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Risk assessment of herbal preparations containing *Withania somnifera* (Ashwagandha)

RIVM letter report 2024-0029
J. A. de Heer

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J.A. de Heer (author), RIVM

Contact:
Linda Razenberg
Department of Chemical Food Safety (CVV)
linda.razenberg@rivm.nl

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Synopsis

Risk assessment of herbal preparations containing *Withania somnifera* (Ashwagandha)

Herbal preparations containing *Withania somnifera* are sold in the Netherlands. The herb is better known as Ashwagandha. Food supplements containing this herb are sold in drugstores and online in web shops. *Withania somnifera* tea is mostly sold online. *Withania somnifera* is used by consumers for stress and fatigue, or to improve sleep.

RIVM studied if herbal preparations containing *Withania somnifera* are harmful to health. The herb can induce harmful effects in individuals who are sensitive to it. It is unknown which individuals are sensitive to *Withania somnifera*. As a precaution, RIVM advises consumers not to use herbal preparations containing *Withania somnifera*, especially during pregnancy.

International studies in humans usually focus on the positive effects of the herb. These studies found no harmful effects in individuals who took food supplements containing *Withania somnifera*. Yet, physicians have seen intoxications in individuals who took these food supplements. They have reported harmful effects on the liver and on concentrations of thyroid hormones and cortisol. These individuals took food supplements containing a similar amount of *Withania somnifera* as that which is found in food supplements containing *Withania somnifera* sold in the Netherlands.

Withania somnifera can also be used to make tea. It is not known whether consumers are exposed to the same amount of harmful substances through *Withania somnifer*a tea as through food supplements. No scientific research has been carried out into the effect of *Withania somnifera* tea. In the absence of information, it is assumed that the conclusion for food supplements is also valid for *Withania somnifera* tea.

In traditional practices in oriental countries, including China and India, *Withania somnifera* has been used to induce abortion, amongst others. It is unknown how often this was done and if this approach is currently still used. The effect has not been studied. As a precaution, therefore, RIVM advises consumers not to use herbal preparations containing *Withania somnifera* during pregnancy.

Keywords: *Withania somnifera,* Ashwagandha, food supplements, safety

Publiekssamenvatting

Risicobeoordeling van kruidenpreparaten met *Withania somnifera* (Ashwagandha)

In Nederland worden kruidenpreparaten met *Withania somnifera* verkocht. Het kruid is beter bekend onder de naam Ashwagandha. In de vorm van voedingssupplementen zijn ze zowel in drogisterijen als online verkrijgbaar. *Withania somnifera* thee is vooral online verkrijgbaar. *Withania somnifera* wordt gebruikt bij bijvoorbeeld stress, vermoeidheid of om beter te slapen.

Het RIVM onderzocht of kruidenpreparaten met *Withania somnifera* schadelijk zijn voor de gezondheid. Het kruid kan schadelijke effecten veroorzaken bij mensen die er gevoelig voor zijn. Het is onbekend om welke mensen dat gaat. Het RIVM adviseert daarom uit voorzorg om geen kruidenpreparaten met *Withania somnifera* te gebruiken, en vooral niet tijdens de zwangerschap.

Internationaal onderzoek bij mensen is meestal gericht op de positieve effecten van het kruid. Daarin zijn geen schadelijke effecten gevonden bij mensen die voedingssupplementen met *Withania somnifera* gebruikten. Wel hebben artsen vergiftigingen gemeld bij mensen die deze supplementen hadden ingenomen. Zij melden schadelijke effecten in de lever en op de concentraties van schildklierhormonen en cortisol. Deze mensen hadden voedingssupplementen gebruikt met ongeveer evenveel *Withania somnifera* als de supplementen die in Nederland te koop zijn.

Withania somnifera kan ook als thee worden gebruikt. Het is niet bekend of de gebruiker hiervan dezelfde hoeveelheid schadelijke stoffen binnenkrijgt als bij het gebruik van voedingssupplementen. Er is geen wetenschappelijk onderzoek gedaan naar het effect van *Withania somnifera* thee. Bij gebrek aan informatie wordt aangenomen dat de conclusie voor voedingssupplementen ook geldt voor het gebruik van het kruid in de vorm van thee.

In traditionele gebruiken in oosterse landen, zoals China en India, is *Withania somnifera* onder andere gebruikt om abortus op te wekken. Het is niet bekend hoe vaak dit is gedaan en of dat nog steeds gebeurt. Dit effect is niet onderzocht. Daarom wordt uit voorzorg geadviseerd om tijdens de zwangerschap geen producten met dit kruid te gebruiken.

Kernwoorden: *Withania somnifera,* Ashwagandha, voedingssupplementen, veiligheid

Contents

Summary

Introduction

In December 2020, the Minister for Medical Care and Sport of the Ministry of Health, Welfare and Sport (VWS) announced actions that would be taken to better regulate food supplements and herbal preparations in the Netherlands, thereby facilitating enforcement. One of those actions is to expand the list included in the Herbal Preparations Decree of the Dutch Commodities Act[1] with substances/botanicals that are either forbidden or restricted (i.e. subject to a maximum level) in food supplements or herbal preparations (Van Ark, 2020). In order to determine whether a substance or botanical needs to be included in this list, a risk assessment is warranted. The selection of substances and botanicals chosen for risk assessment was based on the prerequisite that the substances/botanicals are sold on the Dutch market and (widely) used and there are indications for possible health risks, e.g. Rapid Alert System for Food and Feed (RASFF) reports, from enforcement institutes. The current risk assessment is about herbal preparations containing *Withania somnifera*.

Currently, there are no specific restrictions for the use of *W. somnifera* in herbal preparations included in the Herbal Preparations Decree of the Dutch Commodities Act. In addition there are no European legislations for *W. somnifera*.

Withania somnifera (L.) Dunal, also called ashwagandha, belongs to the nightshade family or *Solanaceae.* The plant has been used for over 3000 years in Ayurvedic medicine. *W. somnifera* consists of many phytochemical constituents, including alkaloids and withanolides (DTU, 2020; BfR, 2013).

Previous evaluations

In 2009, the World Health Organisation (WHO) published a monograph on medicinal plants including *W. somnifera* root. A dose of 3 to 6 gram of dried powdered *W. somnifera* root is mentioned in the WHO monograph (not further specified). Furthermore, 250 mg is recommended twice a day when it is used as an antistress agent. According to this monograph, *W. somnifera* was traditionally used to induce abortion (WHO, 2009).

In 2013, the German Federal Institute for Risk Assessment (BfR) published a risk assessment of several plants and herbal preparations, including *W. somnifera* (BfR, 2013; in German). BfR identified safety concerns, due to the adverse effect of *W. somnifera* on the thyroid function and its historical use for the induction of abortion. BfR recommended to include *W. somnifera* root in list C of appendix III of EC Regulation 1925/2006 on the addition of vitamins, minerals and other substances to food (BfR, 2013). This list contains substances whose use in foods is under community scrutiny (EC, 2006). No health-based guidance value was established by BfR.

[1] https://wetten.overheid.nl/BWBR0012174/2020-07-01. Accessed September 2022.

In 2020, the Danish National Food Institute (DTU) published a risk assessment for *W. somnifera* roots (DTU, 2020; in Danish). DTU noted that effects on the thyroid glands and sex hormones were seen in animal studies and in humans. They concluded that a safe use limit for exposure to *W. somnifera* root or extract of roots could not be established on the basis of the available data (DTU, 2020).

Products on the Dutch market

A wide range of herbal preparations (food supplements and tea) containing *W. somnifera* can be found on the Dutch market. Food supplements are available as capsules, tablets or tinctures containing *W. somnifera* extract or as root powder. The tea contains root powder or dried root pieces. In some food supplements, *W. somnifera* is combined with other substances and herbal extracts. Food supplements containing *W. somnifera* claim to reduce stress, improve sleep, give rest and energy, support memory and concentration, support emotional balance, protect cells against free radicals, support airways and improve skin and increase libido.

Exposure

Based on the recommended use of the food supplements, the estimated exposure to *W. somnifera* extract ranged from 50 mg to 2 g per day (0.7 to 29 mg/kg bw per day for a 70-kg person). Based on the recommended use of food supplements containing the root powder, exposure ranged from 1.3 to 2.5 g *W. somnifera* root powder per day (19 to 36 mg/kg bw per day for a 70 kg person). For tea, the exposure to *W. somnifera* powder could only be estimated for one product. When one portion is consumed per day the exposure to *W. somnifera* root powder was 5 g per day (71 mg/kg bw per day for a 70 kg person). It has to be noted that the calculated exposure is solely based on the publicly available information provided by the manufacturers of these herbal preparations.

Biological data

- In animals, constituents of *W. somnifera* were rapidly absorbed in the body (Modi et al., 2022; Srivastava et al., 2013). One of them, withaferin A was distributed throughout the whole body (Wang et al., 2019; Singh et al., 2018). No other constituents were studied. No information was identified about the excretion of constituents of *W. somnifera* or their metabolites in animals. No human data are available.
- After single exposure, no acute toxicity was observed (DTU, 2020; BfR, 2013).
- In two short-term repeated dose toxicity studies with a duration of 28 days, no adverse effects were observed after exposure to doses up to respectively, 1000 and 2000 mg/kg bw *W. somnifera* extract (Balkrishna et al., 2022; Prabu et al., 2013, as cited by DTU). However, in other short-term toxicity studies adverse effects on for example the immune system were observed from doses of 10 mg/kg bw *W. somnifera* extract onwards.
- Indications that *W. somnifera* can alter levels of thyroid hormones and thereby induce excess thyroid activity were found in animal studies and human data.

- No indications for genotoxicity of extracts of *W. somnifera* were found in genotoxicity tests (Modi et al., 2022). However, there was an indication that the constituent withanone might be genotoxic (Siddiqui et al., 2021). The percentage of withanone in the tested *W. somnifera* extract was low (0.004%), but it is unknown if this is comparable to other extracts.
- No chronic toxicity and carcinogenicity studies were identified.
- Reproductive toxicity was studied from day 5 of gestation, as a result the effect in early pregnancy was not studied (DTU, 2020). For other reproductive parameters, the results were contradicting and no conclusions can be drawn, except for sexual maturation, which was induced early in both genders (DTU, 2020)
- Nine case reports were identified, in which thyrotoxicosis (n=1), suppression of adrenal function (n=1) and liver injury (n=7) were reported in humans after consuming food supplements containing *W. somnifera* extract (154 – 1350 mg). The authors of the articles judged the likeliness of the role of *W. somnifera* as definite in one case, highly likely in two cases, probable in two cases and possible in one case. In three case reports the likeliness was not assessed. It has to be noted that causality cannot be proven in case reports.
- The Netherlands Pharmacovigilance Centre (Lareb) received four notifications of liver injury linked to *W. somnifera* products in the Netherlands.
- Twenty-eight clinical trials are available. In these trials, decreased cortisol levels, an effect on sex hormones levels and a larger immune response were reported at doses of 240-675 mg/kg bw *W. somnifera* extract or 5 g *W. somnifera* powder.

Natural Medicines reported several potential interactions of *W. somnifera*, including interactions with antidiabetic drug and thyroid hormones (Natural medicines, 2023).

No safe use level

It was investigated whether the presumption of safety could be applied to *Withania somnifera*. Botanical preparations for which an adequate body of knowledge exists, can benefit from a presumption of safety without any need for further testing (EFSA, 2009; EFSA, 2014). The presumption of safety could not be applied to *W. somnifera* and more information is needed to assess its safety.

Despite the new information identified in this assessment, it was not possible to establish a health-based guidance value (HBGV) for *W. somnifera* or its main constituents, due to a lack of (adequate) toxicological data and unresolved concerns. No data from toxicity studies with a duration longer than 28 days were available. Furthermore, effects on thyroid and sex hormones were found in the short-term toxicity studies and adverse effects on the liver and levels of thyroid hormones and cortisol were observed in case studies. Also, there were unresolved concerns regarding the risk for the unborn child as, according to the WHO, *W. somnifera* was traditionally used to induce abortion. Although no adverse effects were observed in the available reproduction toxicity studies, these did not adequately assess the effect of *W. somnifera* in early pregnancy and can therefore not be used to

draw conclusions on this aspect. In addition, there are indications that a constituent of *W. somnifera* is genotoxic, but the concentration in available *W. somnifera* extract is unknown. The clinical studies cannot be used as a basis for an HBGV, because not all aspects of toxicity were investigated in these studies. As no HBGV could be established, no safe use level could be determined.

The intake levels reported in the case reports described in peer-reviewed papers (77 to 1350 mg/day *W. somnifera* extract) will be considered as the effect level in the risk assessment.

Risk assessment

In the nine human case reports adverse effects, as liver injury, thyrotoxicosis and suppression of the adrenal system, were observed after daily exposures to 77 to 1350 mg *W. somnifera* extract per day.

These levels are in the same range as the estimated exposure for food supplements available in the Netherlands. However, it is unlikely that a large part of the population is at risk for these effects, as the adverse effects were not observed in clinical trials, in which the exposure to *W. somnifera* extract and the duration of use was comparable to the situations described in the case reports. This leads to the conclusion that the sensitive individual could experience adverse effects such as liver injury, thyrotoxicosis and suppression of the adrenal system. It is unknown which individuals are sensitive to *Withania somnifera*.

Furthermore, the possible risk for the unborn child cannot be adequately assessed.

For *W. somnifera* tea no toxicological data are available and the information on the exposure is limited. In the absence of sufficient data, the conclusion for food supplements is assumed to also apply to *W. somnifera* tea.

Conclusion and recommendations

The use of food supplements containing *W. somnifera* that are currently available on the Dutch market may lead to adverse effects such as liver injury, thyrotoxicosis and suppression of the adrenal system in sensitive people. It is unknown which individuals are sensitive to *Withania somnifera*. In addition, according to the WHO, *W. somnifera* was historically used to induce abortions, indicating that *W. somnifera* could be harmful to the unborn child. No suitable reproduction toxicity studies were available to exclude that herbal preparations containing *W. somnifera* can cause this effect.

