



# The Novel Pharmacological Approaches to Coumestrol: Focusing on the Psychiatric and Neurological Benefits and the Newly Identified Receptor Interactions

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## Abstract

For years, the health benefits of coumestrol (CMT) have been investigated by researchers around the world. Anti-oxidative properties of the phytoestrogen which can be extracted from several plant tissues, have already been reported as well as the cancer chemopreventive capabilities. Recently, psychiatric and neurological effects of this natural compound have become of interest to researchers so that strong evidence would support the idea that CMT can exert significant effects on the central nervous system. Pharmacologically, this phytoestrogen would act as a selective estrogen receptor modulator with several-fold more affinity to  $\beta$  sub-types of the receptors (ER $\beta$ ); although other mechanisms of action may be involved. The aim of this review was to gather the recent reports regarding the most important pharmacological benefits of CMT focusing on the psychiatric and neurological ones. Furthermore, the mechanisms of action underlying the pharmacological effects were tried to be clarified more. For this purpose, some keywords such as "Coumestrol", "Pharmacological Effects", "Neurological", "Psychiatric" and "Neuropsychiatric" were searched in popular scientific databases such as Google scholar, Scopus and Pubmed. Then the delegated documentations were brought together, categorized and discussed on this basis. Reviewing the gathered information showed that, apart from the effects on reproduction and the related organs which are mainly conducted through estrogen receptors, CMT has reported to improve various disorders all over the body. Particularly, regarding the neurological and psychiatric systems, the advantages in cerebral hypoxia-ischemia, the Alzheimer's disease, anxiety, depression and cognitive impairments would be the most important ones. Here, other receptors that have shown interactions with CMT (beside estrogen receptors which are the main target), were also reviewed among which insulin receptors, farnesoid X receptors, pregnane X receptors, and constitutive androstane receptors can be mentioned while only the last two seem to be involved in forming the neurological and psychiatric effects.

## Introduction

Coumestrol (CMT) is a natural organic compound in the class of phytochemicals known as coumestans. This phytoestrogen has been recognized in ladino clover and some other sprouting plants such as alfalfa. Although its effects on estrogen receptors would not be as significant as 17 $\beta$ -estradiol, this isoflavonoid has become well-known among the researchers who work on natural molecules with estrogenic properties. It is important to note that, according to the literature, CMT has shown powerful antioxidant activity because of the hydroxyl groups that are also important for the estrogenic activity.<sup>1-3</sup> Interestingly, apart from the popular and well-recognized reproductive effects,<sup>1,4</sup> the molecule has exerted novel pharmacological

benefits in recent years which will be reviewed in the following sections.

The correlation of the central nervous system (CNS) function to the fluctuations in the levels of reproductive hormones have been repeatedly mentioned in the previous studies.<sup>5-7</sup> Particularly, the close link of estrogens with psychiatric and neurological conditions is not a new topic and has always been debated. As an example we can point to the relationship between the fluctuations in estradiol levels and changes in mood, cognition, sleep quality, neuroprotection and anxiety-like behaviors.<sup>8,9</sup> In this regard, the effects of various estrogenic compounds on the psychiatric and neurological conditions have been tested and claimed.<sup>10</sup> Among the compounds, selective estrogen

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receptor modulators, which are well-known as SERMs, have reported to affect the mentioned conditions clearly.<sup>11</sup> Particularly, we can point to the effects of these medicines on anxiety,<sup>12</sup> depression,<sup>13</sup> and cognitive disorders,<sup>14</sup> which have been already claimed.

Therefore, in this article we aimed to review some newly recognized and applicable pharmacological benefits of CMT, the natural modulator of estrogen receptors, with a focus on the psychiatric and neurological ones and accordingly, suggest a hypothesis regarding the possible biological pathways through which the phytoestrogen exerts its effects on the CNS.

