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MiroHealth's phytoestrogenic effect on skin, oestrogen receptors, reproductive system and cancer cells.

Abstract: Anne Selene's MiroHealth range of herbal products contains the Thai traditional herb *Pueraria Mirifica* (*White Kwao Krua)* that contains a wide range of known phytoestrogens that binds to both ERα and ERβ estrogen receptors in both males and females witch acts as an 17β-oestradiol agonist. It is proven to have no acute organ toxicity, no toxicity on skin, skin sensitisation, eye, under UV-A nor on human primary skin. It's shown to have possible effect on reproductive systems in males due to its oestrogenic activities. MiroHealth has antiproliferative effects on certain gynaecological cancer cell lines.

Keywords: Pueraria Mirifica, White Kwao Krua, MiroHealth, phytoestrogens, oestrogen, oestrogen receptors, reproductive system, hormones, cancer, Genistin, Genistein, Daidzin, Daidzein, Miroestrol, Deoxymiroestrol, Puerarin, Mirificoumestan, Kwakhurin, β-sitosterol, Stigmasterol, Spinasterol, Campesterol, Coumesterol, Pterocarpene

Introduction

White Kwao Krua (hereinafter know as WKK) used in MiroHealth from Anne Selene has the scientific name of *Pueraria Mirifica*, Airy Shaw et Suvatabhandu named after Mr. Airy Shaw and Mr. Kasin Suvatabandhu from Chiang Mai (1952) and belongs to the Laguminosae a sub-family of Papillonoidae. WKK is a climber tuberous root found in northern Thailand and are traditionally used for many decades said to have positive effects on both males and females for generating youthful skin, increase memory, thickening and delaying grey hairs, relieving body weakness, increasing appetite, treatment of sleeplessness, breast health and enlargement as well as being rejuvenating.

Several previous studies, first published by Kerr (1932) claiming to found a rejuvenating plant from Siam (Thailand) where its outcome led to further investigations and it was found that isolation of a highly potent, but yet unknown chemical structure (Schoeller, Dohrn & Hohlweg, 1940) could have such and other effects. The plant was later on classified as Pueraria Mirifica (Kashemsanta, Airy Shaw et Suvatabhandu, 1952).

In the past, further chemical structures of WKK has been reported by Jones & Pope (1960), Bounds & Pope (1960), Taylor, Hodskin, Rollett (1960), Benson, Cowie & Hodskin (1961). More recent biological and chemical studies has found WKK to contain isoflavones Genistin, Genistein, Daidzin, Daidzein, Pterocarpene, Puemiricarpene and different other chemicals such as Miroestrol [1], Deoxymiroestrol, Puerarin [2], Mirificoumestan, Kwakhurin [3, 4] β-sitosterol, Stigmasterol, Spinasterol, Campesterol, and Coumesterol [5, 6, 7].

In contrast, Soya beans also contains isoflavones Daidzin, Daidzein, Genistin and Genistein but not the others and long-term consumption of phytoestrogen-rich diets has shown to have lower risk of common cancers such as breast, colon and prostate cancer and reduced risk of artherosclerosis, osteoporosis, deep vain thrombosis, stroke, diabetes and reduced menopausal and andropausal symptoms in both woman and males.

In addition isoflavone phytoestrogens also possess a benefit by a proposed hypothesis of decreasing total cholesterol, LDL-C, triglyceride and increase HDL-C in both normal and hypercholesterolemic conditions [11].

The objectives of this paper was to determine clams that phytoestrogens found in WKK has any effect on reproduction system for both males and females and if it has any effect on Oestrogen Receptors (ERs) also with the view of being a positive agent for breast and other cancer prevention as well as having positive effect on both menopause in woman and andropause in males.

Oestrogenic activity of derivatives found in MiroHealth

Based on research made by Bounds and Pope (1960) [8] on mouse uterine weight bioassay, there was evidence that phytoestrogens found in WKK has a chemical structure and functionality similar to 17β-oestradiol (E2) which was used as the standard oestrogen. However it was evident that its dose-response curve was unlike that of E2 and more like oestriol (E1).

