Toxicology of ginger extracts

In this guide

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Cytotoxicity

51. The cytotoxicity of ginger extracts has been investigated with varied results. Plengsuriyakarn *et al*. (2012) examined cytotoxicity of ethanolic ginger extracts in a cholangiocarcinoma (CCA) cell line 6 (CL-6) model, compared to hepatocarcinoma (HepG2) and normal human renal epithelium (HRE) models, using calcein-AM release and Hoechst 33342 assays to assess cell viability and apoptotic activity. The median inhibitory concentration, (IC50) values for cytotoxicity of the crude ethanolic extract of ginger ranged from 11 – 245 µg/ml across the 3 cell lines and the two assays.

52. Zaeoung *et al.* (2005) reported that the IC50 of aqueous and methanolic extracts of ginger was greater than 39.2 µg/ml against breast (MCF7) and colon (LS174T) cell lines.

53. Abudayyak *et al*. (2015) found the aqueous and methanolic extracts of ginger exhibited no cytotoxic activity when assessed using an MTT test (a colourimetric assay for assessing cell metabolic assay) in the rat kidney, NRK-52E cell line. The chloroform extract resulted in an IC50 value of 9.1 mg/mL.

54. However, it was noted that the inhibitory concentration (IC50) values presented in the studies reviewed were based on a small amount of data, from only a few different cell lines and therefore firm conclusions could not be drawn. Also, the purpose of most of these studies was an attempt to identify possible anti- cancer agents, rather than as an assessment on the safety of ginger as a supplement and therefore relevant endpoints would not have been assessed.

Mutagenicity

55. Nakamura and Yamamoto (1982) found that the juice of ginger rhizome possessed both mutagenic and anti-mutagenic properties, and that 6-gingerol in particular was a powerful mutagen. The group also demonstrated that 6-shogaol was much less mutagenic (strain Hs30 of Escherichia coli) than 6-gingerol (Nakamura & Yamamoto 1983). In a *Salmonella typhimurium* reverse mutation (Ames) assay, the urine of rats fed diets containing 0.5, 1 and 5% powdered ginger for 1 month and exposed to benzo(a)pyrene was found to display a significant reduction in mutagenicity as indicated by a reduced number of TA98 and TA100 revertants at all ginger concentrations (Nirmala *et al*. 2007) when tested in an Ames assay.

56. In another Ames assay, an ethanolic extract of ginger (Soudamini *et al.* 1995) and an essential oil from ginger (Sivaswami *et al*. 1991) demonstrated mutagenic activity in *S. typhimurium* strains TA100 and TA1535 at concentrations of 25-50 mg/plate and 5-10 mg/plate, respectively. Similarly, an ethanolic ginger extract at concentrations between 10 and 200 µg/plate, and gingerol and shogaol were mutagenic in strains TA100 and TA1538 with metabolic activation by rat liver S9 mix, while zingerone did not display mutagenic effects (Nagabhushan *et al*. 1987).

57. Abudayyak *et al*. (2015) found an aqueous ginger extract exhibited mutagenic activity when assessed using the Ames assay on *S. typhimurium* TA98 (in the presence of S9 mix) strain over a concentration range of 0.78–25 ug/mL however, no activity was exhibited on TA100 strain. No activity was observed with the chloroform and methanolic extracts.

58. Based on the available data, ginger showed some mutagenicity in TA100, TA1535, and TA98 strains, but this is low compared with established mutagens. An aqueous extract of ginger was not shown to be mutagenic *in vivo* (Nirmala, Prasanna Krishna and Polasa, 2007).

Acute toxicity

59. An acute toxicity study (Malik and Sharma, 2011) in male Wistar rats showed no signs of toxicity or mortality. The animals were administered doses of 250, 500 and 1000 mg/kg lyophilised ginger powder by gastric gavage. The authors stated that the three dose levels used in the study corresponded to 5, 10 and 20% of the NOAEL of the powder (5000 mg/kg).

