(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING PECTIN EFFECTIVE IN INHIBITING THE MALE REPRODUCTIVE TOXICITY

(57) Abstract: The present invention relates to a pharmaceutical composition comprising a pectin which is effective in inhibiting a male’s reproductive toxicity. The pharmaceutical composition is orally administered in an effective amount of 1μg to 1000 mg per 1kg of the recipient’s weight. It can be used as a prophylactic/therapeutic agent for inhibiting the reduction of the number and motility of sperms, prostate reduction and male’s infertility caused by environmental hormones including dioxin.
PHARMACEUTICAL COMPOSITION COMPRISING PECTIN EFFECTIVE IN INHIBITING THE MALE REPRODUCTIVE TOXICITY

BACKGROUND OF THE INVENTION

5 [Field of the Invention]

The present invention relates to a pharmaceutical composition comprising pectin effective in inhibiting the male reproductive toxicity.

[Description of the Prior Art]

Endocrin system disturbing materials, so-called "environmental hormones" which have recently become an issue and been watched with keen interest in the U.S., Europe, Japan, etc., are chemical materials disturbing the function of the endocrin system and having similar activities as hormones in the body. Examples of such materials are various chemical products for industry, agricultural chemicals such as insecticides and herbicides, organic heavy metals, dioxins generated in an incinerator, hormone analogues such as phytoestrogen, synthetic estrogens useful as medicines such as diethylstilbestrol, and other food or food additives. A particular concern is given to dioxins among such materials.

Dioxin is an organic compound having two benzene rings chemically bonded to two oxygen atoms. A generally available dioxin is dioxin chloride wherein a hydrogen within benzene rings is substituted with a chloride. There are tens of species which are different in number and position of chlorides. The most toxic and stable dioxin is 2,3,7,8-TCDD (tetrachlortribenzop-p-dioxin) having four substituted chlorides. Because the dioxin is easily generated during the burning-out of domestic garbage or the softening of gasoline, it is more dangerous to the environment (see S. Alex. Villalobos, Michael J Anderson, and Peter Kelley, 1996. "Dioxinline Properties of a Trichloroethylene Combustion-Generated Aerosol, Environmental Health Perspectives, 104. p734-p743).

Dioxins are generally absorbed through the digestive duct, skin and respirator. The absorption rates vary depending on species, solvent, dioxin homologue and isomer, materials taken together, administered amount, and age. The absorbed dioxins are generally
accumulated in the liver and adipose tissues, and hardly excreted out of the body.

The toxicity of dioxins is clearly known from experiments on animals. It was reported that dioxins, even in small amounts, cause skin diseases, and toxicity, carcinogenicity and malformation of the liver. Recent experiments on humans also substantiated the harmfulness of dioxins. Particularly, dioxins cause unbalanced hormone secretion, reduced reproductivity and immunity, malformation of genitals, inhibition of growth, and cancers. From such experiments, it was well known that reduction of a male’s reproductivity can be the reduction of the number of sperms, reduction of seminal fluid, decrease of the concentration of testosterone, reduced motility of sperms, increase of the number of modified sperms, genital malformation, prostate diseases, and other problems in tissues relating to reproduction. While the toxicity of environmental hormones has been studied actively, studies on prophylactic/therapeutic agents against the toxicity of environmental hormones have not been sufficiently made yet. It is presently known that extracts from green tea (containing epicatechin and epigallocatechin as active ingredients) and extracts from ginseng are effective in inhibiting dioxins from reducing the reproductivity. In a test, ginseng was administered to guinea pigs. It was detected that the weight loss of didymus, decrease of the size of seminiferous tubule, destruction of geminal epithelium, deformation of germ cells, etc. have been significantly inhibited (see W. Kim, S. Hwang, H. Song, and S. Kim, “Panax Ginseng Protect the Testis Against 2,3,7,8-Tetrachlorodibenzo-p-dioxin Induced Testicular Damage in Guinea Pigs,” BJU International 1999: 83: 842-849).

At present, no publication or patent discloses the use of a pectin in the inhibition of a male’s reproductivity toxicity caused by an environmental hormone.

U.S. Patent Nos. 5,834,442 and 5,895,784 disclose a method of inhibiting the transition of a primary tumor by orally administrating a citrus pectin of a modified pH to a recipient.

WO 9710727 discloses a method of reducing separation of serum, using an enzyme, from an aqueous vehicle containing a pectin.

WO 9631239 discloses a medicament useful in the prevention and treatment of
catharsis and stomach ulcer in a mammal, comprising a mixture of a pectin and a phospholipid.

