments, there are intensified efforts to search for novel drugs from plants or plant products, which could replace or to be used in conjunction with the existing ones (Duke 1983). The pharmaceutical industry has responded to this need by developing new systemic drugs and topical treatments. Globally, there is a patient-driven trend towards “natural” remedies. Some plants have been reported to have anthelmintic efficacy against helminth parasites in vitro (Table 1). According to recent studies, some plants or their parts have also been shown to have cidal activity against schistosomes of *Schistosoma mansoni*, metacercariae of *Hymenolepis diminuta*, *Echinococcus multilocularis* and *E. granulosus*, and larvae of filarid or other nematode parasites (Comely 1990; Satrija et al. 1995; Ghosh et al. 1996; Khunkitti et al. 2000; Sparg et al. 2000; Al-Qarawi et al. 2001; Mølgaard et al. 2001; Singh et al. 2001; Lyddiard et al. 2002; Marley et al. 2003;

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Table 1 List of medicinal plants having anthelmintic efficacy against helminth parasites in vitro

Medicinal plants	References
<i>Acacia auriculiformis</i>	Ghosh et al. (1996)
<i>A. oxyphylla</i>	Dasgupta et al. (2010, 2013b) and Roy et al. (2012c)
<i>Acorus calamus</i>	Nath and Yadav (2016)
<i>Adhatoda vasica</i>	Yadav and Tangpu (2008)
<i>Albizia anthelmintica</i>	Galal et al. (1991a, b) and Koko et al. (2000)
<i>A. lebbek</i>	Galal et al. (1991a)
<i>Allium sativum</i>	Soffar and Mokhtar (1991)
<i>Alpinia nigra</i>	Roy et al. (2009)
<i>Artocarpus lakoocha</i>	Charoenlarp et al. (1981, 1989)
<i>Balanites aegyptiaca</i>	Koko et al. (2000)
<i>Buddleja asiatica</i>	Garg and Dengre (1992)
<i>Carex baccans</i>	Challam et al. (2012), Roy et al. (2012a) and Giri et al. (2015)
<i>Clerodendrum colebrookianum</i>	Yadav and Temjenmongla (2012b)
<i>Diospyros mollis</i>	Maki et al. (1983)
<i>Flemingia vestita</i>	Yadav et al. (1992), Roy and Tandon (1996) and Tandon et al. (1997)
<i>Lasia spinosa</i>	Yadav and Temjenmongla (2012a)
<i>Lysimachia ramosa</i>	Challam et al. (2010)
<i>Mallotus philippinensis</i>	Gupta et al. (1984) and Akhtar and Ahmad (1992)
<i>Matteuccia orientalis</i>	Shiramizu et al. (1993)
<i>Millettia thonningii</i>	Lyddiard et al. (2002)
<i>M. pachycarpa</i>	Roy et al. (2008)
<i>M. reticulata</i>	Fang et al. (2010)
<i>Oroxylum indicum</i>	Deori and Yadav (2016)
<i>Potentilla fulgens</i>	Roy et al. (2010), Roy et al. (2012a, 2012b) and Giri et al. (2013)
<i>Saussurea lappa</i>	Akhtar and Riffat (1991)
<i>Securinega virosa</i>	Dasgupta et al. (2013a, b)
<i>Solanum myriacanthum</i>	Yadav and Tangpu (2012)
<i>Stephania glabra</i>	Tandon et al. (2004); Lyndem et al. (2008); Das et al. (2009)
<i>Streblus asper</i>	Chatterjee et al. (1992)
<i>Strobilanthes discolor</i>	Tangpu et al. (2006)
<i>Teloxys graveolens</i>	Del Rayo Camacho et al. (1991)
<i>Terminalia catappa</i> L.	Anuracpreeda et al. (2016, 2017)
<i>Trichosanthes multiloba</i>	Tandon et al. (2004) and Lyndem et al. (2008)
<i>Uvaria narum</i>	Hisham et al. (1992)

Naguleswaran et al. 2006). Anthelmintic efficacy of some plant-derived components has been found to be comparable or at par with commonly used broad-spectrum drugs like albendazole, piperazine and diethylcarbamazine (Kalyani et al. 1989; Koko et al. 2000; Enwerem et al. 2001; Onyeyili et al. 2001; Temjenmongla and Yadav 2003).