Short term repeat dose studies

60. Rong et al. (2009) evaluated the safety of powdered Japanese ginger (mainly containing 6-gingerol galanolactone and 6-shogaol) by conducting a 35-day toxicity study in rats. Both male and female rats were treated with 500, 1000 and 2000 mg/kg bw/day by gavage. The results demonstrated that oral administration of up to 2000 mg/kg to male and female rats did not result in any increase in mortality, or changes to behaviour, growth, the general condition of the animals (including: changes in skin, fur, eyes, and mucous membranes, occurrence of secretions, excretions and autonomic activity), food and water consumption. At the highest dose tested (2000 mg/kg), ginger led to slightly reduced absolute and relative weights of testes (by 14.4% and 11.5%, respectively). No effects were apparent in the females.

61. The effect of oral and intraperitoneal administration of aqueous extracts of ginger root over 28 days in female rats at two dose levels (50 mg/kg and 500 mg/kg) was examined for haematological, serum and systemic toxicity (Alnaqeeb et al. 2003). Neither oral nor intraperitoneal administration resulted in mortality. Orally administered aqueous ginger extract resulted in increased levels of serum aspartate aminotransferase (AST) and decreased levels of alanine aminotransferase (ALT).

62. Jeena et al. (2011) conducted a sub chronic toxicity study of the essential oil of ginger in Wistar rats following oral administration at co ncentrations of 100, 250, and 500 mg/kg per day once daily for 13 consecutive weeks to assess the oral safety of ginger oil. No mortality was observed. No unusual changes in behaviour or locomotor activity were observed during the

period of the study, nor were any abnormal changes observed in the relative organ weights of liver, kidney, spleen, lungs, brain, and stomach with respect to body weight in ginger oil-treated animals when compared to vehicle control animals.

63. An increase in serum sodium levels was observed in male rats treated with 500 mg/kg per day but in the absence of changes in sodium levels in females, this change was not considered significant. A slight increase in total bilirubin was observed in female rats treated with ginger oil along with a decrease in AST and ALT levels however, there were no significant changes in hepatic function parameters such as alkaline phosphatase, total protein, albumin, and globulin content.

Reproductive and developmental toxicity

In vitro studies

64. Mohammed *et al* investigated the effects of herbal extracts, including ginger and 6-gingerol, on chick embryonic heart micromass and mouse D3 embryonic stem cell systems (ESD3) (2016). The team observed that a different study had concluded that the use of 6-gingerol l remedies in the first trimester of pregnancy may affect foetal development (Park, 2012). However, 6-gingerol-treated primary embryonic chick cardiomyocytes showed no significant changes in contractile and cellular activity or changes in total protein content in comparison to the control. At concentrations of 0.75–6 µM, 6-gingerol treated primary embryonic chick cardiomyocytes exhibited no significant changes in contractile activity, cellular activity or changes in total protein content in comparison to the control. At concentrations of 12.5–50 µM, inhibition in contractile activity was observed at 48h. All high 6-gingerol concentrations, 12.5–100 µM, tested in micromass, significantly altered both the cellular activity and protein content in a dose-dependent manner.

65. The same concentrations of 6-gingerol were used to treat the ESD3, which showed a significant decrease in cardiomyocyte differentiation for all tested concentrations above 0.75 µM. The cellular activity and protein content of stem cell-derived cardiomyocytes also exhibited a significant decrease with increased 6-gingerol concentration exposure.

Animal studies

66. To date, the number of studies on the safety of the use of ginger supplements during pregnancy is limited. The ginger component 6-gingerol, was highlighted to affect some essential embryonic developmental processes, such as the disruption of angiogenesis. Kim *et al*, demonstrated the ability of 6-gingerol to inhibit proliferation and tube formation of primary cultured human endothelial cells in rat aorta by down regulation of cyclidin D and the ability to inhibit tumour growth in mice through its anti-angiogenic activity (Kim et al., 2005).

67. The teratogenicity of EV.EXT 33, a patented Zingiber officinale extract (comprising 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, and 8-shogaol, which made up 1.9 w/w of the extract) was investigated in Wistar rats, (Weidner & Sigwart, 2001). The extracts were administered orally by gastric intubation at concentrations of 100, 333 and 1000 mg/kg, to three groups of pregnant rats from days 6 to 15 of gestation. Their bodyweight, food and water were monitored during the treatment period. The study concluded that treatment with EV.EXT 33 during the period of organogenesis resulted in neither maternal nor developmental toxicity at daily doses of up to 1000 mg/kg bw.