### Search Strategy

At the first step the target keywords were identified based on the purpose of the article. They included “Coumestrol”, “Phytoestrogen”, “Pharmacological Effects”, “estrogen receptors”, “Neurological”, “Psychiatric” and “neuro-psychiatric benefits”. By following the keywords in popular scientific databases such as Google scholar, Scopus and Pubmed, the most informative papers (regarding the aim of the present review) were selected and summarized, then the important contents were extracted. It was tried to select the articles that have been published over the last three decades with direct evidence for the pharmacological effects of CMT. After that, the obtained text was reviewed several times by the team and finally the last section of the manuscript was written to conclude the biological pathways through which CMT exerts the psychiatric and neurological effects.

### The Effects of CMT Outside the CNS

As shown in Table 1, studies have suggested applicable pharmacological effects for CMT each of which can be a suitable target for future research activities. In the following, some of these effects will be mentioned.

#### Osteoporosis

Bones are the structures that are clearly affected by estrogen levels.<sup>15-18</sup> Phytoestrogens are no exceptions and have exerted significant effects on these structures.<sup>19-21</sup> In this regard, as a phytoestrogen whose alleviating effects on osteoporosis (through increasing the osteogenic differentiation and proliferation of bone marrow stromal cells) have been already suggested, CMT seems to be a suitable target for relevant developed studies in future.<sup>22</sup>

#### Cardiovascular conditions

Some cardiovascular effects have also been found for CMT through the reports.<sup>23,24</sup> Particularly, according to the literature, using the natural molecule may be a suitable solution for improving various aspects of the cardiotoxicity that occur following administration of doxorubicin. These aspects include apoptosis, oxidative stress and subsequently cardiac dysfunction. Interestingly, the phytoestrogen did not show any interactions with doxorubicin's anti-cancer effects. Regarding the mechanism, the above mentioned

effects of CMT was associated with PKA/AMPKa pathway.<sup>24</sup>

#### Obesity

Reviewing the literature has shown that CMT would exert anti-obesity effects through developing the activity of the brown adipose tissue. So that, CMT may be effective in the treatment of obesity-related metabolic diseases through elevating the energy expenditure and subsequently balancing the volume of the adipose tissue.<sup>25-29</sup>

#### Inflammation

Apart from causing anti-oxidative and anti-catabolic effects, CMT has also been introduced as an efficient anti-inflammatory agent. In this regard, a study by You *et al.*<sup>30</sup> which was conducted on rat chondrocytes indicated the activity of CMT against IL-1 $\beta$ . Therefore, the anti-inflammatory potential of CMT can be focused in the studies that are trying to find a solution for better treatment of inflammatory diseases such as osteoarthritis.<sup>31</sup>

#### Skin disorders

Rectifying effects of CMT on some skin problems have also been reported. It has been claimed that, administration of the phytoestrogen can be an effective approach to fight against the negative consequences of being exposed to ultra-violet radiations such as skin aging.<sup>32</sup> On the other hand, CMT has also exerted anti-pigmentation effect, so that it may be considered as a cosmetic agent to prevent the complications such as freckles, dark spots, solar lentigo and melasma.<sup>33</sup> Besides, the ability of the phytoestrogen in healing the wounds has been also noted through the studies.<sup>34</sup>

#### Autoimmune responses

Another pharmacological benefit of CMT is the ability to control auto-immune responses. One of these responses which has been shown to be attenuated by CMT, is auto-immune thyroiditis. Researchers have reported that this effect would occur following suppressing the activity of T-helper 1 cells.<sup>35</sup>

Systemic lupus erythematosus which is defined as targeting different organs by auto-antibodies,<sup>36</sup> has been found as the other auto-immune reaction that would be suppressed by CMT. In this regard, a study in 2004 by Schoenroth *et al.*<sup>37</sup>, indicated the efficacy of the phytoestrogen in rectifying the progression rate of the disease to some extent. Furthermore, it has been shown that, the ethyl acetate extract of alfalfa would be able to modify the illness severity in an animal model of systemic lupus erythematosus.<sup>38</sup>

#### Cancer

A significant number of documents indicate the capability of CMT in fighting against cancer. The efficacy of this natural estrogen in attenuation of various types of cancer has been tested and some of the studies have shown