For *W. somnifera* tea, no toxicological data are available and the information on the exposure is limited. In the absence of sufficient data, the conclusion for food supplements is assumed to also apply to *W. somnifera* tea.

As a precaution, RIVM advises consumers, and in particular pregnant women, to not use herbal preparations containing *W. somnifera*.

1 Introduction

1.1 Background

In December 2020, the Minister for Medical Care and Sport of the Ministry of Health, Welfare and Sport (VWS) announced the actions that would be taken to better regulate food supplements and herbal preparations in the Netherlands, thereby facilitating enforcement. One of those actions is to expand the list included in the Herbal Preparations Decree of the Dutch Commodities Act[2] with substances/botanicals that are either forbidden or restricted (i.e. subject to a maximum level) in food supplements or herbal preparations (Van Ark, 2020). In order to determine whether a substance or botanical needs to be included in this list, a risk assessment is warranted. The selection of substances and botanicals chosen for risk assessment was based on the prerequisite that the substances/botanicals are sold on the Dutch market and (widely) used and there were indications for possible health risks, e.g. Rapid Alert System for Food and Feed (RASFF) reports, from enforcement institutes. The current assessment is about herbal preparations containing *Withania somnifera*.

1.2 Information on existing assessments

In 2009, the World Health Organization (WHO) published a monograph on medicinal plants including *W. somnifera* root (WHO, 2009). In the monograph, available information of *W. somnifera* root on antistress response, anti-inflammatory or immune stimulation activity, anti-ischemic activity, antioxidant activity, chemoprotective activity and neuroprotective activity and the effect of *W. somnifera* root on memory and cognition was reviewed. The WHO reported that adverse effects such as nausea, vomiting and diarrhoea can occur after usage of *W. somnifera* root. Furthermore, they indicated that *W. somnifera* root should not be used during pregnancy or breastfeeding as the plant traditionally has been used to induce abortion and not enough data were available to proof the safety of the plant. Also precautions were given for drug interactions with barbiturates and compounds as diazepam and clonazepam. A dose of 3 to 6 gram of dried powdered *W. somnifera* root is mentioned in the WHO monograph, as well as an oral dose of 250 mg twice a day as an antistress agent. No further details on doses were provided (WHO, 2009).

In 2013, the German Federal Institute for Risk Assessment (BfR) published a risk assessment of several plants and herbal preparations, including *W. somnifera* (BfR, 2013; in German). They noted that no indications for adverse effects were found in clinical trials, however they also concluded that possible adverse effects were not systematically measured. BfR identified clinical trials on hypoglycaemic effects and effects on immune response, iron metabolism and growth, aging and anxiety. For hypoglycaemic effects, only studies in people with hyperglycaemia were conducted and the effect in healthy people was unknown. Increased haemoglobin levels were observed in three human studies, although it was not possible to draw further conclusions in

[2] https://wetten.overheid.nl/BWBR0012174/2020-07-01. Accessed September 2022.

terms of health risks. Also several animal studies were identified, in which an increase of thyroid hormones was observed. Also, one case report was identified in which thyrotoxicosis occurred, which could be linked to the intake of food supplements containing *W. somnifera*. According to BfR, the available developmental toxicity studies were not appropriate to assess the effects on early foetal development. Therefore, they recommended to not use these supplements when pregnant or breastfeeding, especially since *W. somnifera* historically was used to induce abortion. Also they identified no information to assess the safety of *W. somnifera* for children. In addition, BfR noted that an interaction with gamma-aminobutyric acid (GABA) agonists could occur after intake of *W. somnifera*.
Overall, BfR concluded that presumption of safety was not possible as there were no data on safe historical use by large populations. Although comprehensive toxicological studies were lacking, they identified safety concerns due to the adverse effect of *W. somnifera* on thyroid function and due to its historical use for the induction of abortion. BfR recommended to include *W. somnifera* root in list C of appendix III of EC Regulation 1925/2006 on the addition of vitamins, minerals and other substances to food (BfR, 2013). This list contains substances whose use in foods is under community scrutiny (EC, 2006).

In 2020, the Danish National Food Institute (DTU) published a risk assessment for *W. somnifera* roots (DTU, 2020; in Danish). DTU identified several animal studies, including several 28-days experiments. It was concluded that 28 days was too short to determine if *W. somnifera* can have harmful effects. Human clinical trials gave some indications that *W. somnifera* affects hormone levels, including an increase of testosterone and luteinizing hormone (LH) and a decrease of follicle-stimulating hormone (FSH), although alterations of hormone levels were studied mainly in men with poor sperm quality or altered sex hormones. Also in animal studies effects on sex hormones were found. However, the effect direction varied for most hormones between the different animal studies. Alterations of sex hormones could potentially pose a health risk. Furthermore, there were indications from animals studies that *W. somnifera* increased thyroid hormone levels, which may result in a risk of developing increased metabolism and a range of adverse symptoms. Also some studies hinted at effects as inhibition of acetylcholinesterase or activation of the immune system. DTU noted that the animal studies pointed to different types of adverse effects. Some of these effects have also been observed in humans during clinical trials, for example the effects on the thyroid glands and sex hormones. DTU concluded that no safe use level could be established for *W. somnifera* root or root extract on the basis of the available data.

Monographs of *W. somnifera* are published by health Canada (Health Canada, 2023) and are available in the natural medicine database (Natural medicines, 2023) and in Hagers Enzyklopädie der Arneistoffe und Drogen (Hagers Enzyklopädie, 2010). Also, EMA discussed *W. somnifera*, but did not prepare a monograph (EMA, 2011).

1.3 Information on existing legislations

Currently, there are no specific restrictions for the use of *W. somnifera* included in the Herbal Preparations Decree of the Dutch Commodities Act. In addition, there are no European legislations for *W. somnifera*.

2 Literature search

The risk assessment for herbal preparations containing *W. somnifera* was conducted using the template for the safety assessment of plant food supplements as a basis (De Wit et al., 2019).

In grey literature[3] a monograph from the WHO (WHO, 2009) and two risk assessments have been found, one from BfR and one from DTU (BfR, 2013; DTU, 2020). In the most recent risk assessment by DTU (2020), a literature search was conducted until august 2019 on Web of Science using keywords as 'withania somnifera' and 'ashwagandha'.

Information from all risk assessments was included in the current risk assessment. To collect all relevant information published after August 2019, a literature search was conducted. The literature databases PubMed and Scopus were searched on the 26th of July 2022. The search strings can be found in Appendix A. In total 318 unique articles were obtained. Based on title and abstract screening, studies relevant to study adverse effects were selected, resulting in 25 articles. Based on the full text, all studies, except one study on a combination of herbal preparations, were included for the risk assessment.

As no information on the toxicokinetics of *W. somnifera* was included in the previously conducted risk assessments an additional global literature search was conducted to identify information on toxicokinetics of (constituents of) *W. somnifera* in humans and animals. The literature databases PubMed and Scopus were searched on the 4th of August 2022. The search string can be found in Appendix B. In total 58 unique articles were obtained. Based on title and abstract, 17 articles were selected for full text appraisal. Based on the full text, eight articles were selected for inclusion in the risk assessment.

Information on the different herbal preparations containing *W. somnifera* available on the Dutch market was collected from different websites in July 2022.

[3] Grey literature refers to research that is either unpublished or has been published in a non-commercial form. Examples include RIVM reports or EFSA's Compendium of Botanicals.

3 Description of the product

3.1 Identity and nature of the source material

The plant *Withania somnifera (L.) Dunal*, also called ashwagandha, belongs to the nightshade family or *Solanaceae*. Synonyms and common names of *W. somnifera* can be found in Table 1. The plant grows in dry areas of tropical and subtropical regions. It is distributed from northern Africa to southwest Asia and also grows in the Mediterranean region. The plant is also cultivated, for example in India (DTU, 2020; BfR 2013).

W. somnifera is a shrub and is 30 to 75 cm high. The roots have a whitish brown colour. The leaves are green simple ovals. The fruit is a small orange-red berry (Bharti et al., 2021).

Table 1 General information of Withania somnifera (DTU, 2020; BfR 2013).

Scientific (Latin) name	Family: *Solanaceae* Species: *Withania somnifera (L.) Dunal*
Synonyms	*Physalis somnifera L.* *Physalis flexuosa L*
Common names	Ashwagandha Indian ginseng Indian winter cherry
Part used	Whole plant Roots (most often)
Geographical origin	Mediterranean region Northern Africa Middle East South Asia
Cultivation	Yes, for example in India

W. somnifera has been used for over 3000 years in Ayurvedic medicine. The root is used in over 200 different preparations including, but not limited to, medicines for infertility, constipation, weakness, rheumatism, loss of muscle strength and memory loss. In traditional Chinese medicine, *W. somnifera* root has been used as an analgesic and antimalarial drug. It is also known that *W. somnifera* has been used to induce abortion in traditional medicine, although there is no clear evidence that supports this effect (BfR, 2013; WHO 2009).

3.2 Manufacturing process

In the risk assessment of BfR (2013) it is mentioned that an ethanol extract is used for some studies. No other information on the manufacturing process was presented by BfR (2013) and DTU (2020).

The company Ixoreal shared some information on the production process of KSM-66, a *W. somnifera* root extract. KSM-66 is manufactured using 'green chemistry' principles, meaning no alcohol nor

other chemical solvents are used[4]. The roots are pre-treated with milk. No other information is available about this manufacturing process.

Food supplements most often contain a root extract of *W. somnifera*, but not in all cases the plant part is specified. Some food supplements contain root powder. *W. somnifera* tea is most often a plant powder of unspecified origin or small pieces of root.

3.3 Chemical composition

Many phytochemical constituents are present in *W. somnifera* (DTU, 2020; BfR, 2013). These phytochemical constituents can be divided in several classes including withanolides, withanamides, flavonoids, polyphenols and alkaloids. The alkaloids and withanolides present in *W. somnifera* are considered to be the most important for its effects (DTU, 2020; BfR, 2013).

The alkaloids found in *W. somnifera* belong to different groups of piperdine and pyrrolidine alkaloids, including anaferin, anahygrin, cuscohygrin and isopelletierin and tropane alkaloids, including tropine and pseudotropine. Currently, 12 different alkaloids have been identified. Reported total concentrations of alkaloids ranged from 0.1 – 4.3% in the roots and 0.2 – 2.1% in the leaves of *W. somnifera* in several studies (Table 2). No concentration data are available for individual alkaloids (DTU, 2020; BfR, 2013).

Currently, 35 different withanolides have been identified, including withaferin A and withanolide A. Withanolides are steroids, which mainly have been identified in members of the family Solanaceae. The reported total concentrations of withanolides ranged from 0.1 - 1.3% in the roots, 0.2 – 3.75% in the leaves and 3.1 – 27.7% in the fruit of *W. somnifera* in several studies (Table 2). Concentrations of individual withanolides were measured in the roots, leaves, stem and fruit of *W. somnifera* (Table 3) (DTU, 2020; BfR, 2013).

The concentrations of polyphenols and flavonoids in *W. somnifera* were measured in root, leaf and fruit (Table 2). Not much information is available for the withanamides in *W. somnifera*, except from the total concentration in fruit (Table 2) (DTU, 2020).

Besides these active components, the root of *W. somnifera* also contains starch, reducing sugar, hentriacontane, dulcitol and withanicil and iron.

[4] https://ksm66ashwagandhaa.com/ksm-66/what-is-ksm-66/

Table 2 The total concentration of compounds per phytochemical classes in Withania somnifera root, leaf and fruit from different studies (DTU, 2020; BfR, 2013).

	Root	**Leaf**	**Fruit**
Polyphenols	1.8%	3.3%	2.2%
Flavonoids	1.5%	3.2%	2.1%
Alkaloids	0.1-0.3% 0.2-1.0% max 4.3%	0.2-0.6% max 2.1%	
Withanolides	1.3% 0.2-1.1%	0.2-2.1% 3.75%	3.1-3.4% 3.1-27.7%
Withanamides			0.5-1.7%

Table 3 The concentration (%) of individual withanolides in Withania somnifera root, leaf and fruit from different studies (DTU, 2020; BfR, 2013).

	Root	**Leaf**	**Fruit**	**Stem**
Withanolide A	0.01-0.4 max 1,47	0.1-1.3 0.01-0.2	0.004	0.3
Withanolide D	0.2			
Withanolinde S		0.2		
Withaferine A	0.001-0.2 0.1-0.9 0.02 0.2	0.1-1.1 0.1 0.6 0.9	0.4	0.2
Withanoside	0.4 max 0.4			
Withanon	0.01-0.6 max 0.04	0.4		
Withastramonolide	max 0.01			
Physaguline	0.02	max 0.1		
Metaferine A	0.002-0.04	max 0.6		
Metanon		max 0.3		
Metanoside		max 0.1		
Metastramonolide		max 0.1		
27-hydroxywithanolid B	0.001-0.1			
27-hydroxywithanon	0.001-0.1 max 0.004	max 0.1		
27-desoxywithaferin A	0.4			
17-hydroxy-27-desoxywithaferin A	0.07			
12-deoxymetastramonolide	0.01-0.1 0.04	0,004-0,3 0.002	0.01	0.4

The concentrations of active compounds in *W. somnifera* from different regions can vary. Furthermore, the commercially grown plants contain higher concentrations of bioactive compounds, for example withaferin A, compared to wild plants (BfR, 2013).

3.4 Selected compound(s) for risk assessment

The alkaloids and withanolides in *W. somnifera* are considered to be the main active compounds. Some food supplements indicate withaferin A as the active compound. Another source identified withaferin A and withanolide D as active compounds (Monograph *Withania somnifera*,

2004). The current risk assessment is based on data about the extracts and not on the individual compounds, as the concentration of these compounds is unknown in available herbal preparations.