68. Shalaby and Hamowieh, (2010) investigated fertility, serum testosterone and acute toxicity of ginger in rats. One hundred and twenty male Sprague Dawley rats, separated into groups of 10, were orally administered either water or methanolic extracts (prepared using 100 g dry ginger roots soaked in 500 ml water or 500 ml methyl alcohol 90%) in graded doses ranging from 5 to 17.5 g/kg bw (gavage doses were not specified). Following dosing, the number of dead mice in each group after 48 hours of observation were recorded. The oral lethal doses (LD50) of the methanolic and water extracts were calculated to be 10.3 and 11.8 g/kg bw respectively. No signs of toxicity were observed at does up to 5 g/kg bw. Both extracts increased fertility index, sexual organ weight, and sperm motility and count after 65 consecutive days (see below).

69. To investigate the effect of ginger extracts on serum testosterone levels, male rats had their fertility reduced by inducing diabetes, a condition shown to reduce male fertility. The aim was to see whether ginger, with its antioxidant and androgenic effects, would restore fertility. Rats rendered diabetic by subcutaneous injection of 120 mg/kg bw alloxan for 3 days, were administered methanolic extracts of ginger for 65 days at doses of 100 and 200 mg/kg bw/d. Testosterone levels increased to 4.08 ± 0.10 and 7.13 ± 0.14 ng/dL (both significant at *P* 0.001) compared to the diabetic control group which had levels of 3.30 ± 0.03 ng/dL. Serum testosterone levels also increased in rats given water extracts (150 and 300 mg/kg bw) to 4.06 ± 0.03 and 5.04 ± 0.08 ng/dL (both

significant at *P* 0.001 when compared to the diabetic control group) respectively.

70. The study also investigated fertility based on the fertility index (for each male this was calculated as the percentage of the number of females that become pregnant in relation to the number of mated females) and spermatogenesis. Rats were orally administered methanolic extracts at doses of 100 and 200 mg/kg bw for 65 consecutive days and water extracts at doses of 150 and 300 mg/kg bw and compared to a diabetic control group.

71. Histopathological examination of the testes of diabetic rats showed mild to moderate degenerative changes of spermatogenic cells, diffuse oedema and incomplete arrest of spermatogenesis. The testes of rats orally administered 300 mg/kg bw of water extract of ginger root showed mild degeneration of spermatogenic cells and slight oedema of interstitial cells. The testes of rats receiving orally 200 mg/kg bw of methanolic extract of ginger root showed nearly normal seminiferous tubules, showing fewer signs of degradation, suggesting a LOAEL of 200 mg/kg bw/day for the methanolic extract. The study concluded that the results suggest the intake of ginger root extract as a drink may be useful for diabetic patients suffering from sexual impotency.

72. The above study has been included for completeness and as any general mechanisms may be more widely relevant: This is consistent with the findings of Hosseini et al (2015)

73. Hosseini et al. (2015, abstract only) investigated the effect of ethanolic ginger extract on serum testosterone, LH and FSH as well an effect on spermatogenic cell lines in male mature offspring rats. In this study, 72 female rats, sorted into 9 groups were orally administered an alcoholic extract of ginger at doses of 50, 100 and 200 mg/kg bw, during their neonatal and perinatal periods and saline was used as a control. Following puberty, LH, FSH, numbers of Sertoli cells, spermatogonia, spermatocytes and spermatids were counted in 8 male rat offspring from each group. Ginger was found to significantly increase testosterone levels and the number of spermatogenic cells and at doses of 100 and 200 mg/kg bw, alcoholic extract of ginger significantly reduced the FSH and LH levels compared to control groups. The authors concluded that “the oral consumption of Ginger during pregnancy and lactation dose-dependently increase the level of testosterone and the number of spermatogenic cells.”

74. Dissabandara & Chandrasekara (2007) also examined the effect of powdered ginger extract administered prenatally on the postnatal development of rats. A period of administration of the dry powdered extract orally at doses of 500

mg/kg/day or 1000 mg/kg/day (control not specified) during days 5 to 15 of gestation resulted in a lower intake of food and water and lower weight gain in dams in the ginger treated group, with some embryonic loss. Growth and physical maturation of the offspring were unaffected. It was concluded that maternal administration of ginger during mid pregnancy resulted in reduced maternal weight gain and increased embryonic loss without affecting the surviving offspring.