It is however interesting to note that the fibrous root from a very young WKK plant has an active about twenty times lower than an older root and the root system builds up its activity fairly slow till it reaches its maximum at about 4th to 6th years with maximum during flowering.

The known highly active phytoestrogens found in WKK, constitutes a range of compounds of widely different chemical structures. However they have molecular size and shape approximating those found in steroid oestrogen's and possess at least two oxygen-containing substances widely spaced within the molecule whereas one of these being Phenolic Hydroxy and the other being a Phenolic Hydroxyl, Alcohol Hydroxyl, Carboxyl or Keto-group.

The chemical structure of Miroestrol cannot be aided with the oestrogen activity of the endocrine organs, but it seams that high activity of those molecular features are present and particularly because, according to Taylor et al. (1960), the distance between the 3-OH and 18β-OH oxygen atoms in Miroestrol, as he measured by X-Ray crystallographic methods, is nearly the same as that found between oxygen atoms of the 3-OH and 17β-OH in E2. In addition if we consider the high activity of E2 and 17α-ethynyloestradiol in which according to Emmens (1940) are true oestrogens, we found that thus evident shows that the Hydroxyl group other than the Phenolic one may be that of a secondary or tertiary alcohol and therefore of a highly active oestrogen.

Furthermore if we look at the same evident and we assuming that only one of the oxygen-containing substitutes in the D-ring of Miroestrol is involved in determining its high activity, this substitutes may be either the 17β-OH or 18β-OH.

The high activity of Miroestrol was shown to be 0.25% of E2 (Jones, Waynforth & Pope, 1961) however later studies shown in private communications by Emmens using intra-vaginal assay in mice, that Miroestrol has an activity of 0.7% of E2. The more recently discovered Deoxymiroestrol was found to have significant activity with its strong oestrogenic effect where 1mg of dried WKK powder was shown to equal 0.52-0.75 microgram ethinylestradiol [23l.

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Isoflavonoids effect on skin.

Since the most resent research conducted between 2000 and 2001 by Chiang Mai University in Chiang Mai, Thailand and Chulalongkorn University in Bangkok, WKK that started as traditional Thai herb, has since become a unique ingredients in commercially available dietary supplements in the form of capsules as well as found in cosmetics, topically applied pharmaceutical products and other products for breast health.

Studies has shown that WKK as dietary supplement is best consumed as dried yellow powder in capsules (100 kg of fresh roots gives about 10 Kg of dried powder), often mixed with other herbs for maximum benefits while breast and skin creams are most usable when they are based on nano-technology to ensure that all active ingredients reaches all layers of the skin. Research conducted by Pharmaceutical-cosmetics Raw Materials and Natural Products Research and Development Center (PCRNC), Institute for Science and Technology Research and Developments (IST) together with Faculty of Pharmacy, Chiang Mai University in Thailand [9] concluded that the bioactive compounds Puerarin, Miroestrol, Daidzein and Genistin found in WKK was found in all layers of skin but depended on its WKK concentration.

In a separate studies conducted by Department of Biology, Faculty of Science and Faculty of Pharmaceutical Sciences at Chulalongkorn Univercity and others [10, 11] found no toxicity in WKK where Acute Toxicity Test conducted on five mature male and female mice showed no acute toxicity on liver, kidney, brain, heart, lung, spleen, stomach, intestine, pancreas, adrenal gland, pituitary gland, testis and ovarian and were normal as compared with the compared control group.

The same study made Primary Skin Irritation tests based on six male New Zealand white rabbits showed there were no related changes in erythema and edema, which suggests that WKK extract does not causes skin irritations in the treated rabbits.

Furthermore the same study conducted a Primary Eye Irritation Test on nine New Zealand white male rabbits and did not find any abnormal clinical signs where the pathological change of the cornea, iris, and conjunctiva in the eye irritation test was classified as "0" on both WKK extract rinsed and non-rinsed. The study suggest that WKK extract caused no primary eye irritations on the treated rabbits.

At the same test thirty guinea pigs (Hartley) were tested for Skin Sensitivity Test which showed no clinical signs to suggest that WKK extract causes skin sensitisation or any adverse effect to the treated pigs.

