Prophylactic Effects of Irigenin on Cyclophosphamide-induced Nephrotoxicity and Myelosuppression in Male Rats in Comparison to Vitamin E

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Cyclophosphamide (CPA) is an alkylating agent belonging to the class oxazaphosphorines. It is a potent anticancer agent that is effective against a wide spectrum of malignancies; also it has been used for the treatment of severe manifestations of autoimmune inflammatory disease since the 1960s.

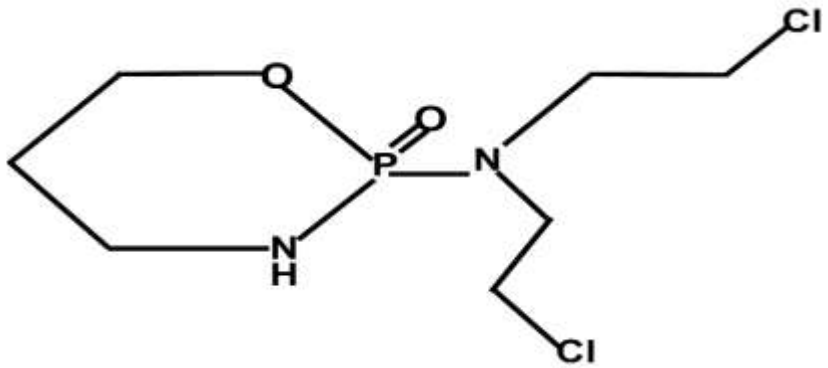


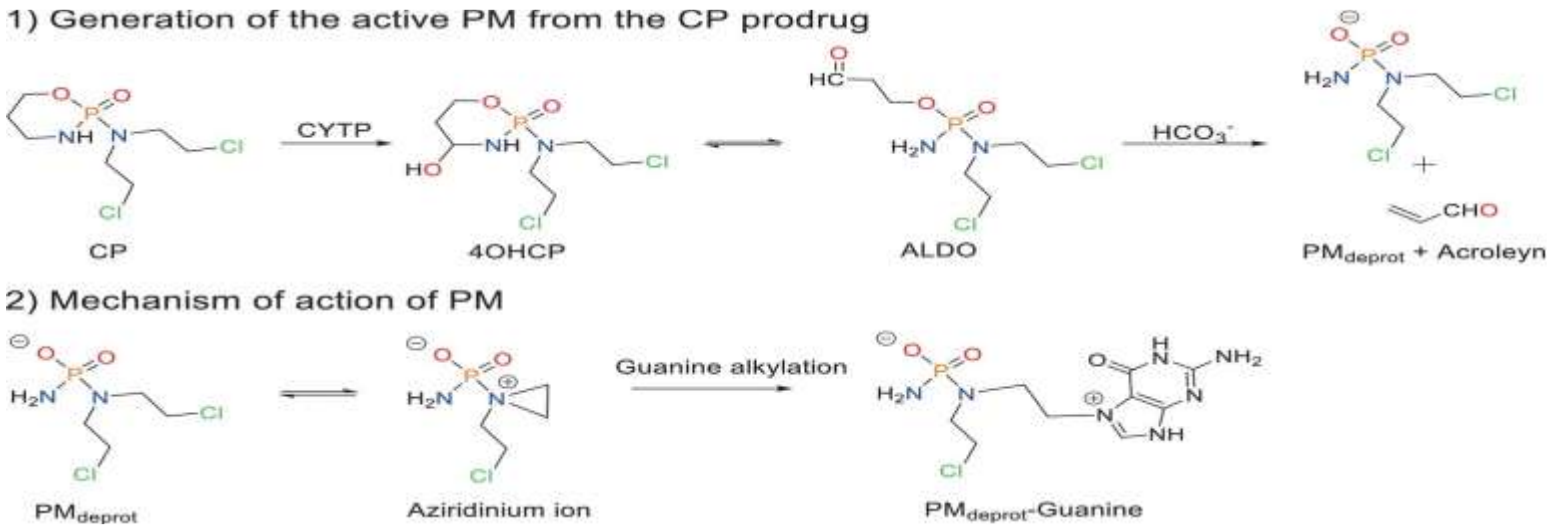
Fig. 1: Chemical structure of cyclophosphamide

Cyclophosphamide (CPA) acts as a prodrug, and its metabolism occurs in the liver to the -active and -inactive form (acrolein).

Moreover, CPA exerts its cytotoxic effects by cross-linking DNA strands, thereby inhibiting cell division and promoting apoptosis in rapidly-proliferating cells; and this mechanism is crucial for targeting cancer cells; but, CPA can produce adverse events, include sterility, nephrotoxicity, suppression of bone marrow, cystitis, and cardiovascular complications.