75. ElMazoudy and Attia (2018) investigated the effect of powdered dried ginger root on the oestrus cycle and implantation in female mice. ICR mice, were orally dosed at 250, 500, 1000, or 2000 mg/kg bw/d aqueous ginger extract. These were investigated in four different experiments: (i) treatment for 90 days and throughout mating and gestation; (ii) 35-days of treatment evaluating the effects on the oestrous cycle; (iii) treatment for 20 days and throughout mating to evaluate pre-implantation loss (antifertility); and (iv) treatment for 20 days and throughout gestation to evaluate post-implantation loss (abortifacient). In the 90-day study, the dams were terminated on gestation day 20. In the mothers one mortality was recorded in the 1000 mg/kg bw/d group on gestation day 18 and two in the 2000 mg/kg bw/d group at gestational day 16. There was a significant reduction in body weight changes in these two dose groups compared to the control group; however, food consumption was comparable.

76. In the study investigating the oestrus cycle, a significant reduction in the numbers of oestrus cycles was observed at the highest dose, with the length of the oestrus cycle in this group being significantly prolonged (10.05 ± 0.8) days compared with (4.99 ± 0.5) days recurrent and successive oestrous cycles in control mice. At the highest dose level, the length of the oestrous cycle was prolonged with a significant decrease in the duration of diestrous-metestrus (luteal) phase and prolonged proestrus-estrus (ovulatory) phase. In the study investigating pre-implantation loss, a significant decrease in the number of corpora lutea was observed at the highest dose. Implantation failure was also increased by 36% compared to the control group and pre-implantation loss at this dose group was also 16.6% higher than the control group. The authors considered that this may reflect a dose-dependent antifertility (anti-implantation) effect.

77. Regarding fertility and developmental outcomes, the female copulation index was significantly reduced at 2000 and 1000 mg/kg bw/d, whereas the female pregnancy index was significantly decreased only at the highest dose. The number of implantation sites and live fetuses in the 2000 mg/kg bw/d group was lower than the other treated and control groups. An increase in fetal resorption

and post implantation loss was also seen in the highest dose group. There was no evidence of fetal malformations however growth retardation, reduced pup weight and delay in the crown-rump length was observed in this dose group as well. Finally, changes in ovarian histopathology were observed at 2000 mg/kg bw/d, following 90 days of treatment. Ovarian follicle atresia was observed. The atretic follicles contained cell debris and there was haemorrhage in the antral cavity.

78. Additionally, degenerated primordial follicles with pyknotic nuclei forming polycystic ovaries were noted. Deteriorated follicles were observed as a detaching of layers of granulosa cells from the basal membrane by dilation of zona pellucida and with evidence of apoptosis; non-visibility of the follicular nuclei was also evident in damaged ova. The authors considered the above observations as evidence that ginger possesses anti-ovulation properties. Overall, the authors concluded that ginger impairs the normal growth of the corpus luteum because of progesterone insufficiency during early pregnancy and that the results suggested that ginger can disrupt the oestrous cycle and blastocyst implantation without teratogenesis. They considered the lowest NOAEL to be 250 mg/kg bw.

79. When evaluating the effect of the aqueous extract of Ginger rhizomes on the sexual parameters of rats. Peneme et al. (2023) initially determined the acute toxicity of the aqueous extract of ginger rhizomes in accordance with OECD guideline no. 423. Rats were given 5000 mg/kg aqueous ginger extract by gavage. No change in the general condition or behaviour of the mice compared with the control batch was observed. No animal mortality was observed after 48 hours or 14 days of observation. This experiment was followed by administering aqueous ginger extract at doses of 300 and 600 mg/kg, 17β-oestradiol at a dose of 1 mg/kg or distilled water, orally to rats for 14 days. A non-significant increase and decrease in body weight was observed at doses of 300 and 600 mg/kg respectively. The authors state that rats treated with 17 β-oestradiol also showed a reduction in body weight, as with the ginger extract at 600 mg/kg, and they considered this to confirm an oestrogenic effect. The eosinophil indices for the 600 mg/kg group were similar to the 17β-oestradiol indicating disruption of the oestrus cycle. A significant increase in oestradiol levels was observed in the rats treated at 300 mg/kg, and a non-significant decrease at 600 mg/kg compared with the control batch. The rat batch treated with the reference molecule 17 β-oestradiol at 1 mg/kg also showed a drop in oestradiol levels.