There are few medicinal plants available in northeastern region of India (Rao 1981). One of them, *Flemingia vestita* Benth (Fabaceae), has usage as anthelmintic against intestinal worms in local traditional medicine. To get rid of worm infections, the natives in the region consume the edible tuberous root (unpeeled) of the plant. To validate the

use of root tuber of *F. vestita* against helminths, the phytochemicals in the ethanolic extract of root tuber peel of *F. vestita* have been isolated and identified. The root tuber peel of *F. vestita* has rich flavone content; genistein (4',5,7-trihydroxyisoflavone) (Fig. 1) is found to be the major active principle present in the root tuber peel, besides other isoflavones—formononetin, pseudobaptigenin and daidzein (Rao and Reddy 1991). Isoflavones are naturally occurring phytoestrogens, notably found in the soybean family. Besides *F. vestita*, genistein is also found in many other plants that belong to the same family or related family like Rutaceae family, *Fortunella obovata* Hort, *Erythrina*

Fig. 1 Structure of genistein (4',5,7-trihydroxyisoflavone), isolated from ethanolic root peel extract of *Flemingia vestita*

variegata, *Millettia reticulata* Benth, *Tetracera scandens*, *Genista sessilifolia*, and Amaryllidaceae (*Puerariae radix*) (Lapčík et al. 2004; Ha et al. 2006; Zhang et al. 2007; Koblovská et al. 2008; Lee et al. 2009; Fang et al. 2010; Bontempo et al. 2013; Mikšátková et al. 2014), and is shown to have various activities. The in vitro anthelmintic efficacy of genistein has been tested against few helminth parasites in order to authenticate traditional usage of *F. vestita* against intestinal worms (Tandon et al. 1997).

The biological functions of isoflavones are well known; genistein, in particular, is an inhibitor of tyrosine protein kinases (Akiyama et al. 1987) and competitive inhibitor of several protein kinase reactions (O'Dell et al. 1991). The phytoestrogen is known to have various other pharmacological activities, such as anti-cancer, anti-diabetic, and anti-oxidant. Isoflavones, genistein and daidzein in particular, have been shown health-promoting benefits in different types of cancers (Barnes and Peterson 1995; Fotsis et al. 1995; Lamartiniere et al. 1995; Wiseman and Duffy 2001; Nagata et al. 2002; Duffy et al. 2007; Satih et al. 2008, 2010; Chemler et al. 2010). Studies on whether genistein has an effect on diabetes or not are very limited; however, animals and humans studies have shown that ingestion of soy protein moderates hyperglycemia (Lavigne et al. 2000; Jayagopal et al. 2002; Choi et al. 2008), suggesting its beneficial role in diabetes. Another important consideration is that genistein modulates pancreatic β -cell function via activation of the cAMP/PKA-dependent ERK $\frac{1}{2}$ signaling pathway (Fu et al. 2010). Additionally, Lee (2006) suggests that genistein is capable of reducing hyperglycemia and diabetic complication via minimization of islet cell loss in pancreas, which is also supported by Yang et al. (2011). Recently, biocomputational study demonstrates that genistein inhibits human cytosolic phosphoenolpyruvate carboxykinase (PEPCK-C) by mixed inhibition mechanism (Katiyar et al. 2015). In various cell lines, the modulatory effect of genistein on PEPCK-C expression has also been demonstrated (Seenappa et al. 2016). Dkhar et al. (2017) demonstrate that genistein represses human PEPCK-C in insulin-independent pathways in HepG2 cell line. Furthermore, it exerts anti-

diabetic effect in type 2 diabetic conditions by enhancing the glucose and lipid metabolism (Park et al. 2006). There has been a surge of curiosity in exploring the antioxidant activities of isoflavones and their analogous metabolites, and genistein is found to be an antioxidant too (Arora et al. 1998; Mitchell et al. 1998; Liu et al. 2004; Rufer and Kulling 2006; Sienkiewicz et al. 2008; Ma et al. 2010). Mitchell et al. (1998) show that S-equol, a metabolite of genistein, is more effective antioxidant than genistein and daidzein. Besides, genistein is reported to have beneficial effects on atherosclerosis and chronic inflammatory diseases for its inhibitory effect on NO production (Sheu et al. 2001), and its possible role in cell cycle is also reported in *Candida albicans* (Lamartiniere et al. 1995; Yazdanyar et al. 2001).