**Table 1.** The pharmacological effects irrelevant to the CNS.

Disease	Effect	Detailed effects	Administration route	Administered dose	Ref.
Osteoporosis	Therapeutic potential	Increasing the osteogenic differentiation and proliferation of bone marrow stromal cells	<i>In vitro</i>	10 <sup>-10</sup> to 10 <sup>-5</sup> M	22
Cardiovascular conditions	Cardioprotection	Affecting PKA/AMPKa pathway	Perfusion to the isolated tissue, <i>in vitro</i>	1, 10 and 100 µmol.L <sup>-1</sup>	23
			Intragastrically	5 mg.kg <sup>-1</sup> .day <sup>-1</sup>	24
Obesity	Anti-obesity potential	Developing the activity of the brown adipose tissue, elevating the energy expenditure and subsequently balancing the volume of the adipose tissue	Oral gavage	100 µg per 20 g mouse	25
			<i>In vitro</i>	1 to 100 µmol.L <sup>-1</sup>	26
			<i>In vitro</i>	10 <sup>-3</sup> to 10 <sup>-1</sup> mM	27
			<i>in vitro</i>	20, 40 and 60 µM	28
Inflammation	Potent anti-inflammatory effects	Activity against IL-1β	<i>Ex vivo</i>	1, 5 and 10 µM	30
			Subcutaneously	10, 50 and 100 mg.kg <sup>-1</sup>	31
Skin disorders	Improving effects	Inhibiting UVB-induced MMP-1 expression and activity	<i>In vitro</i>	5, 10, 20, 40 and 80 mM	32
Autoimmune responses	Suppressing effects	Controlling thyroid-specific autoantibody production, suppressing the activity of T-helper 1 cells, modifying the illness severity in an animal model of systemic lupus erythematosus	Orally	0.8 mg.L <sup>-1</sup> coumestrol in 1% Tween 80 in drinking water	35
			Oral diet	0.1% coumestrol	37
Cancer	Anti-cancer potential	Targeting the relevant kinases	<i>In vitro</i>	20, 40 and 80 mM	42
			<i>In vitro</i>	10, 20, 30, 40, 50, 60 and 70 µM	39
			<i>In vitro</i>	50 µM	40
			<i>In vitro</i>	20 µM	41

interesting results. Various pathways have been suggested for the mechanism through which CMT exert its anti-cancer effects. Among them direct targeting of haspin kinase has been proposed.<sup>30,39-42</sup>

### The Effects of CMT on Neurological and Psychiatric Conditions

As presented in Table 2, some psychiatric and neurological benefits have also been introduced for CMT which will be discussed in detail below.

#### Cerebral hypoxia-ischemia

The ability of estrogens in protecting the neurons is not a novel topic and has been investigated for years.<sup>43-47</sup> This capability has been tested in various models including the in-vivo models for cerebral ischemia. Receptor-dependent pathways seem to be very important in the processes through which estrogen protect the neurons. In this regard, a rat model of neonatal hypoxia-ischemia in 2019 indicated the neuroprotection mediated through the estrogen receptors.<sup>48</sup>

As a natural estrogen, CMT has been found to be persistently able to protect the neurons against cerebral ischemia in male rats. Considering the improvements in the activity of Na<sup>+</sup>, K<sup>+</sup>-ATPase, the observed effect would be explainable.<sup>2</sup>

On the other hand, CMT has shown to have preventing effects on different phases of global ischemia-induced neuron loss in hippocampus of rats. Although the exact underlying mechanism for the neuroprotective effects of CMT is unclear, estrogen receptors are very likely to play significant roles in this regard. Since the involvement of other pathways has been found very probable,<sup>49</sup> performing further mechanistic studies in this regard would be strongly recommended.

#### Alzheimer's disease

CMT also seems to be capable of inhibiting cholinesterase enzyme. This pharmacological property of CMT can be very useful for the treatment of Alzheimer's disease (AD). Besides, CMT has been found effective in inhibiting β-secretase 1 enzyme which is strongly involved in forming the amyloid-β peptides.<sup>50</sup>

It has already been shown that, CMT would elevate the viability of astrocytes which is significantly attenuated in confronting with amyloid-β peptide and lipopolysaccharide. The fact that formation of these two develop AD, is well-understood. Their harmful effects on cerebral cells have been reported to be attenuated by CMT administration. Underlying this effect, the alterations that occur in the levels of tumor necrosis factor-α and some interleukins (such as 1 and 6) seem to be alleviated. The