80. The Committee considered the animal studies to be inconclusive.

81. ElMazoudy and Attia (2018) noted reductions in bodyweight and deaths in mice dosed up to 2000 mg/kg bw/day ginger extract and Alnaqeeb *et al.,*

(2003), observed increases in serum aspartate aminotransferase (AST) in female rats dosed up to 500 mg/kg ginger extract.

82. However, the Committee noted that the database was limited, and the extraction and concentration of ginger varied between the studies. On the basis of the available information, more data would be needed in order to allow for a robust investigation of the effects described above. Therefore, at present, the Committee were unable to determine a point of departure, to reach a conclusion.

Human studies - exposures in pregnancy

83. Willetts et al. examined the effect of ginger on pregnancy induced nausea (2003). 120 women less than 20 weeks pregnant, were given 125 mg ginger extract (EV.EXT35; equivalent to 1.5 g of dried ginger) or a placebo four times per day for 4 days. However, there is some lack of clarity in the description of this study as it is stated in the discussion "Women in the treatment arm of this trial took ginger for 8 days and those in the placebo arm took ginger for 4 days." It is not clear whether this refers to the trial described. Three spontaneous abortions were observed in the group receiving ginger, although one of these had not started taking ginger. One spontaneous abortion was observed in the placebo group.

84. In an observational study conducted by Laekman et al. (2021) 51 pregnant women could freely use ginger tablets with a maximum of 2 tablets of 50 mg EXT.GR10 a day in case of gastrointestinal discomfort during pregnancy. EXT.GR10 is a 10-times concentrated ethanolic extract of ginger root. No strict minimum number of tablets was set, and 44 out of 51 patients (86.3%) took the ginger tablets. The 44 patients took 544 tablets or a mean of 12.4 tablets per patient, with a minimum of 1 and a maximum of 55 tablets. Stillbirth, prematurity, hypertension, and gestational diabetes were reported. There were no serious complications at birth. Four cases of dysplasia of the hip and two minor malformations were recorded in the offspring. Outcomes were compared to the rate in a Flemish population delivering during the same period. Hypertension, low birth weight and premature delivery were 15.9%, 13.6% and 20.5 % respectively in the ginger cohort compared to the representative population where the rates were 5.4%, 5.6% and 5.8% respectively. The author states that there was no relationship between the events affecting the mother and child and the number of EXT.GR10 tablets taken.

Effect on P450 (CYP) Enzymes and Herb-Drug Interactions

85. CYPs are a family of enzymes responsible for the biotransformation of several drugs. Induction or inhibition of CYP enzymes is a major determinant of the occurrence of drug-drug interactions.

In silico

86. Qiu *et al.* (2015) estimated the molecular interactions between 12 main active components (6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, 8-shogaol, 10-shogaol, ar-curcumene, β-bisabolene, β-sesquiphelandrene, 6-gingerdione, (-)-zingiberene, and methyl-6-isogingerol) and human P450 (CYP) 1A2, 2C9, 2C19, 2D6, and 3A4 and attempted to predict the absorption, distribution, metabolism, excretion, and toxicity (ADMET) of the 12 ginger components using computational methods and literature searches. This study suggests that ginger components may regulate the activity and expression of various human CYPs, resulting in alterations in drug clearance and response with a high risk of inhibition of CYP2C9 and CYP3A4.

In vitro studies

87. Ginger extracts and the major components thereof - 6-gingerol (6G), 8-gingerol (8G), 10-gingerol (10G) and 6-shogaol (6S) - were investigated in *in vitro* models and shown to have an inhibitory effect on CYP enzymes CYP3A4, CYP2C9 (Kimura *et al.*, 2010), CYP2C19 (Kim *et al.*, 2012), and CYP1A2 and CYP2C8 with IC50 values as low 1μM, (e.g., 6-shogaol on CYP1A2; Mukkavilli *et al.*, 2014).

