Annex A - Mechanism of Action and Toxicity Studies

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Mechanism of Action

In vitro and animal studies

13. The mechanism by which raspberry leaf may exert its alleged therapeutic effects during pregnancy is poorly understood (Bowman *et al*., 2021). Hastings-Tolsma *et al*. (2022) hypothesised that raspberry leaf's role in promoting parturition may be related to: its inflammatory, vasodilatory and antioxidant effects; its ability to promote apoptosis in cervical and myometrial cells; and the effects of the isoflavone genistein. However, limited and at times contradictory evidence was given to support these claims.

14. Six studies were identified which had investigated the effects of raspberry leaf extract(s) on uterine and other types of smooth muscle *in vitro* and or in animals (Burn and Withell, 1941; Beckett *et al*., 1954; Bamford *et al*., 1970; Rojas-Vera, Patel and Dacke, 2002; Zheng *et al*., 2010; Olson and DeGolier, 2016). However, results from these studies were highly variable, with some reporting a stimulatory effect and others reporting a relaxant effect. These differences have been attributed to differences in raspberry leaf preparations, dosages, extraction methods, animal tissue, the pregnancy status of the uterus/uterine tissue, baseline muscle tone, and whether or not the raspberry leaf was tested *in vitro* or *in vivo* (Bowman *et al*., 2021).

Human studies

15. A case series by Whitehouse (1941) reported the effects of 1.30-2.59 g crude raspberry leaf extract or 20 oz. 5 % raspberry leaf tea, administered via a uterine bag, on the uterine muscle of three post-partum women. Based on the findings, it was concluded that: “the main effect [of raspberry leaf was] relaxation of the uterine muscle.”

Toxicity studies

In vitro and animal studies

Acute toxicity

16. No adverse effects were observed in mice when aqueous raspberry leaf extract containing the equivalent of 2 g extract, was orally administered to mice (Burn and Withell, 1941). However, death was observed in mice following intravenous administration of 4 g/mL aqueous raspberry leaf extract (Burn and Withell, 1941) and in chicks following intraperitoneal administration of a raspberry leaf extract containing the equivalent of 0.1 g leaf (with cyanosis and heart dilation occurring in mice given an equivalent intraperitoneal dose of the same extract) (Beckett *et al*., 1954).

Subacute toxicity

17. Only one study was identified which had assessed the sub-acute toxicity of raspberry leaf (Yang *et al*., 2019). During the study, different raspberry leaf preparations were administered to eight-week old, ICR male mice by oral gavage over two weeks at a dose of 100 mg/kg/bw/day (containing 15-55 % gallic

acid-equivalent polyphenols). The preparations included an ethanolic raspberry leaf extract (RLE); an ethanolic extract subjected to high temperature and high pressure treatment (RLE-H); and a raspberry leaf powder (RLP).

18. The study assessed body weight, adiposity, relative organ weights (heart, lung, liver, spleen, kidney, testis, fat pad) and time to exhaustion in a swimming test. None of the mice given RLP, RLE or RLE-H died or exhibited visible signs of disease over the two-week study period (Yang *et al*., 2019). Final body weight, adiposity index and body mass index in the RLE and RLE-H groups were statistically significantly decreased compared to the control and RLP groups. Relative weights of testes were statistically significantly increased in all treated groups compared to the control group. Exhaustion swimming times in the RLE and RLE-H group were statistically significantly increased compared to the control and RLP groups. Adverse intestinal flatulence was observed in the RLE and RLE-H groups, which the authors suggested may have been due to the high intakes of ellagic acid in the groups receiving these extracts. In a repeated study, RLE with pectin and sodium alginate with boiling water was prepared into a gelled food for the mice. Similar effects were observed as for RLE in the initial study, except that body weight was not decreased and intestinal flatulence was not observed.

Cytotoxicity

19. An aqueous raspberry leaf extract and “five-seeds” formulation were both found to exhibit cytotoxicity towards HEK 293 and Chang liver (HeLa derivative) cells in vitro, at concentrations of 1-100 mg/mL (Teo et al., 2021). The “five-seeds” formulation contained aqueous extracts from raspberry leaf, **Lycium barbarum, Cuscuta chinensis Lam, Schisandra chinensis and Plantago asiatica** in a 1:1:1:1:1 ratio. The formulation exhibited higher IC50 values than the raspberry leaf extract alone when tested on HEK 293 cells (33 mg/mL vs. 21.2 mg/mL) and Chang liver cells (38.5 mg/mL vs. 20.1 mg/mL). The proportion of SubG1 (apoptotic) Chang liver cells was also found to be considerably lower following treatment with a 25 mg/mL formulation compared with when they were treated with an equivalent concentration of raspberry leaf extract (10.17% vs. 30.37%). Therefore, the authors concluded that the “five seeds” formulation appeared to have modulated the toxicity of the individual herbs used to make it.

