Toxicity studies - Raspberry leaf tea

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Acute toxicity

16. No adverse effects were observed in mice when aqueous raspberry leaf preparation containing the equivalent of 2 g extract, was orally administered to mice (Burn and Withell, 1941). However, following intraperitoneal administration of extract equivalent to 0.1 g of raspberry leaf to mice, severe cardiotoxicity was observed (extreme cyanosis and widely dilated hearts) (Beckett et al., 1954). When the extract was administered intravenously to mice, deaths occurred, with an LD50 of 0.4 g raspberry leaf equivalents per mouse (Burn and Withell, 1941). Intravenous administration of extract equivalent to 0.1 g of raspberry leaf to chicks resulted in convulsions and death (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). In this 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 toxicity 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 (gaseous distention) 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 these groups. 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. Teo *et al*. (2021) investigated the toxicity of a traditional Chinese herbal medicine, “Five-seeds”, *in vitro*. The cytotoxicity of extracts of the constituent herbs, *Rubus idaeus* (raspberry leaf), *Lycium barbarum*, *Cuscuta chinensis* Lam, *Schisandra chinensis* and *Plantago asiatica* individually and in 1:1:1:1:1 ratio, as occurs in “Five-seeds”, was determined in human embryonic kidney (HEK-293) and Chang liver (now known to be HeLa derived) cells. All extracts, and the mixture showed some cytotoxicity in both cell lines, with IC50s ranging from 18 to >100 mg/mL of extract. There was evidence that cytotoxicity was, at least in part, due to an increase in apoptosis (proportion of cells in subG1 phase).

Genotoxicity

20. No studies which had investigated the genotoxicity or carcinogenicity of raspberry leaf were identified in the scientific literature.

21. A European Public Assessment Report for one raspberry leaf product registered as a traditional herbal medicine ("Lydiva" capsules) reported that an Ames (bacterial reverse mutation) test and an *in vivo* micronucleus assay in mice had been conducted on the aqueous raspberry leaf extract contained in this product (BASG, 2014). A positive result was reported in the Ames test in *Salmonella typhimurium* strain TA98 in the presence of metabolic activation. It was suggested that this result may be due to flavonoids (e.g. quercetin) in the extract, which are generally considered to be non-genotoxic *in vivo*. The extract was reported to be negative in the *in vivo* micronucleus test in mice.

Reproductive and developmental toxicity

22. Limited numbers of studies were available which had investigated the reproductive or developmental effects of raspberry leaf during pregnancy. Of those identified, three involved pregnant mice or rats given raspberry leaf extracts orally, from the point mating 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.

23. In the first study, female Wistar rats were administered 10 mg/kg bw/day commercially available raspberry leaf extract from Canada or an equivalent dose of the raspberry leaf components kaempferol or quercetin (Johnson *et al*., 2009) throughout pregnancy until parturition. Pregnancy outcomes were assessed in the parental (P) generation, and reproductive development and fertility were then assessed in the female F1 offspring. Raspberry leaf exposure during pregnancy in the P generation was associated with a significant increase in the length of gestation by 1.6 days. There was also a non-statistically significant reduction in pregnancy success rate (78% compared to 100% in the control group). A significant reduction in time to vaginal opening was observed in the F1 offspring. In the F2 offspring, although there was no significant difference in birthweight, the proportion of pups considered to be growth restricted (having a birthweight more than 2 standard deviations below the mean birthweight of the control group) was significantly increased, at 9.8% compared to 0%. 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 increases gestation length and results in altered reproductive

development and function in the offspring…[which] raise concerns about the safety of this herbal preparation for use during pregnancy."

24. In the second study, from the same laboratory as the first, female Wistar rats were given 10 mg/kg bw/day of raspberry leaf extract from the same commercial source as used by Johnson et al. (2009) or an equivalent dose of the raspberry leaf components kaempferol, ellagic acid or quercetin from the day of mating to the day of parturition (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 representing the most common hepatic P450 enzymes (CYPs). Maternal consumption of raspberry leaf tea extract resulted in slightly 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 for these three enzymes 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.