SUMMARY OF THE INVENTION

The applicant discovered after several experiments that a pectin is effective in inhibition of a male’s reproductive toxicity caused by environmental hormones including dioxins.

Pectins are contained in large amounts in primary cell walls and intermediate lamella of a plant. The main physiological activity of pectins is to connect and protect cell structures and to affect the water retention power. A pectin consists of partial methyl esters of acids of polysaccharide and their sodium, potassium, calcium and ammonium. The methyl ester group which is closely related to the functionality of the pectin can be easily hydrolyzed by alkali, and has various functions according to the rate of hydrolysis.

Generally, a pectin is easily hydrated and has a strong moisture absorbency. Also, a pectin forms a hydrophilic colloid solution in water and apparently has a high viscosity.

Pectins are currently used in many industries such as confectionery, food additives, dairy food and cosmetics. A particular attention should be paid to the application in the medical field. Pectin is recognized as a prevention food with various therapeutic activities. One of the discovered activities of pectin is the prevention of poisoning by a heavy metal or a radioactive metal. According to experiment results, a pectin has an ability to form a superior complex with a heavy metal such as lead, Mercury, cadmium, molybdenum and manganese, and with a radioactive metal such as cobalt, strontium, cesium, zirconium and ruthenium. Therefore, a pectin prevents metal poisoning and produces a superior effect during a long-term accumulation of metals within the body.

In addition, a pectin reduces cholesterol in serum and expedites the peristalsis of intestines, thereby removing constipation. It also reduces the blood glucose level in a patient suffering from diabetes, and is effective in reducing weight and treating gastrointestinal disorders. Such effects have been proven by experiments. At present, pectins are used in the above indications and other applications of pectins are being studied.
The present invention can use pectins extracted from citruses, lemons, oranges, apples or the like, but is not limited to those pectins only. It is preferred to use a pectin extracted from a citrus. The chemical equation of a citrus pectin is given by

\[
\text{GaLP-galactopyranosa} \\
\text{GaLUA-galacturonopic acid} \\
\text{L-Rhap-L-rhamnopyranosa} \\
\text{L-Araf-L-arabafuronosa}
\]

The term “pectin for medical use” used in this application is distinguished from a general lower pectin, and hereinafter refers to a pectin having the esterification degree (degree of methyl esterification of organic acids of a pectin) of less than 50. However, the present invention is not to be limited to the medical use of a pectin (i.e., inhibition of a male’s reproductive toxicity).

The pectin used in the present invention can be prepared by a method disclosed in the applicant’s earlier application, Korean Patent Application No. 10-1999-45200 (filed on October 19, 1999). According to the method, a pectin is obtained by drying shells of a citrus, apple, orange, lemon or the like, cutting the dried shells into minute pieces, hydrolyzing and extracting a pectin, and concentrating and drying it by a membrane filler. The method also comprises the purifying step of treating the pectin sequentially with alkali and acid and removing moisture with alcohol.
The present invention, using a highly purified pectin prepared by the above method, can produce a superior effect in inhibiting a male's reproductive toxicity caused by environmental hormones including dioxins.

The term "male's reproductive toxicity" used in this application means reduction of the reproductivity of a male such as reduction of the number of sperms, reduction of seminal fluid, decrease of the concentration of testosterone, reduced motility of sperms, increase of the number of modified sperms, genital malformation, prostate diseases, and other problems of the tissues relating to reproduction.

To achieve the primary object of the present invention, a pharmaceutical composition comprising a pectin, more preferably, a pectin for medical use, is provided.

A preferred example of the invention provides an oral pharmaceutical composition comprising a pectin for use in inhibiting a male's reproductive toxicity caused by environmental hormones. Therefore, a composition comprising a pectin can be prepared in a liquid formulation form such as gel, solution or suspension, or in a solid unit dose form such as a compressed tablet or a capsule. Methods of preparing such a composition in a liquid formulation or a solid unit dose form and vehicles used for the methods are publicly known in the relevant art. A dispersing agent, emulsifier, surfactant, flow controlling agent, preservative, modifier or the like can be additionally used within the scope of the present invention.

Though the applicant suggests that the pharmaceutical composition of the present invention be preferably in an oral administration form, nothing described in this application should be interpreted to narrow the administration path of the pharmaceutical composition. Also, a combined therapy is considered in the present invention. That is, it is possible to administer a pectin together with ingredients (e.g., ginseng extracts and green tea extracts) known to be effective in protecting reproductivity from environmental hormones. The pectin used in the present invention can be administered with a pharmaceutically acceptable carrier, preferably with a non-toxic carrier suitable for a particular administration method. The pectin can be formulated to be suitable for a known administration.
Pectins can be formulated in various forms other than the above described medicament form, such as food including health supplement food and additives.