Animal studies

88. Several reports have been published on the pharmacological properties of ginger, with varying results. Studies have examined the herb-drug interaction in animal models, (Okonta *et al.*, 2008; Egashira *et al*., 2012) although some studies have questionable results.

89. A study into the effect of ginger on the pharmacokinetics of metronidazole was reported by Okonta *et al*., using rabbits (2008). In a two-phase study, five healthy local strain rabbits (3 females, two males) were each given 3 mg/kg oral metronidazole. Following a 2-week washout period, the rabbits were given 1 ml/kg of ginger extract orally daily for 3 days and immediately given 3 mg/kg metronidazole per oral on the third day. Ginger significantly increased the absorption and plasma half-life and significantly decreased the elimination rate constant and clearance of metronidazole.

90. Egashira *et al.*, reported the interaction between ginger juice and tacrolimus in rats (2012). Tacrolimus (0.6 mg/kg) was administered intraduodenally in male Sprague-Dawley rats 1 h following oral administration of 10 mL/kg 50% ginger juice or water. CYP3A enzymes metabolize tacrolimus in the intestine as well as in the liver and the author states that ginger has been reported to change the activity of CYP3A4. Pre-treatment with ginger juice was found to significantly increase tacrolimus blood concentrations compared to those in animals pre-treated with water or orange juice.

91. The possible herb-drug interaction of ginger crude extract (GCE) on glibenclamide and insulin was investigated by Al Omari *et al.*, along with its hypoglycaemic and antihyperglycemic effects in normoglycemic- and streptozotocin-induced (STZ) diabetic rats (2012). Ginger crude extract was administered to normoglycemic male rats as a single dose (1 day) and as a daily dose for 1 week. STZ diabetic rats were treated with the same GCE concentrations (25, 50 and 100 mg/kg bw) together with glibenclamide (5 mg/kg bw) or insulin (1.2 IU/kg bw).

92. Single administration of ginger crude extract resulted in a significant decrease in blood glucose level (BGL) in normoglycemic rats after 1 and 2 hours (50 mg/kg bw). In STZ- diabetic rats ginger crude extract (25 and 50 mg/kg bw) decreased non-fasting BGL (N-FBGL) significantly at 1.5, 2.5, 3.5 and 4.5 hours. Glibenclamide (5 mg/kg bw) in combination with ginger crude extract at doses 25 or 50 mg/kg bw resulted in a significant reduction in the N-FBGL by 26.3% and 25.1% respectively after 4.5 hours, compared to glibenclamide alone which exhibited a 7.9% reduction.

Human studies

93. Human data showed possible interactions with medicines, including antibiotics, immunosuppressants, and anticoagulant medications. Although, in some cases, multiple concomitant medications were being used therefore, the effects observed cannot necessarily be directly attributed to ginger supplementation (Rubin *et al*., 2019).

94. Conversely, whilst investigating the effects of ginger on the pharmacokinetics or pharmacodynamics of warfarin and the effect of ginger on clotting status, Jiang *et al.,* (2005), found that neither the pharmacokinetics nor pharmacodynamics of warfarin were affected in healthy males who were treated with a single 25 mg dose of warfarin, following 7 days of pretreatment with ginger tablets (3 tablets, 3 times per day, each capsule containing extract equivalent to

0.4 g of ginger rhizome powder). Furthermore, ginger had no effect on international normalized ratio (INR) or *ex vivo* platelet aggregation in response to arachidonic acid.

Anti-platelet aggregation activity

In vitro studies

95. Srivastava (1986) reported an effect of ginger extracts on *in vitro* platelet aggregation. Ginger extracts in water, n-hexane, chloroform, and ethyl acetate were shown to inhibit platelet aggregation using arachidonic acid (AA), epinephrine, adenosine diphosphate (ADP), and collagen as agonists.

Animal studies

96. A study by ElMazoudy and Attia (2018) linked follicular failure to haemorrhagic effects in a study investigating the effect of aqueous ginger extract on the oestrus cycle and implantation, in female mice. The authors concluded that ginger impairs the normal growth of the corpus luteum and that the results suggested that ginger can disrupt the oestrous cycle and blastocyst implantation without teratogenesis. They considered the lowest NOAEL to be 250 mg/kg bw. The COT noted that this might be worth further investigation. However, it was also noted that other factors could be contributing to the results observed and the study results were inconclusive.