25. In the third study, C57BL/6N Tac mice received *ad libitum* access to water bottles containing 1.78 or 2.66 mg/mL raspberry leaf extract, from the day of mating throughout pregnancy (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 locomotor activity, 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.

26. A fourth reproductive study was identified, involving immature Sprague-Dawley rats, in which possible mechanisms for the reported contraceptive effect of raspberry leaf were investigated (Graham and Noble, 1955). Extracts of fresh or dried raspberry leaf (equivalent to 0.8 - 18 mg

raspberry leaf) were mixed *in vitro* with pregnant mares' serum diluted to a concentration of 100 I.U. per 0.1 mL gonadotrophin and the mixtures incubated for two hours at 37^{O}C. The authors reported that when rats were injected subcutaneously with 0.5 mL of the extracts, containing the equivalent of 100 I.U. of mare's gonadotrophin, they exhibited marked reductions in ovarian weight within three days compared with the controls (given pregnant mare's serum only). Based on the findings, the authors concluded that raspberry leaf "possessed an appreciable amount of [*in vitro* anti-gonadotrophic] activity." It should be noted that GnRH antagonists have been developed clinically due to their potential benefit in assisted reproduction. GnRH antagonism can also have other varied biological effects, dependant on exposure and stage of pregnancy. There is no significant evidence of major congenital malformations in fetuses caused by GnRH antagonists or GnRH agonists.

Drug-herb interactions

27. A study by Langhammer and Nielsen (2014) found that ethanolic raspberry leaf extracts prepared from commercially available capsules were capable of inhibiting recombinant human CYP enzymes *in vitro*. These included CYP1A2, CYP2D6 and CYP3A4, with IC50 constants ranging from 44-81 µg/mL. The authors concluded that clinically relevant systemic CYP inhibition could be possible for raspberry leaf, and that it might cause clinically relevant inhibition of intestinal CYP3A4. If raspberry leaf inhibits these CYPs *in vivo* it may affect drugs that are metabolised by these enzymes.

28. Holst *et al*. (2011) administered a self-completed survey of herbal remedy use during pregnancy, which looked at parallel use of other pharmaceuticals. The survey was given to 1,037 expectant mothers who were at least 20 weeks pregnant and who presented at an antenatal clinic at Norfolk and Norwich University Hospital. Of the 578 respondents (response rate 55.7%), 232 (40.1%) reported using both herbal remedies and pharmaceuticals during pregnancy. It is unclear what the other pharmaceuticals were or whether the women experienced any adverse effects. However, the authors commented that four women reported simultaneous use of iron and a tannin-containing herb (raspberry leaf, chamomile or valerian). They noted that tannin-containing supplements may interfere with iron absorption, which should be taken into account in anaemic patients. However, no studies were identified in the present review which had evaluated the effects of raspberry leaf tea on iron absorption.

Human studies

29. Several human studies on the safety of raspberry leaf use during pregnancy were identified (Parsons *et al.*, 1999; Simpson *et al.*, 2001; Nordeng *et al.*, 2011). This included several case reports of adverse effects experienced by pregnant women or their newborns after taking raspberry leaf, ranging in severity from petechiae to acute liver injury (UKTIS data; MacPherson and Kilminster, 2006; Wedig and Whitsett, 2008; EMA, 2014; Cheang *et al.*, 2016; Koenig, Callipari and Smereck, 2021). However, limited information was available in these case reports about the doses taken, and it was uncertain whether the adverse effects described were related to raspberry leaf consumption or to other factors, such as the use of other herbal products described in several of them (MacPherson and Kilminster, 2006; Wedig and Whitsett, 2008; Koenig *et al.*, 2021). In some cases, the authors attributed the adverse effects to other products consumed, such as evening primrose oil (Wedig and Whitsett, 2008) or blue cohosh (MacPherson and Kilminster, 2006).

30. Two publications noted that there had been some suggestion in the lay press that raspberry leaf might promote human miscarriage or abortion (Simpson *et al.*, 2001; Johnson *et al.*, 2009). However, the authors of both papers concluded that the evidence to support this was very limited.