3.5 Stability

In the risk assessment of BfR (2013) the stability of dried and wet extract of *W. somnifera* was briefly mentioned. The dried extract became lumpy after 5 months of storage in 30℃, with a relative humidity of 65%. In the wet extract, withaferin A and withanolide A degraded fast at high temperatures and high relative humidity.

3.6 Use and use levels

Food supplements containing *W. somnifera* are widely available online and can also be bought in most common drugstores in the Netherlands. Table 4 gives an overview of several food supplements containing *W. somnifera* available on the Dutch market (based on an internet search on Dutch websites, July 2022). Food supplement are available as capsules, tablets or tinctures containing *W. somnifera* extract or as root powder. The intake per unit and recommended usage varies strongly between the food supplements. Around half of the food supplements contain only *W. somnifera*, whereas the other half contains a combination of *W. somnifera* and multiple different ingredients, including vitamins, minerals and other plant extracts. Food supplements containing *W. somnifera* claim to reduce stress, improve sleep, give rest and energy, support memory and concentration, support emotional balance, protects cells against free radicals, support airways, improve skin and/or improve libido.

On some webpages warnings are listed for specific groups of people for which the respective supplements is not suitable (Table 4).

Besides food supplements, *W. somnifera* tea is also available, however the tea seems to be only available online. Table 5 gives an overview of the available tea products on the Dutch market. The tea contains root powder or dried root pieces. For some products instructions, claims or warnings are given (Table 5). The method of preparation can differ, as one product instructs to drink the *W. somnifera* powder with the liquid whereas normally tea powder or leaves are removed before drinking.

Table 4 Examples of food supplements containing Withania somnifera available on the Dutch market

Ingredients	**Amount of *W. somnifera* per unit**	**Recommended use per day**	**Maximal amount of *W. somnifera* per day**	**Claims**	**Warnings**
Ashwagandha extract, passion flower extract (100 mg), valerian root extract (120 mg)	50 mg	1 capsule	50 mg extract	Yes[5]	Use is not advised for children and during pregnancy and breastfeeding
Ashwagandha extract, ginger extract (150 mg), turmeric powder (100 mg), Vitus Agnus Castus extract (5 mg), Biotin (2008 µg), vitamin B6 (1,4 mg), zinc (10 mg), iron (5 mg)	50 mg	1 capsule	50 mg extract	Yes[6]	Use is not advised for children and during pregnancy and breastfeeding
Powdered ashwaganda root	Powder	½ - 1 tea spoon (1.3-2.5 g)	2500 mg powder	Yes[7]	No
Ashwagandha extract (100 mg), D-aspartate (193 mg), stinging nettle extract (52,5 mg), vitamin B6 (2,1 mg), vitamin D3 (3,57 µg), selenium (30 µg), zinc (3,25 mg)	100 mg	2 capsules	200 mg extract	No	This supplement is not suitable for children under 18. This supplement contains stinging nettles. Ask advise from a doctor or pharmacist.
Withania somnifera root extract	300 mg	1 capsule	300 mg extract	Yes[8]	Before use ask advise from a doctor in the case of pregnancy, breastfeeding, use of medication and illness
Withania somnifera root extract	500 mg	1-2 or 4 capsules	2000 mg extract	Yes[9]	No

[5] Ashwagandha supports focus and concentration.
[6] Ashwagandha helps with regulating emotional balance around menstruation.
[7] Ashwagandha relieves temporary stress, gives rest and relaxation
[8] Ashwagandha supports in times of fatigue, contributes to emotional balance and is an anti-oxidant.
[9] Ashwagandha, at higher dosage from 2 g, supports airways and libido, supports in stressful situations and helps to keep skin healthy.

Table 5 Examples of tea containing Withania somnifera available on the Dutch market

Ingredient	User manual	Claim	Warning
Pieces of *W. somnifera* root	Add 5 teaspoons of product to 300 ml of boiling water maximal 2 times a day.	Yes[10]	Yes[11]
W. somnifera powder	Add 5 g of product to 70-100 ml cold water or 200 ml milk or soy or rice drinks	Yes[12]	No
W. somnifera powder	No instructions	No	No

[10] Ashwagandha root is an adaptogen.
[11] We recommend not to take Ashwagandha tea on an empty stomach, as this could lead to nausea. Do not use this product during pregnancy and breastfeeding. Ask advise from a doctor when diagnosed with ulcers or hemochromatosis.
[12] Ashwagandha supports mental and physical wellbeing and memory. It helps relax and gives support during periods of stress.

4 Exposure: extent and duration

4.1 Exposure from use of herbal preparations

Based on the recommended use mentioned in Table 5, the exposure to *W. somnifera* from food supplement use was estimated. For food supplements containing an extract the estimated exposure ranged from 50 mg to 2 g *W. somnifera* extract per day (equal to 0.7 to 29 mg/kg bw per day for a 70-kg person). For food supplements containing the root powder exposure was estimated to range from 1.3 to 2.5 g *W. somnifera* powder per day (equal to 19 to 36 mg/kg bw per day for a 70-kg person).

For the tea, user instructions are not available for all products. For two products instructions are given to use 5 gram of powder (71 mg/kg bw for a 70 kg person) or 5 teaspoons of dried root in pieces for one portion. The user could take multiple portions per day.

4.2 Possibility of additional/combined human exposure

No other products containing *W. somnifera* are available on the Dutch market.

4.3 Information on historical use of the ingredient

The intake of *W. somnifera* in traditional uses is unknown.

5 Biological data

5.1 Toxicokinetics

In the risk assessment of DTU (2020) no information on the absorption, distribution, metabolism and excretion of constituents of *W. somnifera* was presented. In the risk assessment of BfR (2013) it was mentioned that there were no available data on the pharmacokinetics or the pharmacodynamics of *W. somnifera* and its constituents.

A global literature search for toxicokinetic information on *W. somnifera* was conducted. Several studies reporting toxicokinetic parameters of constituents of *W. somnifera* were identified in literature. In the section absorption, only studies were included in which a *W. somnifera* extract was administered orally. In the section distribution, also studies were included in which constituents of *W. somnifera* were administered.

5.1.1 *Absorption*

Mice were orally administered to *W. somnifera* extract at 200 mg/kg bw. Blood samples were collected before and at 5 time points between 1 and 12 hours after administration. The highest maximum concentration (C_{max}) of withaferin A after oral administration was 30 ng/mL and was reached after one and a half hour. The half-life ($t_{1/2}$)was 2 hours. The C_{max} of 12-deoxywithastramonolide (12-DW) was 0.1 µg/mL and was reached after 4.6 hours. The $t_{1/2}$ was 6.6 hours (Srivastava et al., 2013).

Sprague-Dawley rats were orally exposed to 500 mg/kg bw of *W. somnifera* extract. Blood samples were collected before dosing and at 11 time points between 15 minutes and 24 hours post-dosing. In this study the metabolites withaferin A, withanoside IV (WIV), 12-DW and withanolide A were measured. The C_{max} of the metabolites was 124, 14, 58 and 7 ng/mL for withaferin A, WIV, 12-DW and withanolide A, respectively and was reached after 0.25, 0.8, 0.3 and 0.3 hours respectively. The $t_{1/2}$ was 3, 1.1, 1.7 and 0.7 hours respectively for withaferin A, WIV, 12-DW and withanolide A (Modi et al., 2022).

Dai et al. (2019) found that withaferin A had a good permeability in Caco-2 cells.

No human data was identified.

5.1.2 *Distribution*

Based on results from *in silico* predictions, Modi et al. (2022) reported that withanolide A, withanolide B, withanone, 12-deoxywithastramonolide and withaferin A could pass the blood-brain barrier and withanoside IV and withanoside V could not.

The distribution of withaferin A was studied in several studies.

Dai et al. found that 73% of withaferin A was transformed or bound to tissue after passing the liver (Dai et al., 2019).

Within 5 minutes after intravenous administration (2 mg/kg), withaferin A was distributed widely in rats. The concentrations found in certain tissues was higher compared to the concentration found in plasma. The highest concentration was found in the lungs, followed by the liver, kidneys, spleen, heart and brain. The presence of withaferin A in the brain means that the compound can pass the blood-brain barrier. Withaferin A had a high passive permeability at pH 4 and 7. Also plasma protein binding ability was high; 86% at 5 µM (Singh et al., 2018).

Wang et al. (2019) studied the distribution of withaferin A (0.5, 1.5 and 4.5 mg/kg) after oral gavage in rats. Concentration of withaferin A was highest in the stomach, followed by the heart, lungs, kidneys, intestine, spleen and liver. The maximal concentration in tissue was reached around 30 minutes later than the maximal concentration in blood plasma (Wang et al., 2019).

No human data was identified.

5.1.3 *Metabolism*

Modi et al. (2022) found that some withanosides and withanolides are substrates for cytochrome P450 3A4 (CYP3A4) using *in silico* predictions (probability of 0.72-0.73). Experiments with withaferin A in rat and human liver microsomes showed the formation of 7 metabolites in microsomes from human origin and 4 metabolites in microsomes from rat origin (Dai et al., 2019). The metabolites were a result of hydroxylation, hydrolysis and hydro-generation of the parent compound withaferin A or metabolites (Dai et al., 2019).

No human data was identified.

5.1.4 *Excretion*

No information on the excretion of *W. somnifera* constituents was identified.

5.2 Toxicological data

Below, the toxicological data included in the assessments by BfR (2013) and DTU (2020) are shortly summarized followed by new data identified in the literature search.

5.2.1 *Acute toxicity*

DTU Fødevareinstituttet

Two acute oral toxicity studies in female rats were described (Patel et al., 2016; Prabu et al., 2013 as cited by DTU). In both studies, no adverse effects or deaths were observed for 14 days after a single dose of 2 g/kg bw *W. somnifera* root extract (0.04% and 4.5% withaferin A) given via oral gavage. In one of the studies, which was performed according to the applicable OECD guideline according to the authors, also macroscopic pathology was conducted on day 15 and no effects were observed. The oral median lethal dose (LD_{50}) was > 2000 mg *W. somnifera* root extract/kg bw.

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Two oral studies were identified in mice (Malik et al., 2007; Ghosal et al., 1989 as cited by BfR, 2013). No effects were observed in the animals after exposure to single doses up to 2 g/kg bw of *W. somnifera* root or leave extract or a single dose of 1 g/kg bw sitoindoside IX and X, constituents of *W. somnifera*.

Literature search
Gupta et al. (2022) orally exposed BALB/c mice to 0, 50, 300 or 2000 mg/kg bw withaferin A. According to the authors, this study was performed according to OECD guideline 423. The mice were observed every 30 minutes in the first 4 hours, then a few times until 24 hours post-dosing and once a day until 14 days post-dosing. After 14 days, the mice were killed and blood samples and organs were investigated. There were no signs of toxicity in any of the dose groups, except from a dose-dependent decrease (but not statistically significant) in white blood cell count (Gupta et al., 2022).

Liu (2022) predicted LD_{50} values for 75 withanolides using *in silico* models. For two third of the 75 withanolides the LD_{50} values were estimated to be below 100 mg/kg bw for rats. For example, for withaferin A and sitoindoside IX the predicted LD_{50} values were 96.5 and 30.4 mg/kg bw, respectively. The study author did not compare the results to LD_{50} values from animal studies. The results from Liu (2022) were not in line with results from experimental animals studies. The results from the animal studies are considered to be more relevant.

Table 6 Results of oral acute toxicity studies with W. somnifera extract and its constituents.

Test compound	Species; Sex	LD_{50} (mg/kg bw)	Reference
W. somnifera root extract (4.5% withaferine A)	Rat; F	>2000	1
W. somnifera root extract (0.043% withaferine A)	Rat; F	>2000	2
W. somnifera root extract (0.08% withaferine A)	Mice; NR	>2000	3
Sitoindoside IX and X	Mice; NR	>1000	4
Withaferin A	Mice; F	>2000	5

F, female; NR, not reported
1. Patel et al., 2016, as cited by DTU 2. Prabu et al., 2013 as cited by DTU. 3. Malik et al., 2007, as cited by BfR 4. Ghosal et al., 1989 as cited by BfR, 2013 5. Gupta et al., 2022

Summary
Table 6 summarizes the results of the studies of acute oral toxicity with *W. somnifera* extract and/or its constituents. No effects were observed after acute exposure up to 2 g/kg bw of *W. somnifera* extract or withaferine A or 1 g/kg bw of sitoindoside IX and X. Based on this information it was concluded that *W. somnifera* shows low acute toxicity.

5.2.2 *Short-term and sub-chronic toxicity*

DTU Fødevareinstituttet

Several short-term toxicity studies were identified in rats and mice. Patel et al. (2016) exposed male and female rats to doses of 0, 0.5, 1 or 2 g/kg bw of *W. somnifera* root extract by oral gavage daily for 28 days According to the authors, the study was performed corresponding to the applicable OECD guideline. DTU noted that Patel et al. (2016) used the guideline from 2001 instead of the more recent guideline from 2008. In the study of Patel et al. (2016) statistically significant differences were found for several haematological and clinical-chemical parameters, but the values of the dose groups were still within normal limits for the species according to the author. DTU (2020) noted that the two highest doses showed statistically significant increases for haemoglobin concentration in females and packed cell volume and mean corpuscular volume in males. At the highest dose also increased haemoglobin concentration in males and an increased number of white blood cells and lower prothrombin in females were observed. DTU did not conclude whether these findings were adverse in their opinion. Overall, DTU concluded that longer studies than 28-days studies are needed to study the harmful effects of *W. somnifera*.

Prabu et al. (2013) also exposed male and female rats to doses of 0, 0.5, 1 or 2 g/kg bw of *W. somnifera* root extract by oral gavage daily for 28 days. According to the authors, the study was performed corresponding to the applicable OECD guideline. It was concluded that there were no adverse effects and a NOAEL of 2000 mg/kg bw per day for *W. somnifera* extract was derived. In this study not a single parameter was statistically significantly different between the control and dose groups. DTU (2020) noted that a Tukey's test was used to test for significance, where normally a Dunnett's test would be used.