Researchers reported that, nephrotoxicity and myelosupression are major problems associated with CPA chemotherapy; and, oxidative stress (OS) is reported to play an important role in CPA-induced organ damage، but, other mechanisms of CPA-induced toxicity could have a role, such as inflammatory potentials, and apoptosis.

Insights on cyclophosphamide metabolism and anticancer mechanism of action: A computational study



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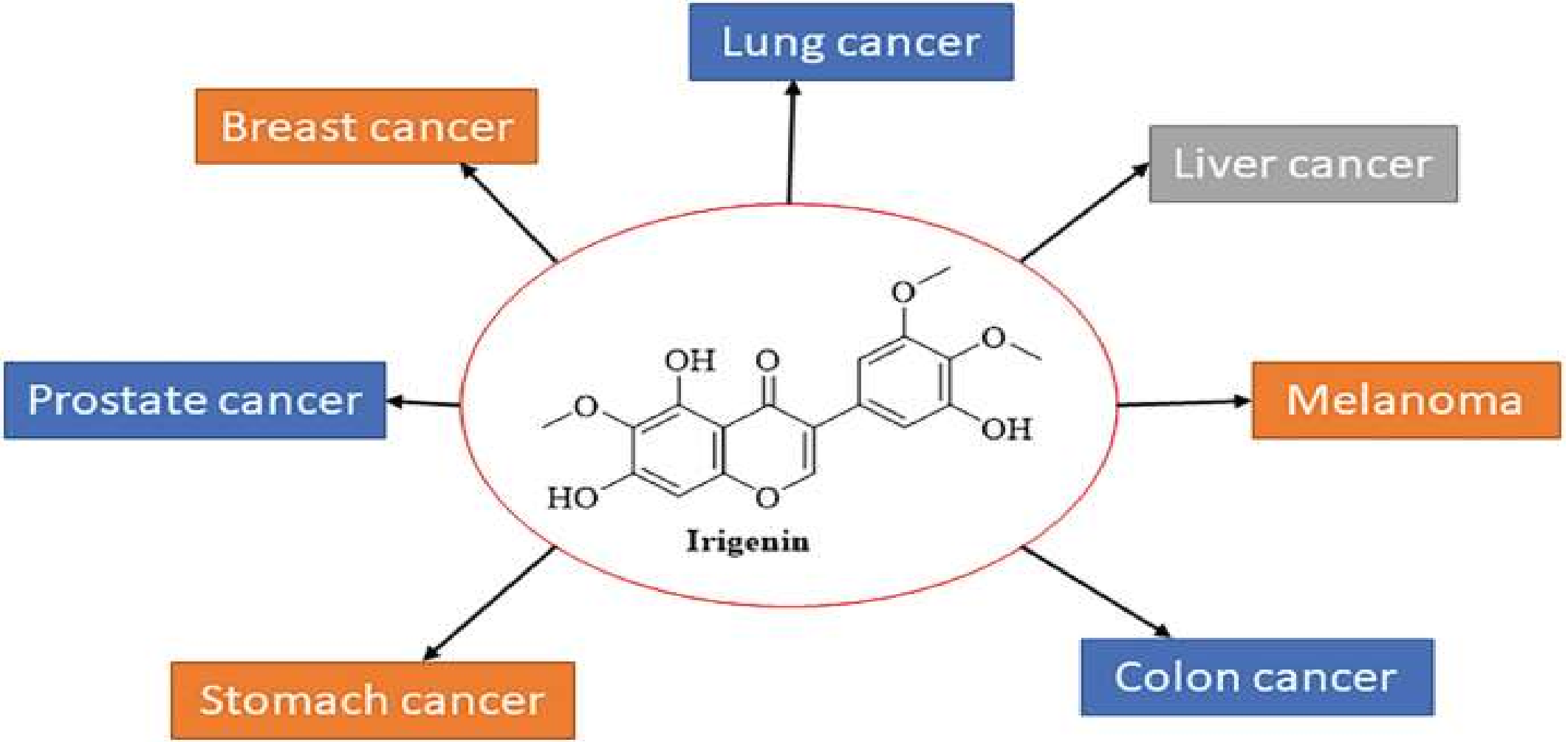
Irigenin

is an O-methylated isoflavone, **a type of flavonoid. isolated from various natural plants, including the rhizomes of the leopard lily** (*Belamcanda chinensis*) and *Iris kemaonensis*. **Crocus leaf, and Ukrainian Iris.**

Irigenin has been isolated from several plant species but majorly has been reported in the genus Belamcanda (Iridaceae) which is a monotypic genus having species, Belamcanda chinensis (L.) DC., distributed mainly in China, Japan, India, Korea, and eastern parts of Russia.