31. One of the main human safety studies that the COT considered in its evaluation was a retrospective cohort study by Parsons *et al.* (1999). The study was conducted at hospitals in Sydney, Australia. It included a convenience sample of 57 postnatal women who reported using raspberry leaf during pregnancy and a control group of 51 women randomly selected from the hospital database who stated that they had not used raspberry leaf during pregnancy. The groups were otherwise considered comparable. The women in the study who consumed raspberry leaf reported having done so either as tea (56.1%), tablets (40.4%) or a combination of all three - tea, tablets and tinctures (3.5%) - from as early as eight weeks' gestation for 1-32 weeks. Doses ranged from 1-8 cups of tea or tablets daily, but the tincture appears to have been a single dose, possible taken by one woman (this information is unclear in the paper). No further information was provided about the doses taken or how they were prepared.

32. Parsons *et al.* (1999) identified no adverse effects associated with raspberry leaf consumption, based on information from the hospital obstetric database and participants' medical records. There was no significant difference in maternal blood loss, babies' Apgar scores at five minutes of age, pre-labour

maternal diastolic blood pressure or transfer to a special/intensive care baby unit. Nor was there any significant difference in the length of gestation, likelihood of labour augmentation, incidence of meconium liquor, need for an epidural or length of each of the three stages of labour. Raspberry leaf users had a shorter mean duration of the first stage of labour compared with the control group, though this was not statistically significant. There was also a trend towards raspberry leaf users being less likely to require an artificial membrane rupture, caesarean section, forceps or vacuum birth. The authors concluded that: “the findings suggest that the raspberry leaf herb can be consumed by women during their pregnancy…to shorten labour with no identified side effects for the women or their babies” (Parsons *et al.*, 1999).

33. The other main human safety study that the COT considered was a double-blind, randomised, placebo-controlled trial carried out by Simpson *et al*. (2001). The study, which was carried out by the same group, in Sydney, Australia, aimed to evaluate the safety and efficacy of raspberry leaf tablets in shortening and easing labour when consumed from 32 weeks’ gestation. The sample consisted of 192, low-risk nulliparous women (mostly Caucasian) with a healthy pregnancy, who were randomised to receive either a placebo or raspberry leaf tablets containing 2.4 g extract daily with food in two separate, 1.2 g doses (n=96 women per group).

34. There were no adverse effects that could be directly attributed to the raspberry leaf, except possibly constipation, which was exclusively observed in four of the raspberry leaf participants (Simpson *et al.*, 2001). Similarly, there were no significant differences between the raspberry leaf and placebo groups with respect to other safety outcomes, including maternal blood loss; maternal diastolic blood pressure; newborn Apgar score at five minutes; presence of meconium-stained fluid; or newborn birth weight (Simpson *et al.*, 2001). The babies in the placebo group tended to have a higher average Apgar score at five minutes, with a narrower spread of measures, but this difference was not statistically significant. A slightly higher proportion of babies from the raspberry leaf group was admitted to the Neonatal Intensive Care Unit or Special Care Nursery within 24 hours of birth (5.2% compared to 3.7%). No statistical evaluation of this difference was presented; however, chi-square analysis shows that there was no statistical difference between the two groups (P=0.4). The authors noted that the frequency of admissions in the raspberry leaf group was still below the average admission rate for term babies born within the participating hospital at the time of the study.

35. There were no statistically significant differences reported with respect to any of the efficacy outcomes, such as emergency caesarean rate (Simpson *et al.*, 2001). However, based on the findings, it was concluded that a raspberry leaf dose of 2.4 g/day appeared to be safe for mother and baby.

36. The COT also considered data collected by the UK Teratology Information Service (UKTIS) to be of importance in its evaluation. Since its inception in 1983, UKTIS had received six reports of accidental or “therapeutic” raspberry leaf exposure (tea or tablets) during pregnancy. Limited information was available about the dose or timing of exposure but pregnancy outcomes for the six women were normal, except for one, who gave birth to a child with cerebral palsy following a delayed delivery. This would have been incidental, and not due to the consumption of raspberry leaf. One of the women had accidentally consumed large quantities of 400 mg raspberry leaf tablets after mistaking them for sweets (no further information is available on the dose taken), and experienced nausea and diarrhoea but no pregnancy-related effects. She gave birth to a normal, liveborn infant at 40 weeks.