In other short-term toxicity studies (ranging from 5 days to 4 weeks) in mice and rats changes were observed for several parameters. In female mice exposed to 0, 10, 30, 100 mg/kg bw per day of *W. somnifera* root extract orally for 15 days, cellularity and plaque forming cells in lymphoid organs and type 4 immunological reaction increased in all dose groups after being immunized on day 9 (Malik et al., 2007, as cited by DTU). It was not reported whether *W. somnifera* was administered via the diet or using oral gavage. In the lowest and middle dose groups also spleen and thymus weights were elevated. The differences were only statistically significant for the thymus and no elevation was observed in the highest dose group. DTU (2020) noted that an increased immune response is an adverse effect, which is also seen in the studies in mice by Siddiqui et al. (2012), as cited by DTU and Kuswaha et al. (2012), as cited by DTU and a study in rats (Rasool & Varalakshmi, 2006, as cited by DTU). These three studies were not further discussed by DTU. In some of these studies, the effects were observed at all dose levels.

In two other studies using oral doses of *W. somnifera* root extract, changes in behaviour in mice (100 and 200 mg/kg bw per day) and in the forced swimming test (at 100, 200 and 500 mg/kg bw per day, but not at 0 or 50 mg/kg bw per day for 5 days) in rats were observed (Gupta & Rana, 2007; Kumar & Kalonia, 2007 as cited by DTU). It was not reported whether *W. somnifera* was administered via the diet or

using oral gavage. These behavioural changes were interpreted as an reduction of anxiety and an antidepressant effect.

In a study exposing male albino rats to 0 or 250 mg/kg bw per day *W. somnifera* extract via drinking water for 4 weeks, decreased plasma cortisol levels, increased liver weights, decreased adrenal weights. All these changes were statistically significant (Sharma et al., 1986, as cited by DTU). No histopathological changes were observed. DTU (2020) noted that the quality of this study was low, since the study design had limitations and not all data was reported correctly.

In a study exposing rats daily to approximately 5 g of powdered *W. somnifera* in feed for 10 to 14 days, 5 out of 6 rats showed centrilobular hydropic degeneration in the liver, 4 out of 6 rats showed peribronchial and perivenous oedema in the lung and 5 out of 6 rats showed intratubular vascular congestion, tubular casts and tubular degeneration (Arseculeratne et al., 1981, 1985, as cited by DTU).
In one study, dose-dependent inhibition of acetylcholinesterase (AChE) (up to approximately 70%) in the brain of rats with scopolamine-induced Alzheimer's was found after daily oral exposure to *W. somnifera* root extract for 10 days (Visweswari et al., 2014, as cited by DTU). DTU (2020) noted that no more information was available and that no other studies were found in literature, which studied AChE inhibition after *W. somnifera* exposure.

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Several studies were identified in rats and mice. Studies performed by Sharma et al. (1986) and Arseculeratne et al. (1981) were also included in the risk assessment of DTU (2020) and are described above.

In rats with chemically induced Alzheimer's symptoms, oral exposure to 50 mg/kg bw per day of *W. somnifera* root extract (equimolar concentrations of withaferin A and sitoindoside) for 14 days increased concentration of acetylcholine, choline acetyltransferase activity and binding of muscarine receptors in frontal cortex and hippocampus (Bhattacharya et al., 1995, as cited by BfR). It was not reported whether *W. somnifera* was administered via the diet or using oral gavage. This effect was not observed after exposure to 20 mg/kg bw per day of *W. somnifera* extract, in control animals or in animals without chemically induced Alzheimer.

Two studies showed improved blood glucose levels and glycated haemoglobin levels and/or insulin levels and insulin sensitivity in rats with induced diabetes after exposure to 100-400 mg/kg bw per day of *W. somnifera* root extract orally (Answer et al., 2008, as cited by BfR) and by unknown route (Udayakumar et al., 2009, as cited by BfR). Also, 200 mg/kg bw per day *W. somnifera* root extract for 8 weeks increased haemoglobin concentration in the blood of diabetic rats to values of non-diabetic controls. Route of administration is not mentioned (Udayakumar et al., 2009, as cited by BfR). Several other studies investigated the effect of *W. somnifera* extract on haemoglobin levels in blood. However, studies using the same dose (100 mg/kg bw per day) and length (15 days) showed inconsistent results in mice, where the blood haemoglobin

concentration increased in one study (Ziauddin et al., 1996 as cited by BfR) but not in the other (Diwanay et al., 2004, as cited by BfR).

Literature search
Additional short-term and sub-chronic toxicity studies were identified. In a study of Gupta et al. (2022) BALB/c mice were orally administered 0, 10, 70 or 500 mg/kg bw per day withaferin A by gavage for 28 days. According to the authors, the study was performed following the OECD guideline 407. The mice were observed daily for signs of toxicity and weighed every week. At the end of the experiment, blood samples were collected for haematological and biochemical investigations and organs were collected for histopathology. No mortality or other signs of toxicity were observed in any of the dose groups. Body weight was not different between the dose groups. No statistical differences were observed in the haematological and biochemical investigations, organs weights and histopathology. The authors considered 500 mg/kg bw per day withaferin A as the NOAEL, which was the highest dose tested (Gupta et al., 2022).

In a study of Balkrishna et al. (2022) Sprague Dawley rats (n=10) were administered 0, 100, 300 or 1000 mg/kg bw per day of *W. somnifera* whole plant extract by oral gavage for 28 days. According to the authors, the studies were performed according to OECD guideline 407. The rats were monitored daily for clinical abnormalities and twice a day for morbidity and mortality during the study. Once a week, body weight and feed consumption were measured and a detailed clinical observation was conducted, including changes of the skin, fur, mucous membranes and eyes, observations of autonomic activity and occurrence of secretions and excretions. Ophthalmoscopic examinations were conducted in the control and highest dose group in week 4 of the study. On day 29 and 43, blood samples were collected for haematological and clinical chemical investigations. In the last week of treatment, urine samples were collected and colour and clarity of the urine and the parameters bilirubin, glucose, protein, pH and specific gravity were evaluated. At the end of the study, gross pathology was performed and organs were weighted. In the control and highest dose group also microscopic pathology was performed. No mortality, abnormal clinical symptoms, ophthalmoscopic changes or changes in urinalysis parameters were observed in any of the study groups. No differences were observed in body weight or feed consumption between the groups. Some haematological parameters including mean corpuscular volume (male), haematocrit (female), white blood cell count (male) and platelet count (male) showed statistically significant differences. However, these results could not be linked to the treatment, as these findings were only observed in the mid-dose group. Mean corpuscular volume and mean corpuscular haemoglobin concentration were statistically significantly different in females at all dose levels, but some doses showed a statistically significant increase and other doses a statistically significant decrease. In both males and females of the high dose group, prothrombin time was statistically significantly decreased, although the values were within the range of normal values for this rat species. Some clinical chemistry parameters including total bilirubin levels (female), cholesterol levels (male) and Na^+ levels (male) were statistically significantly different, but the effects were not dose-dependent and

therefore not considered to be treatment-related. In males, total bilirubin and albumin levels were increased at the highest dose level, but the values were in the normal range of this rat species. The same was seen for Na^+ levels in females. No gross or histopathological changes were observed and organ weights were similar among the different groups. The authors derived a NOAEL of 1000 mg/kg bw per day, the highest dose tested (Balkrishna et al., 2022).

In a double-blind study of Kaur et al. (2022), stressed dogs were given 15 mg/kg bw per day *W. somnifera* root extract (n=12) or placebo (n=12) via their regular meal at night for 4 weeks. The dog owner was asked to report adverse effects in the dog. Furthermore, blood samples were collected to measure concentrations of haemoglobin, platelet count, alanine transaminase, alkaline phosphatase, serum creatinine, and total thyroxine (T_4). Dog owners reported no adverse effects of the treatment in dogs included in the treatment group. In the treatment group, the haemoglobin concentration was statistically significantly increased (+7%), the respiratory rate was statistically significantly reduced (-16%) and the T_4 concentration was significantly increased (22%) at the end of the trial compared to start of the trial. These parameters were not statistically significantly different between treatment and control groups, except for the respiratory rate. All haematological and biochemical parameters were within the normal range in the treatment and control groups. Stress and anxiety responses were statistically significantly reduced in treatment dogs compared to control (Kaur et al., 2022).

Table 7 Results of the short-term and sub-chronic toxicity studies.

Test compound	Species; sex	Dose[a] (mg/kg bw per day)	no. per group[b]	Route	Duration (days)	Effect	Reference
Withaferin A	Mice; F	0, 10, 70 or 500	5	Gavage	28	No adverse effects were observed, NOAEL of 500 mg/kg bw per day was derived	Gupta et al., 2022
W. somnifera root extract	Mice; NR	100, 200	NR	Oral	NR	Changed behaviour**	Kumar & Kalonia, 2007, as cited by DTU
W. somnifera root extract	Mice; F	0, 10, 30, 100	6	Oral	15	Increased immune response in all dose group**	Malik et al., 2007, as cited by DTU
W. somnifera extract	Mice; NR	NR	NR	NR	NR	Effect on immune system,** no further information available	Siddiqui et al., 2012, as cited by DTU
W. somnifera extract	Mice; NR	NR	NR	NR	NR	Effect on immune system,** no further information available	Kuswaha et al., 2012, as cited by DTU
W. somnifera root extract	Mice; NR	100	NR	NR	15	Increased haemoglobin concentration**	Ziauddin et al., 1996, as cited by BfR
W. somnifera root extract	Mice; NR	100	NR	NR	15	No effect on haemoglobin concentration	Diwanay et al., 2004, as cited by BfR
W. somnifera root extract	Rat; M/F	0, 500, 1000, 2000	12	Gavage	28	No adverse effects were observed, NOAEL of 2 g/kg bw per day was derived.	Prabu et al., 2013 as cited by DTU
W. somnifera whole plant extract	Rat; M/F	0, 100, 300 or 1000	10	Gavage	28	No adverse effects were observed, NOAEL of 1 g/kg bw per day was derived.	Balkrishna et al., 2022
W. somnifera root extract	Rat; M/F	0, 500, 1000, 2000	12	Gavage	28	No adverse effects were observed	Platel et al., 2016, as cited by DTU

Test compound	Species; sex	Dose[a] (mg/kg bw per day)	no. per group[b]	Route	Duration (days)	Effect	Reference
W. somnifera whole plant in liquid	Rat; M	0, 250	23, 35	Drinking water	28	Decreased plasma cortisol levels*, increased liver weight* and decreased adrenal weights*	Sharma et al., 1986 as cited by DTU
W. somnifera dried whole plant	Rat; NR	± 5 (g)	6	Diet	10-14	Centrilobular hydropic degeneration in the liver, peribronchial and perivenous oedema in the lung, intratubular vascular congestion, tubular casts and tubular degeneration was observed in most animals	Arseculeratne et al., 1981, 1985, as cited by DTU
W. somnifera root extract	Rat; M	NR	5	Oral	10	Inhibition of acetylcholinesterase in the brain of rats with scopolamine-induced Alzheimer's**	Visweswari et al., 2014, as cited by DTU
W. somnifera root powder in liquid	Rat; NR	NR	NR	NR	NR	Effect on immune system,** no further information available	Rasool & Varalakshimi, 2006, as cited by DTU
W. somnifera root extract	Rat; NR	0, 50, 100, 200, 500	7	Oral	5	Changes in forced swimming test at three highest concentration**	Gupta & Rana, 2007, as cited by DTU
W. somnifera root extract	Rat; NR	0, 20, 50	NR	Oral	14	Highest dose increased ACH levels, ChAT activity and binding of muscarine receptors in rats with Alzheimer symptoms**	Bhattacharya et al., 1995, as cited by BfR
W. somnifera root extract	Rat; NR	200, 400	NR	Oral	NR	Improved blood glucose, glycated haemoglobin and insulin levels and insulin sensitivity in rats with induced diabetes**	Anwer et al., 2008, as cited by BfR

Test compound	Species; sex	Dose[a] (mg/kg bw per day)	no. per group[b]	Route	Duration (days)	Effect	Reference
W. somnifera root extract	Rat; NR	100, 200	NR	NR	64	Lowered blood glucose and glycated haemoglobin levels in rats with induced diabetes**	Udayakumar et al., 2009, as cited by BfR
W. somnifera root extract	Rat; NR	200	NR	NR	64	Haemoglobin concentration in diabetic rats increased to the level in non-diabetic rats**	Udayakumar et al., 2009, as cited by BfR
W. somnifera root extract	Dog; M/F	0, 15	12	Diet	28	Reduced respiratory rate*; Increased haemoglobin* and T_4 concentration*	Kaur et al., 2022

M, male; F, female; NR, not reported; ACH, acetylcholine; ChAT choline acetyltransferase
[a] Dose does not include control group.
[b] Total number per test group includes both male and female animals.
*effect is statistically significantly different
**unknown if differences were statistically significant.