For oral administration, formulations should contain a pectin in an amount sufficient to produce the inhibitory activity against a male's reproductive toxicity caused by environmental hormones including dioxins. Therefore, the pharmaceutical composition of the present invention can contain a pectin in an amount of 1μm to 1000 mg per 1kg weight. The therapeutically effective amount of pectin is determined depending on the recipient's age, sex, state, diet, etc. The "therapeutically effective amount" means an amount effective to obtain a desired result according to the present invention without any pharmacologically adverse side effects. The desired result is the inhibition of the weight loss of epididymis, reduction of the number or motility of sperms, etc.

The pectin can be administered in a single unit dose form or a divided multiple dose form.

**BRIEF DESCRIPTION OF THE DRAWINGS**

FIG. 1 is a graph illustrating the experimental groups.

FIG. 2 is a graph illustrating the weight of epididymis showing the protective effect of a pectin against a male's reproductive toxicity caused by TCDD.

FIG. 3 is a graph illustrating the weight of seminal vesicle showing the protective effect of a pectin against a male's reproductive toxicity caused by TCDD.

FIG. 4 is a graph illustrating the motility of sperms showing the protective effect of a pectin against a male's reproductive toxicity caused by TCDD.

FIG. 5 is a graph illustrating the number of sperms showing the protective effect of a pectin against a male's reproductive toxicity caused by TCDD.

**DETAILED DESCRIPTION OF THE INVENTION**

The applicant performed the following experiments to study how a pectin effects a male's reproductive toxicity caused by dioxins.

The following examples illustrate the invention without in any way limiting its scope.
Example 1

Materials and Method

(1) Animal for test

Three groups, each group consisting of ten 4-week old male SD rats (Sprague-Dawley rats), were adapted in the laboratory environment. When the SD rats were 5 weeks old (110-120g), they were used in the test.

(2) Materials for test

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (NIH, USA) stored in the shade was dissolved in acetone, diluted with a corn oil, and abdorminally administered once in the concentration of 15μg/kg. Pectin was dissolved in sterile distilled water and orally administered everyday in the amount of 50mg/kg.

(3) Experimental groups

The experimental groups consists of group (A) to which a pectin was administered from one week before the administration of TCDD, group (B) to which TCDD only was administered, and group (C) to which a physiological saline only was administered. These groups are shown in detail in FIG. 1.

Analysis and Results

(1) Weight of prostate and seminal vesicle

Analysis

The prostate and seminal vesicle of a rat were separated, and weighed after removing the combined adipose tissues.

Results

The weight of the prostate and seminal vesicle was divided by the body weight of the rat. The results of the analysis on 10 rats in each group are as follows:

[Table 1]
<table>
<thead>
<tr>
<th></th>
<th>Administration of pectin and TCDD</th>
<th>Administration of TCDD only</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate/weight (unit: %)</td>
<td>0.0282 ± 0.0053</td>
<td>0.0285 ± 0.0063</td>
<td>0.0361 ± 0.0077</td>
</tr>
<tr>
<td>(average x 100 ± standard)</td>
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</tr>
<tr>
<td>Seminal vesicle/weight (unit: %)</td>
<td>0.2920 ± 0.0482</td>
<td>0.2578 ± 0.0589</td>
<td>0.3355 ± 0.0851</td>
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<tr>
<td>(average x 100 ± standard)</td>
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This is shown in detail in FIGs. 2 and 3.

The groups to which TCDD was administered showed the reduced ratio of prostate and seminal vesicle to the body weight when compared to the control group. When a pectin was administered, the prostate was recovered by 31.2%. However, there was no significant change in the seminal vesicle.

(2) Sperm motility

**Analysis**

The right caudal epididymis was excised and put into a 35 mm Petri dish containing 3 ml of HBSS (Hank’s balanced salt solution) which had been heated at 37.5°C in advance. Two or three excisions were made on the epididymis by a surgical knife (No. 15), keeping away from blood vessels. The Petri dish was covered with a cover and agitated slowly. After pipetting two or three times, one or two drops were dripped on a slide glass heated at 37.5°C. A total of 200 sperms were observed by an optical microscope of x100 rate, and the percentage of the sperms having motility was calculated.

**Results**

The percentage of the sperms having motility of the observed 200 sperms is as follows:

[Table 2]
This is shown in detail in FIG. 4.

In groups to which TCDD was administered, the motility of sperms were remarkably reduced. The motility was improved by 40.5% by the administration of a pectin.