Another test conducted at the same time was Photo-toxicity Test made on twenty male guinea pigs (Hartley) and showed that WKK extract scored an overage of UV-A radiation response as "0" which means non-irritating and exhibits no photo-toxicity to the skin of the treated pigs.

Finally on the same tests a Human Skin Sensitisation Test were conducted on thirty adult woman applying skin patches with WKK extract. The results were analysed by the Naked Eye Evaluation Standard and were found to show 0 to 0.6 index of the primary irritation respectively. The test was evaluated to have no meaningful differences regarding skin irritation.

Isoflavonoids effect on oestrogen receptors.

According to research on WKK an examination and tests [12] show that this compound has an oestrogenic effect similar to E2, which activates both oestrogen receptor ERα and ERβ. Oestrogen mediates growth, development, differentiation, and reproduction (Nilsson et al. 2001) via binding to a specific nuclear receptor protein, which is the oestrogen receptor (ER).

Two genes, namely ERα and ERβ, encode these ERs whereas their functions are to regulate the expression of target genes (Osborne et al. 2001). On ligand binding, the ER undergoes conformational changes and dissociates from the inactive ER-hsp90 complex.

The activated ER enters the nucleus as a homodimer or heterodimer, then binds to a specific DNA sequence, named the Oestrogen Response Element (ERE), and stimulates oestrogen-target gene expression. The two ERs appear to have unique tissue distributions and their own sets of specific functions in the body. More knowledge of these functions might aid in the developments of receptor-specific Selective Estrogen Receptor Modulators (SERMs) (Kuiper and Gustafsson (1997), Barkham et al. (1998) and Nilsson et al. (1998). Recently new therapy using SERMs for post-menopausal woman has been introduced where SERMs are compounds that interact with ERs and have tissue-specific effects distinct from those of E2 that they are oestrogen agonists in certain tissues while act as antagonists in others.

The main benefits of SERMs are that they selectively interacts with specific ERs, coactivators and corepressors in different organ systems giving better risk to benefit ratio than traditionally known HRT/ERT.

Over the past years several attempts and clinical trials have been conducted to find the perfect SERMs but still the benefits from using phytoestrogens like from WKK has increasingly become evident [14]. Resent studies show that phytoestrogens from dietary sources such as WKK improve the impaired endothelial-dependent relaxation [15, 16] and several Thai

universities investigated the effect on endothelial function using WKK [17]. The study concluded that the results provided evidence that WKK modulates the characteristics of E2 response by increasing its minimal and maximal responses.

As a traditional herb, the WKK has been reported to induce menstrual flow in menopausal woman and to induct developments of mammary gland in both females and males as it might also happen to boys as natural developments during puberty whereas swelling and soreness of the breast gradually brought on [13]. Studies have shown that Miroestrol found in WKK also exhibited mammogenic potency in both overiectomized rats and mice by restoring the mammary duct growth as E2 did during puberty [1] as well as experiments conducted in female rats have shown that WKK suppresses lactation and restore mammary gland growth. In male rats, WKK reduces reproductive behaviour and causes weight reduction of testis, epididymis, prostate gland and seminal vesicles [20].

Hormone Replacement Therapy known as HRT is widely used to treat menopausal symptoms in woman and to reduce incidence of cardiovascular diseases associated with menopause (This et el. 2001).

Several studies show that standard HRT or Oestrogen Replacement Therapy (ERT) appears to be associated with increased risk of developing Deep Vein Thrombosis (DVT), stroke or hart attack, breast and ovarian cancer in otherwise healthy woman. (Schairer et. Al., 2001; Lacey et al. 2002). Studies have also demonstrated that Erα, but not ERβ, is required for E2 to exert its neuronprotective effect [24].

To overcome possible problems with HRT/ERT, phytoestrogens derived from plants have emerged as an alternative to HRT/ERT.