Irigenin activity on various types of cancer

Besides, researchers mentioned that, such isoflavonoid exerted anti-inflammatory, anti-oxidative, anti-apoptotic activities in the ***in vitro*** studies .

Objectives

This study is designed to investigate the effects of orally-administered (20mg/kg/day) irigenin for 28 days against a single IP dose of CPA (injected at day 28) against a single toxic dose (150mg/kg) of CPA (in comparison to Vitamin E) on:

1- Oxidative stress (OS) potential in the **kidney tissue homogenate** of rats by utilizing selected parameters of OS **[MDA, reduced glutathione (GSH), glutathione peroxidase (GPx)** enzyme level, by ELIZA method; and **2**- The inflammatory **(TNF-α, IFN-gamma, IL-17)** and the anti-inflammatory markers **(IL-10)** levels **by the qRT-PCR mRNA expression assay.**

3- Levels of apoptotic markers **[caspase-3 (Casp-3), and Fas];** and its effect on the antiapoptotic protein **Bcl-2** level in the **kidney tissue homogenate by Western blot (W.B) analysis.**

4- Measurments of serum urea and creatinine levels.

The **myelosuppression**- induced by a single toxic dose of **CPA** through the estimation of complete blood count **(CBC) [(RBCs), (Hb), (WBCs), platelets.**

5- Histological examination of kidney and BM rats' femur.

Animals and Experimental Design

Fifty (50) apparently-healthy male Albino rats weighing **150-250gm** to be randomized into **5** groups (**10 animals/group)** as follows:

Group I. Rats orally-administered 1% tween 20 dissolved in distilled water (DW) *via* rats 'oral gavage for 28 days. This group represents the **control group**. The 1% Tween 20 is safe when given to rats.

Group II. (The induction group). Rats orally-administered 1% tween 20 dissolved in distilled water (D.W) *via* rats' oral gavage for 28 days and a single IP dose of **CPA (150mg/kg/day)** to be injected on day 28.

Group III. Rats orally- administered **irigenin** at a dose (20mg/kg/day) suspended in 1% tween 20 dissolved in distilled water (DW) *via* rats 'oral gavage for 28 days.

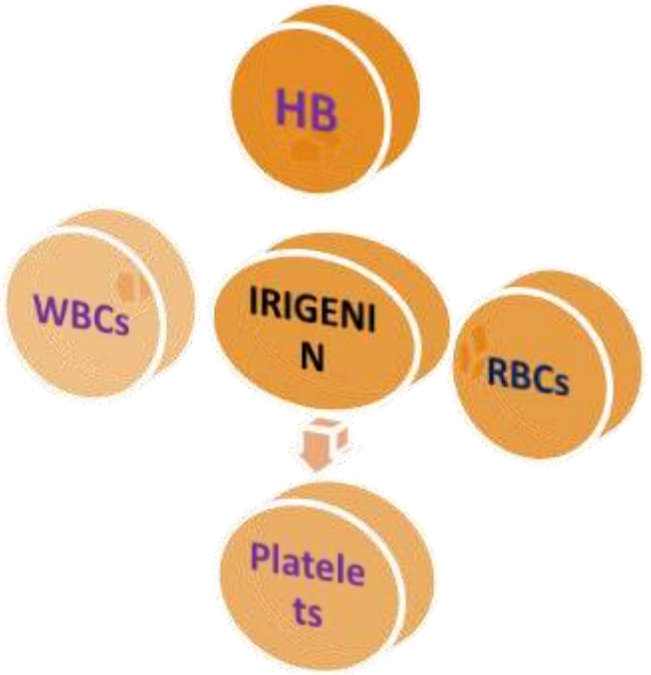
Group IV. Rats orally- administered **Irigenin** at a dose (20mg/kg/day) suspended in 1% tween 20 dissolved in DW *via* rats 'oral gavage for 28 days; and a single IP injection of **CPA (150mg/kg/day)** to be given at day 28.

Group V. Rats orally- administered **Vitamin E** at a dose **(50mg/kg/day)** suspended in 1% tween 20 dissolved in DW *via* rats 'oral gavage for 28 days; and a single IP injection of **CPA (150mg/kg/day)** to be given at day 28.

Twenty-four hours (24hr) after the end of the treatment duration (i.e., at day 29), each male rat will euthanized by diethyl ether then to be sacrificed by cervical dislocation, and then the blood will obtain from the carotid artery from the neck and to be collected.