(3) Number of sperms

Analysis

The right didymus with its skin peeled off was weighed, and put into a conical tube. 20 ml of sterile distilled water was poured into the tube. The didymus was homogenized and treated with ultrasonic for 5 minutes. 2 ml was taken and diluted five times by adding 8 ml of sterile distilled water. The diluted didymus was moved to a hemocytometer, and the number of sperms was counted through an optical microscope at x400. The expression is:

\[
\text{number of sperms} \times \text{square factor} \times \text{hemocytometer factor} \times \text{dilution factor/weight of didymus} = \text{number of sperms} \times 1 \times 104 \times (20 \times 5)/\text{weight of didymus}
\]

Results

[Table 3]

<table>
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<th></th>
<th>Administration of pectin and TCDD</th>
<th>Administration of TCDD only</th>
<th>Control Group</th>
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<tbody>
<tr>
<td>Number of sperms/weight of didymus [unit: (#/g) \times 10^6]</td>
<td>93.74 \pm 15.40</td>
<td>89.66 \pm 8.61</td>
<td>108.84 \pm 5.59</td>
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</table>
The number of sperms which had been significantly reduced by TCDD increased by 21.3% by the administration of a pectin.

TCDD is known as the strongest environmental hormone among dioxins. This test also showed that administration of TCDD results in remarkable reduction of the number of sperms, decrease of sperm motility, and weight loss of seminal vesicle and prostate. In contrast, a pectin inhibited the function of TCDD as an environmental hormone, and increased the number and motility of sperms and weight of seminal vesicle and prostate. Therefore, it is clear that a pectin effectively protects the decrease of a male’s reproductivity due to environmental hormones including dioxins.

As proved in the above example, a pectin has an inhibitory or prophylactic effect with respect to the weight loss of seminal vesicle and prostate and the reduction of the motility and number of sperms due to poisoning by dioxins. Therefore, a pectin can be used as a prophylactic/therapeutic agent against the decrease of a male’s reproductivity caused by environmental hormones including dioxins.
What is claimed is:

1. A pharmaceutical composition for inhibiting a male’s reproductive toxicity comprising a therapeutically effective amount of a pectin.

2. The pharmaceutical composition of claim 1, wherein the therapeutically effective amount of the pectin is 1μg to 1000 mg per 1kg of weight.

3. The pharmaceutical composition of claim 1 which is orally administered.

4. The pharmaceutical composition of claim 1, further comprising a pharmaceutically acceptable carrier.

5. The pharmaceutical composition of claim 1, wherein the male’s reproductive toxicity includes reduction of the number of sperms, reduction of seminal fluid, decrease of the concentration of testosterone, reduced motility of sperms, increase of the number of modified sperms, genital malformation, prostate diseases, and other problems of tissues relating to reproduction.

6. The pharmaceutical composition of claim 1, further comprising any other active ingredient having an activity protecting the male’s reproductivity.
FIG. 1

EXPERIMENTAL GROUPS

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<tr>
<td></td>
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<td>TCDD</td>
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<td>GROUP B</td>
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<td></td>
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<td>CONTROL GROUP</td>
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FIG. 2

RATIO OF PROSTATE TO BODY WEIGHT IN EACH GROUP (w/w %)

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<th>GROUP A</th>
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<tbody>
<tr>
<td>Value</td>
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<tr>
<td>Value</td>
<td>0.014</td>
<td>0.015</td>
<td>0.016</td>
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FIG. 3

RATIO OF SEMINAL VESICLE TO BODY WEIGHT IN EACH GROUP (w/w %)

FIG. 4

SPERM MOTILITY IN EACH GROUP
FIG. 5

NUMBWE OF SPERMS IN EACH GROUP
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

**IPC7 A61K 31/715**

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC7 A61K 31/715, C08B 37/06

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Patent and applications for inventions since 1975

Korean Utility models and applications for Utility models since 1975

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CAS ON LINE(STN), CA ON CD, MEDLINE, PAJ, FPD

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>JP 11-209741 A (SAKATA SHIGENOBU) 3 AUGUST 1999 see claims 1, 2 and pages 2, 3.</td>
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<td>CHAHOUID et al., 'Reproductive toxicity and pharmacokinetics of 2,3,7,8-tetrachlorodibenzo-p-</td>
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<td>dioxin. 1. Effects of high doses on the fertility of male rats', In: Arch Toxicol 1989, Vol.63 No.6,</td>
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<td>on humoral antibody production and cell-mediated activities in mice', In: Areol. Toxicol., Suppl.</td>
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  *"P" document published prior to the international filing date but later than the priority date claimed

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

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Faximile No. 82-42-472-7140

Authorized officer

KIM, Ji Soo

Telephone No. 82-42-481-5606

Form PCT/ISA/210 (second sheet) (July 1998)
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