The WKK root has shown to be one of the most important crude ingredients in traditional herbs used in northern Thailand to avoid the said conditions ad are also consumed as traditional "Thai vine" based on WKK and its said to also have a rejuvenator, aphrodisiac effect as well as pharmacological activity. (Bredbury and White (1954); Cain (1960); Harada and Ueno (1975); Qiceng (1980); Lai and Tang (1989); Keung and Vallee (1998);

Some flavones such as Genistein and Daidzein activates the CI-channels where Genistein and Apigenin were reported to possess a stimulatory effect on sodium, potassium, and chloride ion-cotransporters in renal epithelial cell line whereas Equol, a metabolic compound of Daidzein by intestinal bacteria, is also a potent inhibitor of Na-K-CI cotransporter.

When Miroestrol and Deoxymiroestrol enters oestrogen receptor ERα and ERβ it will modulate the effect on the oestrogen receptor and in fact will supplement when the estrogenic activity is low and dilutes if the receptor estrogenic activity is high. In other words Miroestrol has no effect on oestrogen levels in the body as it only has influence on the oestrogen receptors. What makes WKK active its content of additional phytoestrogens such as Daidzein, Genistein, Puerarin, Mirificin. Puemiricarpene, Mirificoumestan, Kwakhurin, β-sitosterol, Stigmasterol, Campesterol, and Coumesterol. It is therefore proven that WKK in fact acts on both ERα and ERβ as an E2 agonist. This proves that the oestrogenic activity in WKK binds to both ERs.

Healthy young males produce significant high levels of endogenous E2, primary arises from aromatase conversion of testosterone (25-40 ug/24 hrs at a study state of 2-3 ng/dl [25].

With the dependence of cell type, distribution and relative expression levels of ERα and ERβ very and either of the ERs subtypes can be predominantly expressed in E2 targeted tissues and differential expression of ERs in tissues between males and females may account for any gender differences of E2 action. [26]

ERs are present through immunoblotting in both man and woman vascular smooth muscle cells [27] and E2 inhabits growth factors-inducted proliferation and migration in smooth muscle cells in both genders but woman's vascular smooth muscle cells expresses more ERα than ERβ while in the same time, both ERs subtypes are equally relevant in males [28].

Recognising that high dose of E2 might cause negative cardiovascular effects compared to clinically beneficial vasodilation (refers to the widening of blood vessels resulting from relaxation of smooth muscle cells within the vessel walls) and blood lipid modulation

obtained at physological levels, the problem remains in determining optimal doses requirements in ageing men with varying states of E2 deficiency. Therefore phytoestrogens found in WKK that retain not only E2's favourable effects on the cardiovasculature but also with biological actions on different tissues could be used instead of E2-based HRT/ERT.

There have been reports showing that both Genistein and Daidzein were extracted in the urine as conjugated metabolite. Genistein were found as 15% in females while 47% in males whereas Daidzein was found extracted in 24% in females and 66% in males [18]. Studies show that intake of Genistein and Daidzein gives measurable quantities of free Genistein and Daidzein and are present in the circulation with half-life (t1/2) of 3.2h for females and 4.2h for males. The elimination of half-life values for total Genistein and Daidzein in men were 9.2h and 8.2h in females. It is by this proven that the elimination rates of isoflavones from the circulation are effected by sex [18]. In another study [19] shows that changes in serum levels of hormones were detected on day 3 after intake of WKK. Also Miroestrol found in WKK activated the transcriptional activities of both oestrogen receptor ERα and ERβ, however when concentration were increased, it were as active as E2 for ERα and a little less active than E2 for ERβ. This finding also shows that WKK contains phytoestrogens that activates both ERα and ERβ as an E2 agonist but it's also shown in another study that WKK have a higher binding affinity to the ERβ than the ERα [22] where ERβ is expressed in both the vagina and pituitary gland which may conclude that the expression of ERβ in vagina is higher than in the pituitary.

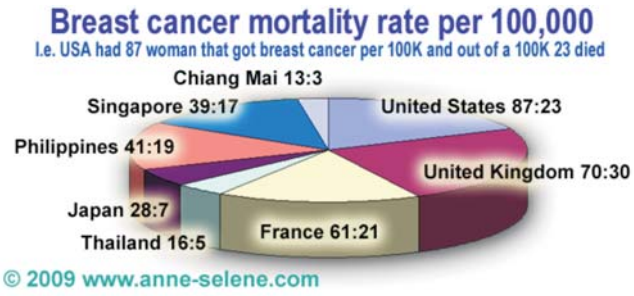
The results of other studies also support the hypothesis that low levels of ER are necessary for high levels of male prosocial behaviour, and provide the first direct evidence that site-specific ER expression plays a critical role in the expression of male prosocial behaviours.