Summary
Table 7 summarizes the results of the short-term and sub-chronic toxicity studies presented in this section. Three 28-day toxicity studies, performed according to OECD guideline, in rats with 0-2 mg/kg bw per day *W. somnifera* extract and one 28-day toxicity studies in mice with 0-500 mg/kg bw per day withaferin A did not show adverse effects, only variation in some parameters which were still within normal limits according to the authors. In three of these studies a NOAEL was derived by the study author. Gupta et al. (2022) derived a NOAEL of 500 mg/kg bw per day for withaferin A. Furthermore, Balkrishna et al. (2022) derived a NOAEL of 1000 mg/kg bw per day and Prabu et al. (2013), as cited by DTU derived a NOAEL of 2000 mg/kg bw per day for *W. somnifera* extract. In all three studies the NOAEL was the highest dose tested.
In other non-guideline short-term toxicity studies (ranging from 5 days to 4 weeks) in mice and rats statistically significant changes were observed in several parameters, but parameters were mostly only studied in a single study. DTU (2020) identified effects such as changes in organ weights, liver and lung cells, immune response and AChE activity. However these parameters were only studied in one study. Most of the studies identified by BfR (2013) found changes of several parameters in animals with induced diseases such as Alzheimer's or diabetes. However, it is unknown if *W. somnifera* extract would induce the same changes in healthy individuals. In the literature search, one additional non-guideline short-term toxicity study was identified. In this study, reduced respiratory rate and increased haemoglobin and T_4 concentration were observed in dogs after a high dose of *W. somnifera* root extract.

5.2.3 *Genotoxicity*

No genotoxicity studies were described in BfR (2013) and DTU (2020).

Literature search
Modi et al. (2022) performed an *in silico* study, which predicted negative results for the AMES test for different constituents of *W. somnifera*.

In addition, Modi et al. (2022) performed a chromosomal aberration assay with *W. somnifera* extract following the OECD 473 guideline, according to the authors. The specific type of cells used for this experiment was not mentioned. The *W. somnifera* extract was quantified and contained Withaferin A (0.96%), Withanoside V (0.91%), Withanoside IV (0.77%), Withanolide A (0.51%), 12-Deoxy-withastramonolide (0.30%), Withanolide B (0.16%) and Withanone (0.004%). *W. somnifera* extract was tested at concentrations of 0.25, 0.5 and 1.0 mg/mL with and without metabolic activation. The negative control showed 5% chromosome aberrations with and without metabolic activation. For the positive control the percent chromosome aberrations were 52 and 21% for cyclophosphamide (with metabolic activation) and mitomycin C (without metabolic activation), respectively. The different doses of *W. somnifera* extract showed chromosomal aberrations of 5% with and without metabolic activation. The authors concluded that *W. somnifera* extract did not induce chromosomal aberrations (Modi et al., 2022).

Furthermore, in a *Salmonella typhimurium* reverse mutation assay (AMES) test *W. somnifera* extract was tested using strains *S. typhimurium* TA 1537, TA 1535, TA 98, TA 100, and TA 102 with and without rat S9 mix. The doses tested were 0.016, 0.051, 0.158, 0.501 and 1.582 mg/plate. The authors concluded that none of the doses of *W. somnifera* extract showed a statistically significant increase in colony numbers (Modi et al., 2022). The authors did not mention which compounds were used as positive and negative controls. Furthermore, the results were not given in the report.

Siddiqui et al. (2021) studied a possible mode of action of *W. somnifera* in a DNA cleavage assay and a competition assay. The study focused on withanone, a withanolide of W. somnifera with structural features more often associated with adverse drug reactions. It was found that withanone can form non-labile DNA adducts with nuceolotides deoxyguanosine, deoxyadenosine, deoxycytidine (but not deoxythymidine) and DNA, in particular under conditions of limited glutathione (GSH) levels. It was found that withanone can form adducts with GSH, which could be a detoxification pathway. However, when GSH and DNA were present simultaneously, withanone could still form adducts with the DNA. The formation of adducts occurs at both the epoxide functional group and the electrophilic Michael acceptor of withanone with primary amines of other molecules. The DNA adducts caused by withanone can effect DNA transcription, replication and repair eventually leading to cell death.

Summary
Two *in vitro* genotoxicity studies, a chromosomal aberration assay and a bacterial reverse mutation assay with *W. somnifera* extract were available as well as an *in silico* study. None of the experiments indicated a risk for genotoxicity. However, the reporting was limited for both *in vitro* assays. Furthermore, one study showed that withanone, a constituent of *W. somnifera* extract may form DNA adducts, in particular under conditions of limited glutathione levels.

5.2.4 *Chronic toxicity and carcinogenicity*

No chronic toxicity and carcinogenicity studies were described in BfR (2013) and DTU (2020) and none were identified in the literature search.

5.2.5 *Reproduction and developmental toxicity*

No reproduction and developmental toxicity studies were described in BfR (2013). DTU (2020) described several reproduction and developmental toxicity studies. No additional studies were identified in the literature search.

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Most of the studies were conducted in male rats and investigated the effect of *W. somnifera* on sex hormones, sex organs, sperm quality and mating behaviour.

Kiasalari et al. (2009) administered feed with 0 (n=8) or 6.25% (n=11) dried *W. somnifera* root daily to male rats for 4 weeks. Sex hormone levels were measured in the blood after 4 weeks. Oestrogen levels in

serum were not affected. Follicle-stimulating hormone (FSH) levels were statistically significantly decreased, whereas progesterone, testosterone and luteinizing hormone (LH) levels were statistically significantly increased (Kiasalari et al., 2009 as cited by DTU).

Ganu et al. (2010) administered a target dose of 0, 100, 200 and 400 mg/kg bw per day dried *W. somnifera* root via drinking water to male rats (6 per group) daily for 4 weeks. At the end of the treatment period, body weight was measured and sperm cells and organs were collected from 4 animals per group. Body weight was statistically significantly increased in the highest dose group compared to the controls. The number of sperm cells was statistically significantly increased in alle groups dosed with *W. somnifera* compared to the controls. The weights of the testis and prostate were statistically significantly higher in the mid and high dose group compared to the controls. The same article describes a study in mice. Male mice (n=6) were orally administered a single dose of *W. somnifera* (100-400 mg/kg bw). After 30 minutes mating behaviour was increased (Ganu et al., 2010 as cited by DTU).

Sahin et al. (2016) administered 0 or 300 mg/kg bw per day of *W. somnifera* root extract via drinking water to male rats (7 per group) for 8 weeks. After the treatment period, mating behaviour, number of sperm cells and testosterone levels were all statistically significantly increased compared to the controls. Other parameters such as weight of prostate, seminal vesicles, testis and epididymis did not change (Sahin et al., 2016 as cited by DTU).

Abdel-Magied et al. (2001) administered 0 or 470 mg/kg bw per day *W. somnifera* leaf extract by gavage to young male rats (aged 20 days) for 6 days. Formation of sperm cells was seen in the *W. somnifera* group but not in the control group. Testosterone and FSH levels were statistically significantly lower compared to the controls. No change in LH levels was observed (Abdel-Magied et al., 2001 as cited by DTU).

Singh et al. (2013) administered 0, 25 or 50 mg/kg bw per day of *W. somnifera* stem extract by gavage to male rats for 60 days. The rats were mated with female rats (2 females per rat) five days before the end of the trial. The number of pregnant females was dose-dependently reduced from 100% in the control group to 67% and 29% in the 25 and 50 mg/kg dose group, respectively. In both dose groups the formation of sperm cells was stopped and the number of sperm cells was dose dependently decreased. In the highest dose group also sperm motility was reduced. Testosterone and FSH levels were statistically significantly lower in the highest dose group. No change in LH levels was observed. DTU noted that only a small number (6 males/dose) of animals was included in this study (Singh et al., 2013 as cited by DTU).

Mali et al. (2008) orally administered 50 mg/kg bw per day *W. somnifera* fruit extract to male rats for 60 days. It was not reported whether *W. somnifera* was administered via the diet or using oral gavage. The number of sperm cells and sperm motility decreased in the dose group. Also the weights of testis, seminal vesicles and other accessory genitalia were reduced and histopathological changes of the testes were observed (not further specified). It is unknown if the

findings at the end of the treatment were compared to pre-treatment values or a control group. DTU noted that important information such as number of rats per group, the inclusion of a control group and quantitative results are lacking in the study description (Mali et al., 2008 as cited by DTU).

Ilayperuma et al. (2002) orally administered 0 or 3 g/kg bw per day *W. somnifera* root extract to male rats (20 per group) for 7 days. It was not reported whether *W. somnifera* was administered via the diet or using oral gavage. Parameters of mating behaviour were decreased 3, 7, 14 and 30 days after treatment compared to pre-treatment values. DTU noted that no data for the parameters was shown (Ilayperuma et al. 2002 as cited by DTU).

Al-Qarawi et al. (2000) administered 0 or 470 mg/kg bw per day of *W. somnifera* leaf extract via oral gavage to young (17- and 25-day old) female rats (10 per group) for 6 days. At the end of treatment a higher body weight was observed in 17-day old rats and higher ovary weights and FSH levels in blood in 25-day old rats. LH levels were not different in both groups of rats. The higher ovary weights in 25-day old rats were a result of development of follicles in the organ, a sign of early sexual maturation (Al-Qarawi et al., 2000 as cited by DTU).

In a reproduction toxicity study Sharma et al. (1986) administered 0 or 100 mg/kg bw per day *W. somnifera* whole plant extract to male and female rats via drinking water for 8 months. The adult animals in the dose group statistically significantly gained more weight compared to the control group. The litter size and number of pregnant animals was not affected. However, the body weights of the offspring were increased in the dose group (70g) compared to the control group (45g) one month after birth. DTU noted the results were not tested for statistical significance, small groups (4 females/2 males) were included in the study and results were given for male and female offspring combined (Sharma et al., 1986 as cited by DTU).

Prabu & Panchapakesan (2015), exposed female rats (25 per group) to 0, 0.5, 1 or 2 mg/kg bw per day of *W. somnifera* root extract by oral gavage from gestational day (GD) 5 to 19. According to the authors, the study was performed following OECD guideline 414. No deaths or toxic effects were observed for mothers or offspring. The only statistically significant difference was an increased body weight and uterus weight of the mothers in the highest dose group. Also, the number of live births was higher in the highest dose group, which could explain the increased body weight and uterus weight (Prabu & Panchapakesan 2015 as cited by DTU).

Summary
The effect of *W. somnifera* on sex hormones in male rats was measured in four different studies. Not all hormones were measured in all 4 studies. Testosterone levels were increased in two studies, whereas a decrease in testosterone levels was observed in the other two studies. LH levels were increased in only one study and remained unchanged in two other studies. FSH levels were reduced in three studies. Oestrogen and progesterone levels were only measured in one study, which

showed no change in oestrogen levels and an increase in progesterone levels. The effect of *W. somnifera* on the weight of the testis was measured in three studies. In one study the weight increased, whereas a decrease was observed in the other studies. Organ weights of the prostate, epididymis, seminal vesicles and accessory genitalia were measured in two studies. In one study the weight of the prostate was increased, whereas the weight of the other organs was decreased, whereas no changes were observed in the other study. Four studies investigated the effect of *W. somnifera* on parameters of sperm quality. In two studies the number of sperm cells was increased, whereas the number of sperm cells and the motility of sperm cells was decreased in the other two studies. The effect of *W. somnifera* on mating behaviour was studied in three studies. In two studies mating behaviour increased, whereas it decreased in the other study. The effect of *W. somnifera* on sexual maturation was studied in both males and females rats. In both genders early sexual maturation was observed. *W. somnifera* induced sperm formation in 20-days old male rats and the development of follicles in ovaries of female rats. The two (limited) studies investigating reproductive and developmental toxicity, did not show adverse effect after *W. somnifera* exposure on prenatal development. The effect of *W. somnifera* in early pregnancy was not studied, as the studies started on day 5 of gestation.

Overall, contradictory results were found in male reproductive studies. The quality of some of the above mentioned studies is poor, although this seems not the (sole) reason for the contradictory results. In the different studies, doses varied and plant material/extracts were from different plant parts. Species differences did not cause the contradictory results as most studies were conducted in rats, except for one study in mice. There are some slight indications that *W. somnifera* root has different effects compared to the other plants parts. However, this could not be confirmed in all studies, so other reasons, for example the poor quality of some studies and different dose ranges, could also cause the contradictory results. Therefore, the specific reason for the contradictory results found in male reproductive studies is unknown.

5.2.6 *Effects on thyroid hormones*

No studies were identified in the literature search.

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In one study healthy rats and rats with chemically induced lower thyroid hormone levels were orally exposed to 0 or 0.5 g/kg bw per day of *W. somnifera* root extract for 1 month (Abdel-Wahhab et al., 2019, as cited by DTU). It was not reported whether *W. somnifera* was administered via the diet or using oral gavage. In rats with hypothyroidism, *W. somnifera* extract statistically significantly increased levels of free and total triiodothyronine (T_3) and T_4. In healthy rats, free T_3 and free and total T_4 levels were increased after exposure to *W. somnifera* extract, however this was not significant.

Another study was identified which measured thyroid hormones in male and female mice after exposure to 1.4 g/kg bw per day of *W. somnifera* root extract by oral gavage for 20 days (Panda & Kar, 1998; Panda & Kar, 1999 as cited by DTU). In males, both T_3 and T_4 levels were

statistically significantly increased. In females only T_4 levels statistically significantly increased.
In roosters, only T_4 levels increased after daily exposure to 20 mg of *W. somnifera* root extract for 30 days (Panda & Kar, 1997 as cited by DTU).

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Panda & Kar (1998) and Panda & Kar (1999) were also described in the risk assessment of DTU (2020) as described above.

Rats with chemically induced lower thyroid hormone levels were orally exposed to 1.4 g/kg bw per day of *W. somnifera* root extract for 15 days (Jatwa & Kar, 2009, as cited by BfR). It was not reported whether *W. somnifera* was administered via the diet or using oral gavage. The study showed statistically significantly increased serum T_3 and T_4 levels in the *W. somnifera* group compared to the control group with chemically induced lower thyroid hormone levels. The serum T_3 and T_4 levels increased to the levels seen in non-treated healthy animals.

Summary
Increased levels of thyroid hormones are observed in rats, mice and roosters after exposure to 20 mg to 1.4 g *W. somnifera* root extract. However in rats, this increase was only significant in rats with chemically induced hypothyroidism but not in healthy animals.

5.2.7 *Human data*
The Dutch Poisons Information Centre (Nationaal Vergiftigingen Informatie Centrum, NIVC) and the Netherlands Pharmacovigilance Centre Lareb were contacted to gather information on *W. somnifera*.