Results


Fas
Irigenin
Caspa
se-3
Bcl-
2


HB
WBCs
IRIGENI
N
RBCs
Platele
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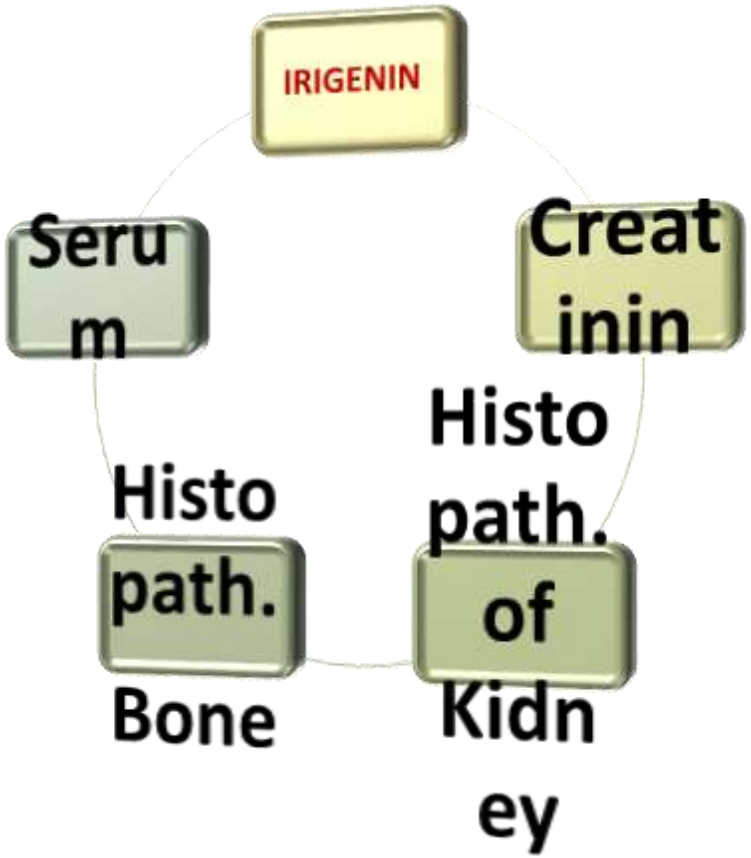
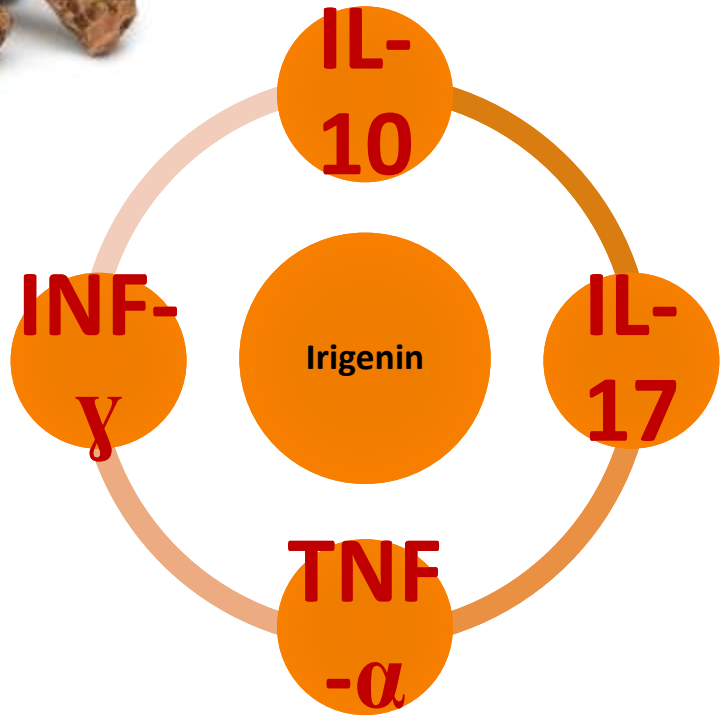

IRIGENIN
Creat inin
Histo path. of Kidn ey
Histo path. Bone
Seru m


IL-10
INF-γ
Irigenin
IL-17
TNF-α

Conclusion

In the present study, it was observed that CPA-induced nephrotoxicity and myelosuppression.

I expect that according to experimental results, pre-treatment with **irigenin** could decrease renal injuries and mylosupression-induced by CPA via the modulation of various markers of OS, inflammation, apoptosis, autophagy, and oxidative DNA damage in the rats.

Thus, the natural based antioxidants and anti-inflammatory properties may have a promising nephroprotective activity in preclinical studies, and might be an effective source for nephroprotective agents.


Thank
Yall