Genotoxicity

20. No studies which had investigated the genotoxicity or carcinogenicity of raspberry leaf were found.

Reproductive and developmental toxicity

21. Limited numbers of studies were available which had investigated the reproductive effects of raspberry leaf during pregnancy. Of those identified, three involved pregnant mice or rats given raspberry leaf extracts orally, from the point breeding was confirmed until parturition (Johnson et al., 2009; Makaji et al., 2011; Hastings-Tolsma et al., 2022). A range of effects was reported in these studies.

22. In the first study, pregnant nulliparous Wistar rats were administered 10 mg/kg bw/day commercially available raspberry leaf extract in gelatine capsules or an equivalent dose of the raspberry leaf components kaempferol or quercetin (Johnson et al., 2009). Raspberry leaf exposure during pregnancy was associated with a significant increase in the length of gestation, a significant reduction in time to vaginal opening in the F1 offspring, together with significant growth restriction of F2 offspring, compared with untreated controls. There was also a non-statistically significant reduction in pregnancy success rate. Compared with the control group, dams given quercetin had significantly increased weight gain during pregnancy. Johnson et al. (2009) concluded that: “in Wistar rats, exposure to raspberry leaf extract throughout pregnancy [increased] gestation length and [resulted] in altered reproductive development and function in the offspring…which [raised] concerns about the safety of this herbal preparation for use during pregnancy.”

23. In the second study, pregnant nulliparous Wistar rats were given 10 mg/kg bw/day of another commercially available extract (containing the equivalent of 0.2-0.4% quercetin and kaempferol and 2-7% ellagic acid) or an equivalent dose of the raspberry leaf components kaempferol, ellagic acid or quercetin (Makaji et al., 2011). Hepatic microsomes were prepared from offspring on postnatal days (PND) 1, 21, 65 and 120 and used to test the biotransformation rates of eight substrates. Maternal consumption of raspberry leaf tea resulted in increased biotransformation rates for three of the substrates by female offspring at PND120. Similar results were also observed for quercetin and kaempferol. These were considered to be more male profiles, since biotransformation rates were higher in control male than control female offspring. The authors concluded that maternal consumption of either raspberry leaf or some of its components lead to long term alterations in the CYP activity of female offspring. If applicable to humans, they suggested that the long term effects associated with consuming raspberry leaf or its constituents during pregnancy may be inappropriately rapid biotransformation of pharmaceuticals, leading to treatment failures; increased activation of xenobiotics, leading to increased tumour formation; and altered

steroid hormone biotransformation, leading to adverse reproductive health/fertility.

24. The third study gave C57BL/6N Tac mice **ad libitum** access to water bottles throughout pregnancy, containing 1.78 or 2.66 mg/mL raspberry leaf extract (Hastings-Tolsma *et al*., 2022). Compared with untreated controls, both raspberry leaf groups exhibited significant reductions in litter size (viable and non-viable). The high-dose group also exhibited increased fluid consumption and significant reductions in pup weight gain at postnatal days four and five. According to the authors, the changes in the high-dose group were accompanied by a trend towards reduced gestation length, although this was not statistically significant. There were no statistically significant differences in measures of neurodevelopment, assessed through pup locomotive abilities including time to righting, orienting response, cliff avoidance and swimming development. However, the authors suggested there were trends towards effects with maternal raspberry leaf consumption, which were more marked in the high dose group. The authors concluded that when ingested throughout gestation in mice, raspberry leaf may impact length of gestation, fluid intake during pregnancy, litter size and viability, as well as pup development.

25. A fourth reproductive study was also identified, involving immature Sprague-Dawley rats (Graham and Noble, 1955). The authors reported that when the rats were subcutaneously injected with 0.4 mL fresh or dried raspberry leaf extracts and 100 I.U. µg-1 pregnant rat serum they exhibited marked reductions in ovarian weight within three days compared with untreated controls (given pregnant rat serum only). The extracts were mixed with the serum in vitro and contained the equivalent of 0.8-18 mg raspberry leaf. Based on the findings, the authors concluded that raspberry leaf “possessed an appreciable amount of [in vitro anti-gonadotrophic] activity.”