NVIC received several notifications on *W. somnifera*. However, the complaints such as hyperventilation and nauseousness could not be supported by scientific literature.

Lareb also reported several notifications on *W. somnifera*. They recently published an article on four cases of liver injury after use of *W. somnifera* products on their website[13]. In all cases the patient recovered after withdrawal of the food supplements. Two cases of hepatitis were reported by a physician. In both cases, multiple food supplements were used simultaneously, which also contained other ingredients including (several) herbs. Not enough information was available to conclude on the role of *W. somnifera* in the liver injury. In addition, two cases of abnormal liver function were reported by a physician. The first patient, a 60-70 year old female with a cognitive disorder, also used food supplements containing vitamin C and B. There was no history of liver function disorder or alcohol use, however the patient had an Epstein-barr virus infection in the past. Hepatitis A, B, C, E and autoimmune hepatitis were excluded. No tests were conducted for the herpes simplex virus or the cytomegalovirus. It was unknown if the food supplement contained contaminants. The injury was classified as a hepatocellular hepatic injury. The role of *W. somnifera* in the liver injury was judged probable, using the RUCAM (Roussel Uclaf Causality Assessment Method) score. The second patient, a 30-40 year old female, did not take other food supplements, but used the medication lorazepam for

[13] https://www.lareb.nl/news/leverschade-bij-producten-met-ashwagandha

which abnormal liver function is a reported adverse effect. The patient did not drink alcohol and was vaccinated for hepatitis B. The patient had an Epstein-barr virus and a cytomegalovirus infection in the past. Hepatitis C was excluded. Other possible causes were not tested. It was unknown if the food supplement contained contaminants. The injury was classified as a mixed hepatic injury. The role of *W. somnifera* in the liver injury was judged unlikely, using the RUCAM score, but important information was missing.

5.2.7.1 Case reports

No case reports were identified in the risk assessment conducted by BfR (2013).

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A healthy Dutch woman suddenly developed several health complaints and got diagnosed with thyrotoxicosis (Van der Hooft et al., 2005). She used one capsule with *W. somnifera* extract (250 mg) every now and then for a period of 6 weeks without any complaints. When she increased the intake to two capsules (500 mg) per day the health complaints began and she stopped using the food supplement. After 4 weeks, the thyroid hormone levels in blood were normalized and the health complaints disappeared. The supplements was analysed in a laboratory for compounds as iodine and thyroid hormones, but none were found. The patient used no medication or other food supplements. There was no family history of thyroid disorders and the symptoms were not compliant with known causes as Graves' disease, Plummer's disease, subacute thyroiditis or postpartum thyroiditis. Furthermore, the absence of thyroglobulin and microsomal autoantibodies suggests other causes than the above mentioned illnesses. The parameters normalized without the need for medical intervention (Van der Hooft et al., 2005).

Five cases are described by Björnsson et al. (2020) with three male and two female patients from the United States or Iceland. The intake levels varied from 450 to 1350 mg *W. somnifera* per day. The specific plant parts used and the preparation methods were not specified in the article. For four cases, the names of the supplements used were given in the article and the brands reported are currently on the market as *W. somnifera* root extracts. The onset of symptoms occurred 1 to 12 weeks after the first intake. The patients were diagnosed with liver injury and experienced symptoms as jaundice, nausea, abdominal pain, pruritus and fatigue. After quitting the use of the supplements, in three patients liver parameters normalized within 1 to 5 months and in one patient after 9 months. One patient was lost to follow up. The supplements were analysed in a laboratory for toxic compounds (not further specified) and trace metals. Nothing was found, but the presence of *W. somnifera* was confirmed. None of the patients used prescribed potentially hepatotoxic medication. Four of the patients used other food supplements. The ingredients of the food supplements were evaluated and only one ingredient, rhodiola which was present in a supplement used by one patient, was flagged as potentially hepatotoxic. Causes such as acute hepatitis A, B and C (in only one patient) and cytomegalovirus were excluded. One patient had increased antinuclear antibody and smooth muscle antibody. After careful consideration, the role of *W. somnifera* supplements in the liver injuries was judged definite in one case, highly

likely in two cases, probable in one case and possible in one case. The severity of symptoms differed (Björnsson et al., 2020).

Mohan et al. (2020) responded to the above mentioned study. According to these researchers, the hepatotoxic potential of *W. somnifera* presented in the study was only an assumption. In the letter it was pointed out that hepatitis C and hepatitis E were not excluded as a cause of liver injury and that the higher antibodies levels found in one case eliminated drug-induced liver injury (DILI) as a cause of liver injury. Furthermore, as several cases took multiple food supplements the effects could be due to an interaction of different compound. Also, the phytoconstituents present in the food supplements were not well described and often herbal preparations are mislabelled in food supplements (Mohan et al., 2020). The authors of the original article responded to the letter. According to Björnsson et al. (2020) increased antinuclear antibody and smooth muscle antibody could also occur in patients with DILI and did therefore not eliminate DILI as a cause of liver injury. Furthermore, the type of liver injury observed is unlikely for hepatitis and hepatitis E has a low prevalence in Iceland, where three of the cases lived. Also, discontinuation of *W. somnifera* supplements normalized serum parameters, without steroid or immunomodulatory treatment. This is unlikely for autoimmune hepatitis (Björnsson et al., 2020).

The article of Björnsson et al. (2020) refers to one case from Japan, described in an article written in Japanese (Inagaki et al., 2017). In this case a patient used four anti-anxiety drugs in combination with *W. somnifera* for 10 months. After the patient increased the intake of *W. somnifera* two to three fold, symptoms started. The intake level of *W. somnifera* and symptoms were not reported. The liver injury showed some similarities as with injuries described in Björnsson et al. (2020). After the patient quit taking *W. somnifera* the symptoms worsened at first, but normalized in 3,5 months during treatment with ursodeoxycholic acid and phenobarbital. Inagaki et al. (2017) excluded other known causes of liver injury (Björnsson et al., 2020).

Literature search
A 41-year old woman with chronic pain used the tricyclic antidepressant amitriptyline daily to relieve pain (Fry et al., 2022). Her morning cortisol levels was measured at 480 nmol/L. Shortly after the measurements, the woman started taking 860 mg *W. somnifera* extract (21.4 mg withanolides) per day. After 10 weeks a short Synacthen test was performed, where a cortisol level of 287 nmol/L was measured and the response to an intramuscular injection of Synacthen was minimal (T_{30min}: 289 nmol/L and T_{60min}: 328 nmol/L). The doctors questioned why the cortisol levels had dropped and found out about the *W. somnifera* supplements. The woman was asked to stop taking the supplements and another test was performed two weeks later. The response to an intramuscular injection of Synacthen was normal (T_{0min}: 275 nmol/L, T_{30min}: 623 nmol/L and T_{60min} 674 nmol/L). The patient had never taken opioid-based drugs or steroid and stopped taking the oral contraceptive pill a year before. Furthermore, the patient did not drink excessively or smoke. This case linked the usage of *W. somnifera* root extract supplements to the suppression of the adrenal function. The suppression

could be reversed after stopping of supplementation. In the study it was determined that there was no co-exposure with medication or lifestyle. A subnormal adrenal response could lead to adverse effects after an acute stressor as injection or illness (Fry et al., 2022).

A 39-year old female had suffered from jaundice and experienced nausea for one week before seeking medical advice (Ireland et al., 2021). She had not been abroad recently, did not take medication, had been sober for three months and did not show signs of chronic liver disease. Previously, the woman had been drinking excessively for five years. She had been taking a herbal supplement containing 154 mg *W. somnifera* root extract, basil powder and biotin every second day for six weeks to reduce anxiety. Blood tests confirmed a hepatocellular pattern of liver injury. Further analysis ruled out hepatitis A, B, C and E, HIV and Covid-19. Also, caeruloplasmin, immunoglobulins A, G and M, ferritin and alpha fetoprotein levels in the blood were within normal range and acute viral hepatitis was ruled out. Autoimmune hepatitis remained a possible diagnosis, but the authors concluded that DILI was more likely. The food supplement also contained basil powder and biotin, however hepatotoxicity or other adverse effect are not known for these ingredients. Following an assessment method for drug-induced liver injury, *W. somnifera* was scored a probable cause of the injury. As bilirubin levels in the blood continued to rise and severe acute cholestatic hepatitis with confluent necrosis was observed during liver biopsy, the woman was treated with ursodeoxycholic acid. She was also advised to stop using supplements containing *W. somnifera*. During the first two weeks, blood parameters slowly improved. After two weeks the symptoms of nausea and jaundice were resolved. The type of liver injury in this case is different from the cases described in Björnsson et al. (2020), as those cases showed mixed and cholestatic patterns of liver injury and this case hepatocellular patterns. This is not unlikely (Ireland et al., 2021).

5.2.7.2 Clinical trials

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Eight clinical trials were identified with the aim to assess the efficacy of *W. somnifera* root (Tabel 7). None of the studies investigated safety of the supplement as the main objective.

In one trial, 57 healthy men were exposed daily to 600 mg *W. somnifera* root extract or a control for 8 weeks to study effects on muscle strength (Wankhede et al. 2015 as cited by DTU). At the end of the trial, a statistically significant higher change (increase) of testosterone level was observed in the treatment group compared to the controls. However, DTU notes that variation in testosterone levels are high and no statistically significant effect was found when comparing the absolute testosterone values of both groups at the end of the trial. The only other parameter measured in blood was serum creatine kinase, which was statistically significantly lower at the end the trial in the treatment group compared to the control group. All other measured aspect were specifically related to muscle strength and fitness. According to the study authors no serious adverse effects were reported and no difference in the Physician Global Assessment of Tolerability to Therapy form (PGATT) score was observed between the groups. The adverse

effects were assessed using a system made for drug testing. According to DTU, this could affect the rating of the severity of symptoms, as mild adverse effect in drug testing are excepted, which should not be excepted for dietary supplements.
In four other trials, in total 361 infertile males were exposed daily to 675 mg *W. somnifera* root extract or 5 g root powder or a control for 3 months, respectively to measure the effect on different parameters, including testosterone, FSH and LH levels and sperm quality (Gupta et al., 2013; Ambiye et al., 2013; Mahdi et al., 2011; Ahmad et al., 2010 as cited by DTU). Testosterone and LH levels increased in the studies, FSH levels decreased and sperm quality increased. Most changes were statistically significant. In three trials, the measurements or occurrence of adverse effect was not mentioned. Only in one trial it was mentioned that adverse effect were recorded, but the results were not described.

In another trial 57 obese men were administered daily a *W. somnifera* root and leave extract containing 21 mg withanolide (60 mg extract) or a control for 8 weeks to measure the effect on hormone levels (Lopresti et al., 2019 as cited by DTU). No differences in adverse effect were observed between treated and untreated men. It was not mentioned which adverse effects were measured. For the hormone levels, a large daily variation was observed, which made it difficult to draw firm conclusions.

In another trial 50 people with hypothyroidism received daily 600 mg *W. somnifera* root extract or a control for 8 week to measure the effect on thyroid hormones (Sharma et al., 2018 as cited by DTU). The serum levels of TSH, T_3 and T_4 statistically significantly increased compared to the control group towards normal values for these hormones. No adverse effects were reported.

In the last trial, 60 people with bipolar disorder were administered daily 0.5 g *W. somnifera* extract or a control for 8 weeks (Chengappa et al., 2013). Levels of TSH, T_3 and T_4 were measured. Ten out of the 60 participants showed abnormal levels of at least one of the hormones at the start or at the end of the trial and were included to review the effect on thyroid hormones (Gannon et al., 2014 as cited by DTU). After 8 weeks, all included subjects (3) from the treatment group showed increased (7-24%) free T_4 levels. In the placebo group 6 out of 7 included subjects showed decreased (4-29%) and one showed increased (15%) T_4 levels. The differences were not tested for statistical significance. In the participants with normal levels of thyroid hormones, no statistically significant differences were observed between the treatment and control group, even though the increase in free T_4 was slightly higher in the treatment group. The study authors concluded that the results indicate that *W. somnifera* can cause T_4 levels to increase.

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Six clinical trials were identified, one of which was not published (Table 8). In three studies the root of *W. somnifera* was used while in the other studies the plant part used was unknown. In the studies using *W. somnifera* root, the participants received either 3 g of *W. somnifera* powder per day for 30 days or one year or 12 mL of *W. somnifera* extract per day for 5 days (Mikolai et al., 2009; Andallu & Radhika,

2000; Kuppurajan et al., 1980 as cited by BfR). Adverse effects were only mentioned in one of the three trials and were not different between the control and exposed group. However it is unclear which adverse effects were measured. In healthy middle aged males, 3 g *W. somnifera* powder per day for one year resulted in increased haemoglobin levels compared to pre-treatment values (Kuppurajan et al., 1980 as cited by BfR). In 12 people with diabetes, 3 g of *W. somnifera* powder per day for 30 days resulted in statistically significant reduced blood glucose levels compared to pre-treatment values (Andulla & Radhika 200 as cited by BfR). In 5 healthy adults, daily 12 mL *W. somnifera* root extract for 5 days resulted in a statistically significant increase of several immune cells (Mikolai et al., 2009 as cited by BfR). However no control group was included in this study.

In the studies for which the plant part used is unknown, the given doses ranged from 0.5 to 2.5 g of *W. somnifera* extract per day for 6 weeks or 18 months or 2 g of *W. somnifera* powder was given per day for 60 days (Andrade et al., 2009; Andrade et al., 2000; Venkataraghavan et al., 1980 as cited by BfR). Adverse effects were only mentioned in two of the three trials. In one study no difference was observed, but it is unclear which adverse effects were measured. In the other trial (1 - 2.5 g/day for 6 weeks), adverse effects were well reported. Slightly more symptoms as sleep problems, light-headedness and a heavy head were observed in the exposed group, although it was not statistically significantly different from the control group (Andrade et al., 2000 as cited by BfR). In 13 children, daily administration of 2 g of *W. somnifera* powder for 60 days resulted in increased haemoglobin levels and mean corpuscular haemoglobin compared to pre-treatment values (Venkataraghavan et al., 1980 as cited by BfR).