The multifarious genistein as anthelmintic

Considering the multifarious role of genistein in various metabolic activities as stated above and its usage as a cure against worm infections in traditional medicine practices, its probable anthelmintic role has been tested on several commonly occurring helminth parasites. *Raillietina* spp., the cestodes of domestic fowl; *E. multilocularis* and *E. granulosus*, the cestodes of dog; *Fasciolopsis buski*, the giant intestinal flukes parasitizing pig/human; *Artyfechinostomum sufragaryfex*; *Fasciola hepatica*; *Heterakis gallinarum* and *Ascaridia galli*, gut nematodes from fowl; and *Ascaris suum*, the giant round worm from the intestine of pig, have been used for investigations in order to find out the role of genistein as an anthelmintic.

Parasites and paralysis

In vitro treatment of helminth parasites with genistein causes flaccid paralysis in them in a dose-dependent manner (Yadav et al. 1992; Roy and Tandon 1996; Tandon et al. 1997, 2004; Lyndem et al. 2008; Toner et al. 2008). The phytoestrogen inflicts complete immobilization/reversible flaccid paralysis in soft-bodied cestodes and trematodes (e.g. *R. echinobothrida* and *F. buski*) within short time; however, due to the presence of a collagen-rich fibrose surface interface, the nematodes take longer time for the onset of paralysis. These studies indicate that genistein acts transtegumentally on these parasites (Yadav et al. 1992; Roy and Tandon 1996; Tandon et al. 1997).

Effect of genistein on surface architecture and tegumental enzymes

Presuming that genistein may pass through the tegumental interface of the soft-bodied parasites, its effect on the tegumental architecture and tegumental enzymes in

helminth parasites has been studied by several authors. Stereoscan- and transmission-electron microscopic observations reveal that there are tegumental alterations and deformity in trematode and cestode parasites (viz. *A. sufrartiyfex*, *F. buski*, *R. echinobothrida*) when treated with genistein (Roy and Tandon 1996; Tandon et al. 1997; Pal and Tandon 1998a). Alterations in the contour of microtriches and disorganization of the tegumental region are also observed in the parasites exposed to genistein. The inner sub-tegumental region and muscle layers are the sites that are predominantly affected; the former shows pronounced vacuolization in the genistein treated parasites compared to the control parasite (Pal and Tandon 1998a). In trematodes, genistein causes sloughing off or deformation of the spines as well as deleterious effect on the tegumental surface, which may be associated with paralysis and subsequent death of the parasites (Roy and Tandon 1996). Genistein and its derivatives, Rm 6423 and Rm 6426, induce truncation of microtriches, nuclear pyknosis and vesiculations in *E. granulosus* and *E. multilocularis*, causing paralysis in the parasites (Naguleswaran et al. 2006). Rm 6423 also induces interruption of metacystode germinal layer in *E. granulosus* and *E. multilocularis* (Naguleswaran et al. 2006). The phytoestrogen causes morphological and neuromuscular disruption in *F. hepatica* as shown in in vitro; the surface changes comprised swelling and blebbing, especially in the posterior region of the fluke showing disruption to the spines, accompanied by some spine loss (Toner et al. 2008). At different concentrations of genistein (10, 100 and 1 mM), there occurs a significant increase in the frequency and/or amplitude of the somatic muscle strips isolated from *F. hepatica* (Toner et al. 2008), which might lead to paralysis.

The tegumental enzymes (acid phosphatase, alkaline phosphatase, adenosine triphosphatase, and 5'-nucleotidase) play an important role in the metabolism of cestodes and trematodes for their survival in internal milieu of the host's intestine (Roy 1982). When the parasites were exposed to the phytoestrogen in vitro, a decline in activity of these enzymes in the genistein treated parasites was histochemically demonstrated (Pal and Tandon 1998b; Kar and Tandon 2004). These effects may contribute to paralysis and subsequent death of cestode and trematode parasites under genistein treatment.