**Table 2.** Benefits of coumestrol in psychiatric and neurological disorders.

Disorder	Final effect	Detailed effects	Administration route	Administered dose	Ref.
Cerebral Hypoxia-Ischemia	Alleviating the negative consequences	Improving effects on the activity of Na <sup>+</sup> , K <sup>+</sup> -ATPase, preventing effects on different phases of global ischemia-induced neuron loss	Intracerebroventricular	20 µg	2
			Intracerebroventricular	20 µg	49
Cognitive impairments	Improving effects	Beneficial effects on the both spatial and episodic memories	Intraperitoneally	30, 60 and 120 µg.kg <sup>-1</sup> .day <sup>-1</sup>	51
Alzheimer's disease	Attenuating the symptoms	Inhibiting cholinesterase enzyme, inhibiting beta-secretase 1 enzyme, elevating the viability of astrocytes	<i>In vitro</i> , Molecular docking	5 to 200 µM	50
			<i>In vitro</i>	10 <sup>-9</sup> M	52
Anxiety	Countervailing effects	Lowering the cortisol levels, anxiolytic effects in defensive freezing, light-dark transition and Vogel punished drinking tasks	Intraperitoneally	30, 60 and 120 µg.kg <sup>-1</sup> .day <sup>-1</sup>	51
			Bilateral infusions to hippocampus	2 µg.µl <sup>-1</sup> .side <sup>-1</sup>	53
			Subcutaneously	10 µg	54
Depression	Alleviating effects	Alleviating depressive behaviors in the forced swim test	Bilateral infusions to hippocampus	2 µg.µl <sup>-1</sup> .side <sup>-1</sup>	53

named factors are strongly associated with inflammation, the phenomenon that occurs in several pathological conditions including AD. Pharmacologically, CMT exert these protective effects through affecting estrogen receptors, particularly ERβ. The exact molecular pathways still remain unclear and require further mechanistic studies to be clarified.<sup>52</sup>

### Cognitive impairments

In a very recent study we showed that, CMT would be able to reduce the cognitive impairments that are chronically induced by stressful conditions through modulating ERβs in the hippocampus. According to our results, treating the mice with CMT improved the both spatial and episodic memories. As an evidence for receptor dependency of the effects, we can point to the second phase of that research where the changes returned following the administration of a specific ERβ antagonist to a significant extent.<sup>51</sup>

### Anxiety

Our previous study also proved the anxiolytic potential of CMT which was obvious in the data recorded regarding the open-field and the elevated plus maze tests. Furthermore, that research showed that treating the stressed mice with this natural molecule would result in lowering the cortisol levels in the samples obtained from serum. Interestingly, administration of the ERβ antagonist returned the changes significantly indicating the receptor dependency of the effects.<sup>51</sup> Notably, Walf and Frye had pointed the ability of CMT in reducing anxiety prior to our recent research.<sup>53</sup> The anxiolytic effects of CMT were also shown in another research where some behavioral tests such as defensive freezing, light-dark transition and Vogel punished drinking tasks were performed.<sup>54</sup>

### Depression

Alleviating depressive behaviors was the other ability of CMT that Walf and Frye mentioned in their research

where the forced swim test was employed to evaluate the mentioned behaviors in ovariectomized rats. Furthermore, the prominent role of ERβ in ameliorating depression was noted in this research.<sup>53</sup>

### Novel Findings Regarding Receptor Interactions

The fluorescence profile of CMT has been investigated for years and the results have been reported in various studies. A research in which calf uterine samples were used, showed that interaction of CMT with estrogen receptors would lead to significant alterations in the fluorescence profile of the samples. In this regard, although affinity of CMT to these receptors has been reported to be less than 17β-estradiol, the phytoestrogen would bind to the receptors to a considerable extent.<sup>55</sup>