Literature search
Fourteen new clinical trials were identified (Table 7). In six trials no specific parameters to assess the safety of *W. somnifera* were mentioned (Sing et al., 2022; Remenapp et al., 2022; Gopal et al., 2021; Fuladi et al., 2021; Atul et al., 2020 and Gannon et al., 2019). In three of these trials the authors reported that no adverse effects were observed during the study after daily administration of 225 to 500 mg *W. somnifera* extract or 6 g *W. somnifera* powder for 12 weeks or 30 days (Sing et al., 2022; Remenapp et al., 2022 and Atul et al., 2020). Fuladi et al. (2021) reported that no statistically significant difference for the occurrence of gastrointestinal upset and vomiting, headache, fatigue and drowsiness or rare side-effects was seen between the control and treatment group receiving daily administration of 2 or 3 g *W. somnifera* extract for 8 weeks. Gopal et al. (2021) mentioned similar occurrence of mild and temporary adverse effects as abdominal discomfort, abdominal pain and nausea in both the control and treatment group receiving daily administration of 600 mg *W. somnifera* extract for 8 weeks. Also mild adverse effects were observed by Gannon et al. (2019). Loose stool or diarrhoea, epigastric discomfort or stomach pain and somnolence were more commonly reported in the treatment group after daily administration of 1 g *W. somnifera* extract for 12 weeks, although this difference was not statistically different. In all the above mentioned trials other effects were observed including increased lung function (sing et al., 2022) reduced anxiety (Fuladi et al., 2021; Gannon et al., 2019),

reduced depression (Gannon et al., 2019), decrease of menopausal symptoms (Gopal et al., 2021) and improved sleep quality (Atul et al., 2020).

In seven trials, specific parameters to assess safety of *W. somnifera* were mentioned (Gopukumar et al., 2021; Langade et al., 2021; Tharakan et al., 2021; Tiwari et al., 2021; Deshpande et al., 2020; Lopresti et al., 2019; Salve et al., 2019). Tharakan et al. (2021), only measured treatment-emergent adverse events to assess the safety of *W. somnifera*. In the other trials, this was combined with at least the measuring of vital signs and in most cases laboratory testing of haematological parameters (Langade et al., 2021; Deshpande et al., 2020; Lopresti et al., 2019) and/or biochemistry (Gopukumar et al., 2021; Deshpande et al., 2020; Salve et al., 2019). In one study it was mentioned that adverse effect were mild, unrelated to the treatment and not statistically significantly different between the control and treatment group daily receiving 120 mg *W. somnifera* extract for 6 weeks (Deshpande et al., 2020). In the study of Tiwari et al. (2021), one person daily receiving 600 mg *W. somnifera* for 8 weeks experience mild ear pain. All other parameters were within normal limits. In all the other trials no adverse effects were observed and the measured parameters were in a safe and normal range after daily administration of 60 mg to 600 mg *W. somnifera* extract for 30 to 90 days. In all the above mentioned trials other effects were observed including improved sleeping quality (Gopukumar et al., 2021; Langade et al., 2021; Deshpande et al., 2020; Salve et al., 2019, higher immunological response (Tharakan et al., 2021), improved cardiorespiratory endurance (Tiwari et al., 2021) and improved memory (Gopukumar et al., 2021) and reduced stress (Gopukumar et al., 2021; Lopresti et al., 2019; Salve et al., 2019) and reduced anxiety (Salve et al., 2019).

One trial was conducted specifically to test the safety of *W. somnifera* extract (Verma et al., 2021). In the trial 80 healthy males and females were administered 600 mg *W. somnifera* root extract or control daily for 8 weeks. During the study adverse effects were recorded based on self-reporting or identification during clinical examination. Furthermore, blood test were conducted to measure haemoglobin levels, platelet count, neutrophil count, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, TSH levels, T_3 levels and T_4 levels. All the values were within the normal references ranges. Furthermore, values measured in the control and treatment group were not statistically significantly different. No adverse effect were reported by the participants in both the control and treatment group during the treatment period (Verma et al., 2021).

5.2.7.3 Summary

In total 9 case reports were identified from the risk assessment of DTU (2020) and the literature search (Table 8), reporting one case of thyrotoxicosis, one case of suppression of adrenal function and seven cases of liver injury. The intake levels reported in these case reports ranged from 77 to 1350 mg *W. somnifera* extract per day. In the case reports causality could not be proven, but the authors of the articles judged the likeliness of the role of *W. somnifera* as definite in one case, highly likely in two cases, probable in two cases and possible in one

case. In three case reports this method was not used, although the causality was studied in every report.
In addition to the case reports, Lareb reported four notifications of liver injury after use of food supplements containing *W. somnifera*. Two of those cases were described in more detail and the likeliness of the role of W. somnifera was judged probable in one case and unlikely in the other, although for the latter case not enough information was available.

In total 28 different clinical trials were identified from the risk assessment of DTU (2020) and BfR (2013) and the literature search (Table 9). Overall, only some mild adverse effects were observed in the treatment groups of the different studies. In none of the studies the adverse effects were statistically different from the control group. Some of the studies did show reduced levels of cortisol, increased activity of the immune system and increased levels of thyroid hormones, which were perceived as positive effects by the study authors, but from a toxicological perspective are considered adverse. Many of the trials were conducted in people with health problems and most of the trials identified in the literature search were conducted in India. It is unknown if these results also apply to healthy people and people from other regions.

Not all adverse effects observed in the case reports were found in the clinical trials. The reason for this discrepancy remains unknown, as the doses used in the trial and intake levels described in the case reports are similar. Possibly, the duration of some trials was too short, but many trials have a duration in the same range as the onset of symptoms in the case reports. Perhaps other compounds caused the symptoms observed in the case reports, if the supplements taken in the case reports were not purely *W. somnifera* or other supplements or medication were used simultaneously. However, for six cases, the supplements were analysed. In five cases of liver injury, no known toxic compounds (not further specified) or trace metals were found and in the case of thyrotoxicosis the supplements was analysed for iodine and thyroid hormones. Also, for additional food supplements used in the case reports (besides *W. somnifera* supplements) it was determined if the supplement could potentially be involved in liver injury. Only in one case another compound present in the supplements was identified, which could possibly be involved in inducing liver injury. Furthermore, for eight cases it was mentioned that no medication was used or that the used medication could not have caused the effect.

Table 8 An overview of the case reports with food supplements containing W. somnifera extract

	Intake (per day)	**Onset**	**Duration of use**	**Diagnosis**	**Causality**	**Ref**
Healthy female	500 mg	Fast	>6 weeks	Thyrotoxicosis	Unknown	1
45-year old male	450 –1350 mg	7 days	2 weeks	Liver injury	Definite	2
24-year old male		12 weeks	13 weeks	Liver injury	Highly likely	2
62-year old female		5 days	2 weeks	Liver injury	Probable	2
61-year old female	500 mg	17 weeks	16 weeks	Liver injury	Possible	2
21-year old male	Unknown	2 weeks	2 weeks	Liver injury	Highly likely	2
Patient	Unknown	Unknown	Unkown	Liver injury	Unknown	2
41-year old female	860 mg	10 weeks	10 weeks	Suppression of the adrenal function	Unknown	3
39-year old female	77 mg	6 weeks	Unknown	Liver injury	Probable	4

1. (Van der Hooft et al., 2005) 2. (Björnsson et al., 2020) 3. (Fry et al., 2022) 4. (Ireland et al., 2021)

Table 9 Overview of trials with food supplements containing W. somnifera extract.

Study population	Dose	Duration	(Adverse) effects	Ref
DTU				
57 healthy men	600 mg (root extract)	8 weeks	Larger increase in testosterone and larger decrease in serum creatine kinase levels in treatment group compared to controls*. No adverse effects are reported.	1
46 healthy infertile men	675 mg (root extract)	3 months	No information on adverse effects. Increased sperm count and motility and semen volume compared to pre-treatment values *.	2
60 healthy infertile and 60 fertile (control) men	5 g (root powder)	3 months	No information on adverse effects. Changes in several sex-hormones and increased sperm quality in treatment group compared to pre-treatment values *.	3
75 healthy infertile and 75 fertile (control) men	5 g (root powder)	3 months	No information on adverse effects. Changes in several sex-hormones and oxidative biomarkers and increased sperm quality in treatment group compared to pre-treatment values *.	4
180 infertile men and 50 healthy (control) men	5 g (root powder)	3 months	No information on adverse effects. Changes in several sex-hormones and increased sperm quality in treatment group compared to pre-treatment values *.	5
57 healthy obese men	21 mg WG (root and leaf extract)**	8 weeks	The occurrence of adverse effects was reported, but the adverse effects were not specified. The incidence was not statistically significant different between control and treatment groups.	6
50 people with hypothyroidism	600 mg (root extract)	8 weeks	No adverse effects are reported. Serum levels of TSH, T_3 and T_4 increased in treatment group compared to the control group*.	7
60 people with bipolar disorder	500 mg (extract)	8 weeks	Adverse effects such as diarrhoea and sleepiness were reported, but the incidence was not statistically significant different between control and treatment groups. Increase of thyroid hormones in 10 people with abnormal low levels.	8

Study population	Dose	Duration	(Adverse) effects	Ref
BfR				
24 people with diabetes	3 g (root powder)	30 days	No adverse effects are reported. Reduced blood glucose levels in treatment group compared to pre-treatment values *.	9
141 healthy middle aged men	3 g (root powder)	1 year	No information on adverse effects. Increased haemoglobin levels in treatment group compared to pre-treatment values.	10
5 healthy adults	12 mL (root extract)	5 days	No information on adverse effects. Increased immune cells in treatment group compared to pre-treatment values *. No control group was included in this study.	11
26 healthy children	2 g (powder)	60 days	No information on adverse effects. Increased haemoglobin levels and mean corpuscular haemoglobin in treatment group compared to pre-treatment values.	12
50 people with anxiety	0.5 and 1 g (extract)	18 months	No adverse effects are reported.	13
39 people with anxiety	1 – 2.5 g (extract)	6 weeks	Adverse effects, such as sleep problems, light-headedness and a heavy head are reported, but the incidence was not statistically significant different between control and treatment groups.	14

Study population	Dose	Duration	(Adverse) effects	Ref
Literature search				
150 people with sleeping problems	42 mg WG (root and leaf extract)**	6 weeks	Adverse effects, such as fever and headache are reported, but the incidence was not statistically significant different between control and treatment groups. At the end of the trial, parameters for sleep quality were better in treatment group compared to control group*.	15
130 healthy stressed adults	300 mg (root extract)	90 days	No adverse effects are reported. Increased scores for memory, sleep and happiness and decreased cortisol levels in treatment group compared to control group*.	16

Study population	Dose	Duration	(Adverse) effects	Ref
80 healthy/insomnia patients	600 mg (root extract)	8 weeks	No adverse effects are reported. Measured parameters for anxiety and sleep improved in both treatment and control group*.	17
60 healthy stressed adults	240 mg (extract)	60 days	No adverse effects are reported. Larger decrease in cortisol and DHEA-s level in treatment group compared to control group.	18
60 healthy stressed adults	250 / 600 mg (root extract)	8 weeks	No adverse effects are reported. Larger decrease in cortisol and stress, anxiety and sleep scores improved more in treatment group compared to control group*	19
24 healthy participants	21 mg WG (root and leaf extract)**	30 days	No adverse effects are reported. Increased level of immunoglobulins and several immune cells and cytokines in treatment group compared to pre-treatment values *	20
50 healthy athletic adults	600 mg (root extract)	8 weeks	No adverse effect related to the treatment are reported. Increased scores for recovery and antioxidant levels in treatment group compared to the control group*.	21
80 healthy participants	600 mg (root extract)	8 weeks	No adverse effects are reported.	22
66 schizophrenic patients	1 g (extract)	12 weeks	More adverse effects, such as diarrhoea and somnolence reported in treatment group, but the incidence was not statistically significantly different from the control group. Larger decrease in depression score in treatment group compared to control group*.	23
100 perimenopausal women	600 mg (root extract)	8 weeks	Adverse effects, such as abdominal pain and nausea are reported, but incidence was not statistically significantly different between control and treatment groups. Larger increase of estradiol levels and decrease of FSH levels in treatment group compared to control group*.	24
45 patients with anxiety	2 – 3 g (root extract)	8 weeks	Adverse effects, such as gastrointestinal problems and vomiting are reported, but no the incidence was not statistically significantly different between control and treatment groups. Larger reduction of anxiety score in treatment group compared to control group*.	25

Study population	Dose	Duration	(Adverse) effects	Ref
60 elderly with insomnia	6 g (root powder)	30 days	No adverse effects are reported. Scores for sleep quality improved in the treatment group*, but no control group was included.	26
60 healthy stressed adults	225 / 400 mg (root and leaf extract powder)	30 days	No adverse effects are reported.	27
150 COPD patients	500 mg (root powder)	12 weeks	No adverse effects are reported. Larger improvement of lung function and antioxidant status in the treatment group compared to the control group*.	28

1. (Wankhede et al., 2015, as cited by DTU) 2. (Ambiye et al., 2013, as cited by DTU) 3. (Mahdi et al., 2011, as cited by DTU) 4. (Ahmad et al., 2010, as cited by DTU) 5. (Gupta et al., 2013, as cited by DTU) 6. (Lopresti et al., 2019, as cited by DTU) 7. (Sharma et al., 2018, as cited by DTU) 8. (Chengappa et al., 2014 & Gannon et al., 2014, as cited by DTU) 9. (Andulla & Radhika et al., 2000, as cited by BfR) 10. (Kuppurajan et al., 1980, as cited by BfR) 11. (Mikolai et al., 2009, as cited by BfR) 12. (Venkataraghavan et al., 1980, as cited by BfR) 13. (Andrade et al., 2000 & Andrade et al., 2009, not published as cited by BfR) 14. Andrade et al., 2000, as cited by BfR) 15. (Deshpande et al., 2020) 16. (Gopukumar et al., 2021) 17. (Langade et al., 2021) 18. (Lopresti et al., 2019) 19. (Salve et al., 2019) 20. (Tharakan et al., 2021) 21. (Tiwari et al., 2021) 22. (Verma et al., 2021) 23. (Gannon et al., 2019) 24. (Gopal et al., 2021) 25. (Fuladi et al., 2021) 26. (Atul et al., 2020) 27. (Remenapp et al., 2022) 28. (Singh et al., 2022)
*statistically significant effects ***W. somnifera*** extract standardized to 35% withanolide glycoside (WG)

5.3 Interactions

In the risk assessment conducted by BfR (2013) it was briefly mentioned that *W. somnifera* could possibly enhance the effect of GABA agonists. Furthermore, BfR mentioned that *W. somnifera* should not be taken together with alcohol, sedatives or anxiolytics, as combined use could affect the central nervous system. They also mentioned that withaferin A has a similar structure as digoxin, a drug prescribed for heart diseases. As a result of the similarity, withaferin A can disturb clinical measurements, which could lead to wrong diagnoses (BfR, 2013). The WHO (2009) gave precautions for drug interactions with barbiturates and compounds as diazepam and clonazepam.