97. The effect of an aqueous ginger extract on platelet thromboxane-B2 (TXB2) and prostaglandin-E2 (PGE2) production was studied by Thomson *et al*. (2002). Adult female Sprague-Dawley rats were administered an aqueous extract of raw ginger at either 50 mg/kg or 500 mg/kg daily, by either oral gavage or intraperitoneally (IP) for a period of 4 weeks. A dose of 50 mg/kg ginger administered orally, or IP did not result in any significant reduction in serum TXB2 levels when compared to saline-treated control groups but doses at 500 mg/kg significantly reduced TXB2 levels in serum.

98. A non-significant reduction in the level of TXB2 was observed when ginger was injected IP. However, levels were not significantly different from the TXB2 levels in control rats that had received saline. 50 mg/kg of ginger administered orally resulted in serum PGE2 levels being significantly reduced and 500 mg/kg was found to be more effective in reducing PGE2 synthesis. PGE2 levels were reported to be significantly lower than the saline control in rats given

500 mg/kg ginger extract both orally and IP.

Human studies

99. Rubin *et al.* (2019) reported the possible effect of ginger supplementation on the (INR) in a woman taking warfarin. The 70-year-old female, who had been taking clonazepam 1 mg, metoprolol succinate 25 mg, paroxetine 10 mg, phenytoin 30 mg, rosuvastatin 20 mg, warfarin 7.5 mg daily, and warfarin 10 mg once day per week, presented with an INR of 8, an increase from 2.7, one month after taking a "Ginger Rescue," a daily oral, chewable, 48 mg ginger supplement that had no other herbal or active ingredients. A week following cessation of the ginger supplement, the INR declined to 2.6.

100. Ginger, in powder form (5 g per day), was demonstrated to significantly (P0.001) decrease ADP- and epinephrine-induced platelet aggregation in healthy male subjects who were fed 100 g of butter daily for seven days (Verma *et al.*, 1993). Conversely, Bordia *et al.,* (1997) found that 4 g powdered ginger administered daily over the course of 1.5 and 3 months had no effect on ADP and epinephrine-induced platelet aggregation in individuals with coronary artery disease (CAD). However, a single 10g dose of powdered ginger, administered to CAD patients resulted in a significant decrease in induced platelet aggregation.

Effects on blood pressure

Animal studies

101. Ghayur and Gilani (2005) reported that a crude extract of ginger administered intravenously, induced a dose-dependent (0.3–3 mg/kg) decrease in arterial blood pressure of anesthetized Sprague-Dawley rats with an EC50 value of 0.9 ± 0.1 mg/kg (mean ± SEM). In guinea pig paired atria, the crude extract exhibited cardio-depressant activity on the rate and force of spontaneous contractions with EC50 values of 0.57 ± 0.03 and 0.88 ± 0.07 mg/ml (mean ± SEM) for force and rate of contraction, respectively. In rabbit thoracic aorta preparation, when tested on the resting baseline, the ginger extract was devoid of any effect up to the dose of 10 mg/mL. The extract was then tested on high-K+ (80 mM) and phenylephrine (1 µM)-induced contractions. The extract relaxed the phenylephrine-induced vascular contraction at a dose 10 times higher than that required against K+ (80 mM)-induced contraction with an EC50 of 0.92 ± 0.04 mg/ml, compared with an EC50 of 0.11 ± 0.01 mg/ml against K^+-induced contraction.

102. Ca^{2+} channel-blocking (CCB) activity was confirmed when the crude extract shifted the Ca^{2+} dose–response curves to the right, the shift being similar to that obtained with verapamil. It also inhibited the phenylephrine (1 mM) control peaks in normal-Ca^{2+} and Ca^{2+}-free solution, indicating that it acts at both the membrane-bound and the intracellular Ca^{2+} channels. When tested in endothelium-intact rat aorta, it again relaxed the K+-induced contraction (EC50 value of 0.091 ± 0.002 mg/ml) at a dose 14 times less than that required for relaxing the PE-induced contraction (EC50 value of 1.26 ± 0.08 mg/ml). The vasodilator effect of the crude extract was endothelium-independent because it was not blocked by Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) (0.1 mM) or atropine (1 mM) and also was reproduced in endothelium-denuded preparations at the same dose range. These data indicate that the blood pressure-lowering effect of ginger is mediated through blockade of voltage-dependent calcium channels.