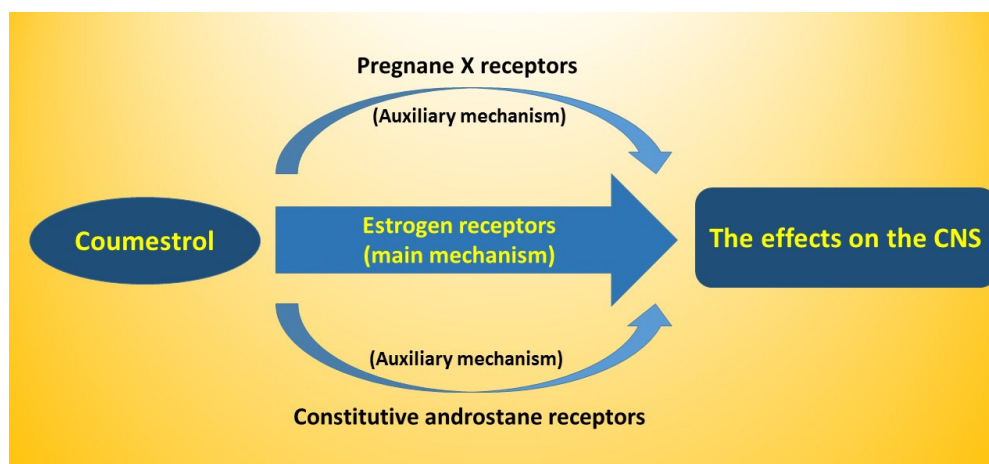
Effects of CMT on estrogen receptors have also been examined in some other tissues. Among them, human breast cancer cells can be mentioned as well as rat mammary tumors. Besides, in the presence of estradiol, CMT seems to compete pharmacologically to reach the receptors.<sup>56</sup>

On the other hand, according to a study by Saijo *et al.*<sup>57</sup> which was conducted in 2011, ERβs would be responsible for the anti-inflammatory effects of CMT in brain microglia.

In sum, although CMT seems to interact both α and β sub-types of estrogen receptors, it has shown stronger affinity to ERβ.<sup>3,58</sup> However, it is important to note that some effects of CMT are reported to be independent of its estrogenic action. A suitable example can be the effects on lipid and carbohydrate metabolism which have been previously examined in ovariectomized rats.<sup>29</sup> Furthermore, the interaction with some other receptors may be involved each of which will be discussed in the following sections.

The previous section of this review, listed the remarkable benefits of CMT in five well-known psychiatric and neurological conditions indicating that, in addition to the other reported tasks, the phytoestrogen can be focused





**Figure 1.** Proposed mechanism for the effects of coumestrol on the CNS.

regarding its effects on the CNS. As mentioned, most of the effects seem to be receptor-dependent and according to the literature, some of them have been confirmed by administration of a specific antagonist. In the following, novel findings regarding receptor interactions of CMT will be discussed and as presented in the Figure 1, the pathways that seem to result in forming the psychiatric and neurological benefits the most, will be proposed.

#### **Estrogen receptors**

Effects of estrogens on the CNS have always been of interest to researchers in different decades.<sup>59,60</sup> In previous sections, it was noted that, along with other benefits, the phytoestrogen (CMT) would be effective in the treatment of some nervous disorders. As discussed, the receptors play important roles in this regard. ER $\alpha$  and ER $\beta$  are two important subtypes of estrogen receptors. ER $\beta$  has been found involved in many important processes in the brain, particularly hippocampus. It has been suggested that the duties of ER $\beta$ s in the hippocampus are similar in both genders and controlling memory, cognition and learning would be the most important ones.<sup>52</sup> Considering this fact on one hand and the proved modulating effect of the phytoestrogen (CMT) on ER $\beta$ s on the other hand, it can be proposed that this subtype of estrogen receptors may play important roles in the development of CMT's effects on the CNS.

#### **Insulin receptors**

The insulin receptors have been introduced as tetramer structures which are formed by disulfide bridges. Naturally, they are activated by binding to insulin,<sup>61</sup> although some other compounds have been also found as the ligands interacting with these receptors. Notably, CMT is one of the ligands and would be able to alter the activity of insulin receptors.<sup>62</sup> In this regard, sphingolipid signaling pathway which subsequently leads to insulin resistance, has been reported to be inhibited by CMT.<sup>63</sup> Another study in 1993 which was conducted on ovariectomized rats has proposed that, CMT would interact membrane insulin receptors of the liver.<sup>64</sup> In this regard, lower levels of insulin were also

reported following administration of CMT.<sup>65</sup> Mostly, the interactions lead to alterations in metabolism,<sup>62-65</sup> while there is no strong evidence of an effect on psychiatric and neurological pathways.