Natural Medicines reported five possible and one probable pharmacokinetic interactions (Natural medicines, 2023). *W. somnifera* might enhance the risk of hypoglycaemia when combined with antidiabetic drugs (possible) or enhance the effect of thyroid hormones (probable), based on some experimental evidence from a clinical cohort study and a lower quality randomized control study, respectively. *W. somnifera* might enhance the risk of hypotension when combined with antihypertensive drugs (possible) or decrease the effect of immunodepressants (possible), based on animal studies or *in vitro* studies. *W. somnifera* might enhance the sedative effects of benzodiazepines (possible) and central nervous system depressants (possible), based on pharmacological theories.

5.4 Derivation of toxicological reference value

No toxicological reference values were derived by BfR (2013) and DTU (2020), because the data did not allow to derive such values.

Despite the new information identified in this assessment, it was not possible to establish a health-based guidance value (HBGV) for *W. somnifera* or its main constituents due to a lack of (adequate) toxicological data and unresolved concerns.

No data from toxicity studies with a duration longer than 28 days were available. Furthermore, effects on thyroid and sex hormones were found in the short-term toxicity studies and adverse effects on the liver and levels of thyroid hormones and cortisol were observed in case studies. Also, there were unresolved concerns regarding the risk for the unborn child as, according to the WHO, *W. somnifera* was traditionally used to induce abortion. Although no adverse effects were observed in the available reproduction toxicity studies, these did not adequately assess the effect of *W. somnifera* in early pregnancy and can therefore not be used to draw conclusions on this aspect. In addition, there are indications that a constituent of *W. somnifera* is genotoxic, but the concentration in available *W. somnifera* extract is unknown.

The clinical studies cannot be used as a basis for an HBGV, because not all aspects of toxicity were investigated in these studies.

In case reports described in peer-reviewed articles liver injury (n=7), thyrotoxicosis (n=1) and suppression of the adrenal function (n=1) were reported after daily intake of 77 to 1350 mg *W. somnifera* extract. In

addition, cases of liver injury after consumption of products containing *W. somnifera* were reported in the Netherlands by Lareb and in the WHO database Vigibase. Liver injury was not found in animal studies or in clinical trials. The occurrence of thyrotoxicosis was supported by evidence from animals studies. However, clinical trials showed contradictory results, as thyroid hormones were increased in two studies, but no change was observed in another study. Cortisol levels were not measured in animal studies, but in two clinical trials reduced cortisol levels were observed. Overall, there was some support from animal studies and clinical trials for the adverse effects found in the case studies. The intake levels reported in the case reports described in peer-reviewed papers (154 to 1350 mg/day *W. somnifera* extract) will be considered as the effect level in the risk assessment.

6 Risk assessment

6.1 Risk assessment

As a first step in the risk assessment, it was investigated whether the presumption of safety can be applied to *Withania somnifera*. Botanical preparations for which an adequate body of knowledge exists, can benefit from a presumption of safety without any need for further testing (EFSA, 2009; EFSA, 2014). This generally means that when there is a history of safe use and the intended use of the botanical preparation in food supplements does not exceed the historical levels of intake, the intended use in food supplements is assumed to be safe. *W. somnifera* has a history of use as Ayurvedic medicine and traditional Chinese medicine, however, safety is not adequately documented and the level of exposure to *W. somnifera* in Ayurvedic medicine and traditional Chinese medicine is unknown.

The presumption of safety cannot be applied to *W. somnifera* and more information is needed to assess its safety. This is in line with what was concluded in the risk assessment conducted by BfR (2013).

Despite the new information identified in this assessment, it was not possible to establish a HBGV for *W. somnifera* or its main constituents, and hence no safe use level for herbal preparations containing *W. somnifera* could be determined.

The intake levels reported in the case reports, described in peer-reviewed papers, are used as the effect level in the risk assessment. In these nine human case reports adverse effects, as liver injury, thyrotoxicosis and suppression of the adrenal system, were observed after daily exposures to 77 to 1350 mg *W. somnifera* extract.

As shown in section 3.5 and 4, *W. somnifera* supplements on the Dutch market contain 50 mg – 2000 mg *W. somnifera* extract. The estimated exposure for supplements available in the Netherlands are in the same range as the intake levels for which adverse effects were observed in the case reports. However, it is unlikely that a large part of the population is at risk for these effects, as the adverse effects were not observed in the clinical trials in which exposure to *W. somnifera* extract and the duration of use was comparable to the situations described in the case reports. This leads to the conclusion that the sensitive individual could experience adverse effects such as liver injury, thyrotoxicosis and suppression of the adrenal system. It is unknown which individuals are sensitive to *Withania somnifera*.
Furthermore, the possible risk for the unborn child cannot be adequately assessed.

For *W. somnifera* tea no toxicological data are available and the information on the exposure is limited. In the absence of sufficient data, the conclusion for food supplements is assumed to also apply to *W. somnifera* tea.

6.2 Interactions

Natural Medicines reported several potential pharmacokinetic interactions of *W. somnifera*, including interactions with antidiabetic drug, antihypertensive drugs, benzodiazepines, thyroid hormones, immunodepressants and central nervous system depressants (Natural medicines, 2023).

Also BfR (2013) and the WHO (2009) mentioned warnings for interactions, although these were not supported by scientific evidence. BfR (2013) warned to not take alcohol, sedatives or anxiolytics together with *W. somnifera* and warned for an enhancing effect of *W. somnifera* on GABA agonists. Also, a structural similarity between *W. somnifera* constituents and the drug digoxin was mentioned. Due to the structural similarity, diagnostic measurements in heart disease patients could be disturbed when digoxin and W. somnifera are taken together. The WHO (2009) gave precautions for drug interactions with barbiturates and compounds as diazepam and clonazepam.

Patil et al. (2014) did not observe statistically significant CYP3A4 inhibitory activity of *W. somnifera* extract *in vitro* using human CYP3A4 isoenzyme. Also, no inhibitory activity of *W. somnifera* or the constituents withaferin A, withanolide A, sitoindosides VII-X and withanoside I-IV on CYP3A4 was observed *in vitro* in rat and human microsomes (Savai et al., 2013). CYP1A inhibitory activity was not observed in human and rat liver microsomes for *W. somnifera* extract or some of its constituents (Savai et al., 2015). However, there are indications that *W. somnifera* extract has a small inhibitory effect on CYP1A *in vivo* in Wistar rats (Savai et al., 2015).
Modi et al. (2022) did not find inhibitory activity of several withanosides and withanolides for CYP1A2, CYP2C9, CYP2D6, CYP2C19 and CYP3A4 using *in silico* predictions (probability of 0.73-0.96).

6.3 Sensitive/vulnerable groups

As shown earlier, there are indications that sensitive individuals could experience adverse effects after exposure to *Withania somnifera*. It is unknown which individuals are sensitive to *Withania somnifera*.

In addition, pregnant women may be a vulnerable group, as there are indications that *W. somnifera* is harmful to the unborn child. It has to be noted that this effect could not be adequately assessed in the risk assessment.

6.4 Uncertainties

6.4.1 *Exposure*

The information on the exposure to *W. somnifera* via intake of herbal preparations is limited, especially for tea.

Furthermore, the amount of active substances can differ per *W. somnifera* extract and only for some extracts the content is further specified. It is also unknown if the exposure to active substances via intake of *W. somnifera* extracts or *W. somnifera* powder and root parts differs. For the risk assessment only information about *W. somnifera*

extract was available. This could lead to an under- or overestimate of the exposure.

6.4.2 *Toxicity*

There are indications that *W. somnifera* may be harmful to the unborn child. Some studies in pregnant animals are available in which no effects were observed, but in none of the studies the animals were exposed during the first days of pregnancy. Therefore, the possible toxicity for the unborn child cannot be adequately assessed.

In addition, there are indications that withanone, a constituent of *W. somnifera,* may be genotoxic. This is in contrast to the results of genotoxicity tests with *W. somnifera* extract (0.004% withanone). As mentioned above, the amount of substances in *W. somnifera* extract can differ. Therefore, it is unknown if the *W. somnifera* extract tested in genotoxicity tests is comparable to available extracts in the Netherlands.

Furthermore, there are indications that *W. somnifera* can affect reproductive parameters in male animals. However, the results of the studies were contradictory, so no conclusion can be drawn about the effect of *W. somnifera* on reproductive parameters

In animals, toxicity studies after longer exposures (>28 days to chronic) are not available. In humans, only two clinical trials had a duration above 3 months. Therefore, the toxicity of *W. somnifera* could be underestimated during long-term use of the food supplements.

The risk assessment was based on case reports and clinical trials in humans. In the case reports the causality cannot be proven and all but one of the clinical trials focused on positive effects.

7 Conclusions and recommendations

The use of food supplements containing *W. somnifera* that are currently available on the Dutch market may lead to adverse effects such as liver injury, thyrotoxicosis and suppression of the adrenal system in sensitive people. It is unknown which individuals are sensitive to *Withania somnifera*. In addition, according to the WHO, *W. somnifera* was historically used to induce abortions, indicating that *W. somnifera* could be harmful to the unborn child. No suitable reproduction toxicity studies were available to exclude that herbal preparations containing *W. somnifera* can cause this effect.

For *W. somnifera* tea, no toxicological data are available and the information on the exposure is limited. In the absence of sufficient data, the conclusion for food supplements is assumed to also apply to *W. somnifera* tea.

As a precaution, RIVM advises consumers, and in particular pregnant women, to not use herbal preparations containing *W. somnifera*.

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Appendix A Literature search toxicity

Pubmed

Selected from October 2019

(("withania somnifera"[Title/Abstract]) OR (ashwagandha[Title/Abstract]) OR ("W. somnifera"[Title/Abstract])) AND (("clinical study"[Title/Abstract]) OR ("case report"[Title/Abstract]) OR ("clinical trial"[Title/Abstract]) OR ("case study"[Title/Abstract]))

13 results

(("withania somnifera"[Title/Abstract]) OR (ashwagandha[Title/Abstract]) OR ("W. somnifera"[Title/Abstract])) AND (("acute toxic*"[Title/Abstract]) OR ("subacute toxic*"[Title/Abstract]) OR ("short term toxic*"[Title/Abstract]) OR ("sub chronic toxic*"[Title/Abstract]) OR ("chronic toxic*"[Title/Abstract]) OR ("adverse event"[Title/Abstract]) OR ("adverse effect"[Title/Abstract]) OR ("negative health effect"[Title/Abstract]) OR (gentox*[Title/Abstract]) OR (carcinogen*[Title/Abstract]) OR (reprotox*[Title/Abstract]) OR ("developmental toxic*"[Title/Abstract]))

8 results

Scopus

TITLE-ABS-KEY ((("withania somnifera") OR (ashwagandha) OR ("W. somnifera")) AND (("clinical study") OR ("case report") OR ("clinical trial") OR ("case study"))) AND (LIMIT-TO (PUBYEAR , 2022) OR LIMIT-TO (PUBYEAR , 2021) OR LIMIT-TO (PUBYEAR , 2020) OR LIMIT-TO (PUBYEAR , 2019))

71 results

TITLE-ABS-KEY (("withania somnifera") OR (ashwagandha) OR ("W. somnifera")) AND (("acute toxic*") OR ("subacute toxic*") OR ("short term toxic*") OR ("sub chronic toxic*") OR ("chronic toxic*") OR ("adverse event") OR ("adverse effect") OR ("negative health effect") OR (gentox*) OR (carcinogen*) OR (reprotox*) OR ("developemental toxic*")) AND (LIMIT-TO (PUBYEAR , 2023) OR LIMIT-TO (PUBYEAR , 2022) OR LIMIT-TO (PUBYEAR , 2021) OR LIMIT-TO (PUBYEAR , 2020) OR LIMIT-TO (PUBYEAR , 2019))

230 results

Appendix B Literature search toxicokinetic parameters

PubMed

(("withania somnifera"[Title/Abstract]) OR ("w. somnifera"[Title/Abstract]) OR (ashwagandha[Title/Abstract])) AND ((toxicokinetic*[Title/Abstract]) OR (pharmacokinetic*[Title/Abstract]))

26 results

Scopus

TITLE-ABS-KEY ((("withania somnifera") OR ("w. somnifera") OR (ashwagandha)) AND ((toxicokinetic*) OR (pharmacokinetic*)))

57 results

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