Miroestrol and Coumestrol enhances **both** ERα and ERβ-mediated transactivation, whereas other phytoestrogens, preferentially enhanced only ERβ-mediated transactivation which shows WKK has a beneficial effect on lipid metabolism, which may result from the activation of gene transcription through selective binding of phytoestrogens to ERα and ERβ [29].

Phytoestrogenes effect on breast cancer cells.

In USA the breast cancer rate in 1998 was about 87 per 100,000 women with a mortality rate of about 23 per 100,000 women. For the same year the figures was for UK about 70:30, for France 61:21 but for Thailand the figures was as low as 16:5. Compared with other Asian countries like Japan had 28:7, Philippines 41:19, Singapore 39:17 where countries like Brazil, New Zealand, Denmark, Finland etc. had figures similar to France. In Norway there was 675 woman that died of breast cancer in 2006 which were still down from 823 woman in 1996.

In the same time, one out of six American men develop prostate cancer which is > 218,000 men were diagnosed with the disease in 2007, according to the American Cancer Society, and about 27,000 died from it.



From the above chart, we can see that the lowest numbers of breast cancer in Thailand is seen in the northern provinces of Chiang Mai with about 13:3 and it was only China that could show same figures.

The development of breast cancer are in the western world estimated to be by the age of 25 about 1:20,000; by the age of 30 about 1:2,500; by the age of 40 about 1:220; by the age of 45 about 1:93; by the age of 50 about 1:50 and by the age of 55 about 1:33. Then it increases to 1:9 by the age of 85.

Both males and females in Asian countries consume about four times more food and dietary supplements products containing phytoestrogens like i.e. WKK and soy products, including steamed fresh soybeans then people in other Western countries. It is well known that soy contains phytoestrogens isoflavones Daidzin, Daidzein, Genistin and Genistein while that WWK in addition contains several

other phytoestrogen such as Puemiricarpene, Miroestrol [1], Deoxymiroestrol, Puerarin [2], Mirificoumestan, Kwakhurin [3, 4] β-sitosterol, Stigmasterol, Spinasterol, Campesterol, and Coumesterol [5, 6, 7].

However even though WKK has been used in Thailand as a rejuvenating herb for more than 100 year, there is relatively few people outside Thailand that knows this tuberous root of WKK is also known to the locals as a herb to prevent breast cancer as this herb contains several valuable phytoestrogens where most important being Miroestrol and Deoxymiroestrol that has a very similar chemical structure as found in E1 and E2.

Research conducted in Thailand [21] has shows that regular consumption of WKK could in fact benefit as a prevention of developing MCF-7 and other breast cancer cells or could also be used as therapeutic treatment of pre-existing once. This may also give answer to why there is so few cases of breast and prostate cancer in the province of Chiang Mai.

To understand the effect of taking WKK, Miroestrol and Deoxymiroestrol is about 3000 times more potent than the estrogenic activity shown in soy Isoflavones. Further more, WKK has more than 1000 times the estrogenic activity of i.e. other plants like Red Clover, Soy and Yam whereas Miroestrol and Deoxy-miroestrol has an activity of 0.7% of E2 or 1mg of dried WKK powder is comparable with 0.52 to 0.75 microgram ethinylestradiol [23l.

To test the cytotoxic effect of known isoflavones, sterols and coumarins from WKK on ERs and breast cancer cells MCF-7, ZR-75-1, MDA-MB-231, SK-BR-3, HeLa and Hs578T, tissues from breast, ovarian, and cervical cancer cells were used [12]. They were then analysed for their chemo-preventive activity in gynaecological cancer *in vitro*, and the result shows that WKK's effect on ERα, ERβ and cancer cells were determined as such that Spinasterol found in WKK has antiproliferative effects on certain gynaecological cancer cell lines such as MCF-7, MDA-MB-231, 2774 and HeLa.

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