#### **Farnesoid X receptors**

Significant number of physiological functions in organs such as the liver and intestine have been attributed to farnesoid X receptors (FXRs) which have been suggested to be strongly involved in regulating the homeostasis and metabolism throughout the body.<sup>66</sup> Particularly, they play key roles in the metabolism of bile acids.<sup>67</sup> The activity of these receptors has been also found effective in improving the non-alcoholic steatohepatitis.<sup>68</sup> Among the ligands of FXRs, we can point to CMT which is an herbal estrogen and has shown interactions with these receptors. Mostly, the outcomes are related to glucose and lipid metabolism,<sup>26</sup> while direct effects on the CNS have not been reported. Although, there is not much evidence in this regard.

#### **Pregnanane X receptors**

The pregnane x receptors (PXR) which are mostly found in the intestine and liver tissues, have been known as one of the key regulators of some important transporters and enzymes. Particularly, the role of these receptors in regulating the activity of CYP450 enzymes have been noted.<sup>69,70</sup>

Many herbal compounds have shown interactions with these receptors.<sup>71</sup> Interestingly, the effect of CMT on PXR has also been reported by Wang and colleagues in 2008.<sup>72</sup>

On the other hand, reviewing the literature indicates that the PXR in the brain would play roles in controlling behavior and some biological procedures such as apoptosis.<sup>73</sup> Therefore, PXR may provide an auxiliary pathway that contribute to some psychiatric and neurological effects of CMT (beside ER $\beta$ s which strong evidence supports their involvement).<sup>48,51-53</sup>

#### **Constitutive androstane receptors**

The activity of constitutive androstane receptors (CARs) has been detected and reported in many crucial pathways

throughout the body, particularly those responsible for detecting and metabolizing xenobiotics. Studies on this receptors have reported significant levels of activity even without binding to a ligand, although the interaction of some molecules with CARs have been claimed and proved.<sup>74</sup> CMT is one the molecules that apart from its main target, binds to the mentioned receptors and subsequently exerts some changes in their activity.<sup>72</sup>

Same as PXR's the expression of CARs in some regions of brain has been reported.<sup>73</sup> Although lack of information is well felt in this regard, affecting the CNS by CMT through CARs (as another auxiliary pathway) does not seem illogical.

### Conclusion

Coumestrol, the phytoestrogen which can be extracted from some plants such as ladino clover and alfalfa, belongs to a group of biological chemicals called coumestans. It is well-known for exerting some reproductive effects through beta-type estrogen receptors, the spectrum of pharmacological properties for coumestrol is not limited to sexual organs, though. This review aimed to gather the most informative reports regarding the pharmacological aspects of the phytoestrogen with focus on the psychiatric and neurological benefits as well as the possible receptor interactions. As presented, reviewing the literature shows that the compound has always been of interest to researchers for better treatment of various medical conditions including osteoporosis, obesity, inflammation, skin photo-aging, autoimmune thyroiditis and cancer as well as some cardiovascular conditions. Furthermore, the present article reviewed potential capacity of this phytoestrogen in alleviating some psychiatric and neurological problems such as Alzheimer's disease, cerebral hypoxia-ischemia, anxiety, depression and some sorts of cognitive impairments. Here, we also gathered the reported information about the receptors that CMT seems to interact with including estrogen receptors, insulin receptors, farnesoid X receptors (FXRs), pregnane X receptors (PXR's), and constitutive androstane receptors (CARs). Notably, in this review, it was proposed that beside estrogen receptors, part of the psychiatric and neurological effects of CMT may be relevant to the activity of PXR's and CARs, the receptors that would be interesting targets for the researchers in this field.

### Author Contributions

KF prepared the draft of the article and SSE revised the contents; AMN and JM interpreted the studies and led the work. The whole authors were involved in discussing the results and preparing the final manuscript.

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### Conflict of Interest

The authors report no conflicts of interest.

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