Effect of genistein on the nervous coordination system

Esterases (non-specific esterase and acetyl cholinesterase) have significant role in nervous coordination of helminths. In order to find out the effect of the phytoestrogen on these components of helminth parasites, *R. echinobothrida* and *F. buski* were treated with genistein. Besides alterations and deformity in their tegumental architecture, the

genistein treated helminth parasites exhibit changes in the activities of nonspecific esterases and acetylcholine esterase with respect to their respective controls (Pal and Tandon 1998c; Kar and Tandon 2000). Acetylcholine, in particular, is known to be involved in muscular coordination and anchoring function; therefore, the neuromuscular disruption caused in genistein exposed *F. hepatica* may explain the onset of paralysis in helminths (Toner et al. 2008).

The free amino acids [comprising of aspartate, threonine, serine, glutamic acid, glutamine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, lysine, histidine, arginine, phosphoserine, taurine, citrulline, ornithine, beta-alanine, and gamma-amino butyric acid (GABA)] have been demonstrated in helminth parasites (Tandon et al. 1998; Kar et al. 2004). There was a change in the free amino acids level in the helminth parasites treated with genistein (Tandon et al. 1998; Kar et al. 2004). The helminth parasites treated with genistein were found to have an enhancement in GABA and citrulline levels (Tandon et al. 1998; Kar et al. 2004). The change in the citrulline level may be involved in nitric oxide (NO) release and resultant paralysis in *F. buski*, the giant intestinal trematode (Kar et al. 2004). An increased in ammonia concentration, released by the parasites, in the host microenvironment may prove to be lethal for survival of the intestinal parasites.

NO, a unique gaseous neural messenger, is known to have a dual role—protective and toxic in organisms—and it has been reported to have anthelmintic effects (Mahmoud and Habib 2003). In helminth parasites, nitric oxide synthase (NOS) activity, accountable for NO production, has been demonstrated in neural tissues by NADPH-diaphorase histochemical staining (Bascal et al. 1995; Gustafsson et al. 1996, 1998; Lindholm et al. 1998; Terenina et al. 1999; Gustafsson et al. 2001; Tandon et al. 2001). There was an increase in NO level in neural tissues of the trematode parasite *F. buski* treated with genistein, which may have assisted the onset of paralysis in the parasite (Kar et al. 2002). Not only in the trematode parasite, but also in the cestode parasite, *R. echinobothrida*, the phytoestrogen enhances NOS activity and cGMP concentration in the parasite tissue (Das et al. 2007, 2009).

Effect of genistein on Ca^{2+} homeostasis in cestode

Ca^{2+} plays a key role in muscle contraction (Nelson and Cox 2012) and possibly involves in paralysis in helminth parasites (Day et al. 1992; Redman et al. 1996); hence, it is imperative to understand the role of genistein on Ca^{2+} homeostasis in helminth parasites. It has been seen that a significant amount of Ca^{2+} was found in a cestode, *R. echinobothrida*, besides other metal ions such as

Fig. 2 Diagrammatic representation of possible role of phosphoenolpyruvate carboxykinase (PEPCK*) in glucose metabolism in cestodes. (Excerpted from Smyth and McManus 1989)

magnesium, iron, zinc, lead and chromium (Das et al. 2006). Following the genistein treatment, the Ca^{2+} homeostasis is disturbed in the parasite, and the Ca^{2+} concentration is decreased (39–49%) in the parasite tissues at the paralysis time; while, there is an increase in Ca^{2+} efflux (91–160%) into the media by the parasite (Das et al. 2006). Changes in the Ca^{2+} concentration at onset of paralytic state may be correlated with rapid muscular contraction caused by genistein in the cestode parasite. This hypothesis was also postulated by Toner et al. (2008), who observed that genistein changed the amplitudes of somatic muscle strips in *F. hepatica*.