Effect on Prostaglandins

In vitro

103. Ginger extracts, along with many gingerols and shogaols have been shown to suppress prostaglandin synthesis in vitro, through inhibition of cyclooxygenase (Jolad et al. 2005; Pan et al. 2008; Dugasani et al. 2010).

104. Lantz *et al.* (2007) investigated the anti-inflammatory effect of ginger extracts and the principal components thereof (6-, 8- 10-gingerols and 6-, 8-, 10-shogaols) in an *in vitro* model, U937 cells, differentiated and exposed to lipopolysaccharide (LPS) from Escherichia coli (1 µg/ml). Extracts containing predominantly gingerols were found not to be cytotoxic, while shogaols were found to be cytotoxic at concentrations above 20 µg/ml. Crude extracts of ginger inhibited LPS-induced PGE2 (IC50 0.1 µg/ml) production but were much less effective at inhibiting TNF-α (IC50> 30 µg/ml). Extracts containing either predominantly gingerols or shogaols were highly active at inhibiting LPS-induced PGE2 production (IC50 0.1 µg/ml). Extracts containing predominantly gingerols inhibited LPS-induced COX-2 expression while shogaol containing extracts had no effect on COX-2 expression.

105. Jolad *et al.* also demonstrated the inhibitory effect of gingerols on LPS-induced PGE2 production in HL-60 cells stimulated with 1 µg/ml of LPS (2004). None of the compounds tested were shown to be cytotoxic.

Animal studies

106. The Committee noted the potential effect of ginger on the prostaglandin pathway, in particular Cyclooxygenase-1 (COX1) and Cyclooxygenase-2 (COX2) inhibition and how this may affect early pregnancy. One study examining the effects of ginger extracts on prostaglandin E2 (PGE2) production *in vitro* (Lantz *et al.* 2007) demonstrated that crude organic extracts (dichloromethane-methanol, 1:1 v/v) of ginger were capable of inhibiting PGE2 production and that the compounds may act at several sites. The most potent effect on lipopolysaccharide (LPS) induced prostaglandin production was noted at less than 0.1 µg/ml. It was noted that the half maximal inhibitory concentration (IC50) values for a range of components were given, and it was demonstrated that the components mainly acted on COX-2. The COT concluded further studies would be needed to determine the role of decreased prostaglandin levels in the early termination of pregnancy.

107. The composition of ginger extracts also appears to vary according to whether ginger is fresh or dried. Suekawa *et al.,* (1986, abstract only) demonstrated that (6)-shogaol, a principal component mainly found in dried ginger, inhibited carrageenan-induced swelling of rat hind paw, AA-induced platelet aggregation in rabbit and prostaglandin PGI2 release in rat aorta, suggesting a potential inhibitory action on cyclooxygenases (COX) in both platelets and aorta tissue.

Effect on animals with induced diabetes

108. Luo et al. (2022) determined the effects of ginger on gestational diabetes in rats. In this study, 40 adult female rats were divided into 4 equal groups: pregnant rats, pregnant rats with diabetes, pregnant rats consuming ginger powder (100 mg/kg, by gavage), and pregnant rats with diabetes consuming ginger powder. The results of this study showed that one of the mechanisms of physiological metabolic adaptations during pregnancy is a change in the expression of mTORc1, SREBP-1c, PPAR-α, and PPAR-g genes. Disruption of their expression can lead to metabolic disorders and hyperglycaemia and even in advanced cases cause gestational diabetes. However, the results in the groups receiving ginger showed that ginger can significantly improve the metabolic status by modulating the expression of these genes. There were no reported adverse effects resulting from the administration of ginger when compared to the control.

109. Streptozotocin induced rats were utilized as a diabetic model and received 200 or 400 mg/kg/day ginger extract for eight weeks. (Raoufi et al., 2023) Ginger at both levels ameliorated the levels of glucose, testosterone, and MDA. At the higher dose group elevated the levels of insulin, 17β-oestradiol, and progesterone were seen.