Effect of genistein on carbohydrate metabolism

Helminth parasites, especially trematodes and cestodes, mainly derive their energy from simple carbohydrate molecules (Smyth and McManus 1989). With respect to carbohydrate metabolism in cestode parasite, genistein was found to alter glycogen metabolism in *R. echinobothrida* by activating the active form of glycogen phosphorylase and inhibiting the active form of glycogen synthase, thus promoting utilization of glycogen (Tandon et al. 2003). Activities of key enzymes of glycolysis—hexokinase, phosphofructokinase, PEPCK, pyruvate kinase (PK), lactate dehydrogenase, malate dehydrogenase and malic enzyme—in the cestode parasite were also altered at the time of paralysis, suggesting genistein does not influence glucose utilization towards aerobic reactions in the parasite (Das et al. 2004a). With respect to gluconeogenic pathway in *R. echinobothrida*, there was a decline in glucose 6-phosphate dehydrogenase activity and pyruvate carboxylase and PEPCK activities were altered in the cestode

parasite under genistein treatment, whereas, the fructose 1,6-bisphosphatase activity remained unchanged (Das et al. 2004b). The glucose level was declined and the malate content and lactate efflux was increased in the genistein treated cestode parasite, suggesting an increased energy demand in the cestode parasite due to genistein treatment (Das et al. 2004c). Change in carbohydrate metabolism by altering activities of key enzymes of glycogen metabolism and glycolysis could be a function of energy demand of the cestode parasite due to genistein treatment (Tandon and Das 2007), which is also evident in some trematode parasites (El-Ansary et al. 2007).

The PK/PEPCK branch point (as shown in Fig. 2) in glycolysis of cestode parasites has been considered as a plausible target for anthelmintic action (Reynolds 1980), since PEPCK plays different role in cestodes and their vertebrate hosts, and both the enzymes use phosphoenolpyruvate as their substrate (Smyth and McManus 1989). In view of understanding the functional differences between PK and PEPCK, the PK/PEPCK branch point has been studied in various helminths (Bueding and Saz 1968; Prichard 1976; Moon et al. 1977; El-Ansary et al. 2007).

The phytochemicals (including genistein) from *F. ves-tita* showed modulatory effect on the purified PEPCK from the cestode parasite, *R. echinobothrida* (Das et al. 2013). Genistein and daidzein showed non-competitive inhibition with respect to the substrate (phosphoenolpyruvate) for PK and PEPCK enzymes from the cestode, *R. echinobothrida*. Further, K_i of these compounds for PK and PEPCK from the cestode parasite has been determined. K_i for genistein is 0.26 and 0.17 μM for PK and PEPCK enzymes from the parasite, respectively; whereas, K_i for daidzein is 0.19 and 0.29 μM , respectively (Das et al. 2015).

Fig. 3 **a** Non-competitive inhibition of rePEPCK (PEPCK from *R. echinobothrida*) by genistein as determined by Lineweaver–Burk plot (LB plot). The plot was computed by taking different concentrations of PEP (0.01–8 mM) in presence of various concentrations of genistein {(filled star) control, (filled circle) 5 μ M, (filled square) 10 μ M, and (filled nabla) 20 μ M}. K_i (0.15 μ M) of genistein for

rePEPCK was determined from the replot (inset) with $R^2 = 0.9972$. **b** Competitive inhibition of gdPEPCK (PEPCK from *Gallus domesticus*) by genistein was calculated by LB plot, showing K_i of 0.20 μ M for gdPEPCK with $R^2 = 0.9990$ (inset). **c** Near-UV CD spectra (250–350 nm) of rePEPCK in absence/presence of genistein (20 μ M)

Effect of genistein on PEPCK activities and their conformation

Considering differential activity of PEPCK in the cestode parasite and its host and in search for potential modulators for the parasite PEPCK, genistein and daidzein were tested on activity of rePEPCK (PEPCK from *R. echinobothrida*) and gdPEPCK (PEPCK from its host, *Gallus domesticus*). Genistein and daidzein inhibit the parasite PEPCK in a non-competitive manner; whereas, both the compounds inhibit the host PEPCK in a competitive manner (Ramnath et al. 2017). K_i of genistein and daidzein for the parasite PEPCK were found to be 0.15 and 0.26 μ M, respectively (Fig. 3a); however, genistein and daidzein showed K_i of 0.20 and 0.35 μ M (Fig. 3b), respectively, for host PEPCK carboxylation reaction, and 0.31 and 0.64 μ M, respectively, for host PEPCK decarboxylation reaction, representing genistein is more inhibitory than daidzein

(Ramnath et al. 2017). Using biocomputational approach, mixed inhibition of genistein for human PEPCK-C has been reported by Katiyar et al. (2015).

The effect of genistein and daidzein on conformation of PEPCK has also been analyzed by Far-UV CD spectra analysis using CD spectrophotometer to understand their effect on the structure of PEPCK. The tertiary structure of PEPCK, both from the parasite and its host, remained unchanged in presence of these compounds (Fig. 3c). This suggests that genistein inhibits PEPCK without changing its conformation and may interact via different sites (Ramnath et al. 2017).

Differential binding of genistein with PEPCKs

There is little structural information available for the parasite PEPCK, and recently, PEPCK gene (NCBI GenBank acc. no. KC252609.1) from *R. echinobothrida* has been

Fig. 4 a Probable interactions of genistein with chicken mitochondrial PEPCK (mPEPCK). The interacting residues are shown as thin stick model, whereas genistein as thick stick model. Surface structure shows genistein and PEP compete for the same pocket of chicken mPEPCK (**b**), whereas different pocket in the case of rePEPCK (**c**)

isolated and analyzed by Dutta et al. (2016). In general, the PEPCK active site is large and encompasses a number of binding pockets, like PEP binding, GTP binding (Johnson and Holyoak 2012). In search of possible modulators for the parasite PEPCK, several plausible modulators (genistein, diadzein, etc.) have been tested on *R. echinobothrida* PEPCK model (Dutta et al. 2016). Out of which, genistein has been shown to have the lowest binding energy (CDocker energy) of 53.20 kcal/mol. From biocomputational study, it has also been revealed that in the host PEPCK, genistein binds adjoining to the substrate-binding site (Fig. 4a) by interacting with R89 through a hydrogen bond and with S288 and R407 through hydrophobic interactions. The role of R89 for optimal PEPCK activity in rat PEPCK-C has been explained by Johnson et al. (2016).

In order to examine the differential binding of genistein to PEPCKs from the cestode parasite and its host, surface models were prepared using Discovery Studio 4.1 software (Fig. 4b, c) as described by Dutta et al. (2016). From the biocomputational studies, it has been observed that genistein overlaps with substrate binding site of the host PEPCK, indicating a competitive inhibitor for its substrate (phosphoenolpyruvate). However, in the case of parasite PEPCK, genistein overlaps with nucleotide-binding site of the enzyme, indicating a non-competitive inhibitor for its substrate, which holds true as in the enzyme kinetics studies by Ramnath et al. (2017). This differential binding

affinity of genistein to the cestode parasite and its host PEPCKs need to be authenticated using X-ray crystallographic studies of the complexes.

Biological relevance of genistein in cestodes

To understand the biological significance of the lead modulator, genistein, predicted from biocomputational studies, the cestode parasite, *R. echinobothrida*, was exposed to various concentrations of genistein. It was observed that onset of paralysis in the genistein treated cestode parasite occurred within 8 h of incubation, depending upon the concentrations of genistein; whereas, the control parasite survived up to ~ 96 h (Ramnath et al. 2017). PEPCK activity in the genistein-treated cestode parasite was also decreased correspondingly to the dosage of genistein, signifying PEPCK plays an important role for the parasite's survival (Ramnath et al. 2017).

Conclusion

From these studies, it appears that genistein primarily act transtegumentally as a vermifugal with several metabolic functions, e.g. amino acid metabolism, carbohydrate metabolism, in the helminth parasites being the plausible secondary targets in helminth parasites. The anthelmintic

activity of the phytochemical from *F. vestita* thus provides a lead towards the development of a newer vermifuge, if not a vermicide. This review may form a basic platform for in vivo investigations of genistein to establish the natural phytoestrogen as an anthelmintic.

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Author's contribution VT and BD prepared the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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