

Anti-carcinogenic and anti-inflammatory potential of potato glycoalkaloids

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Publications

- Anti-carcinogenic

Kenny et al (2013) Cytotoxic and apoptotic potential of potato glycoalkaloids in a number of cancer cell lines. *Journal of Agricultural Science and Applications* **2**, 184-192

- Anti-inflammatory

Kenny et al (2013) Anti-inflammatory properties of potato glycoalkaloids in stimulated Jurkat and Raw 264.7 mouse macrophages. *Life Sciences* **92**, 775-782

Glycoalkaloids

- The Solanaceae plant family includes aubergine, tomato and potato.
- Glycoalkaloids are secondary plant metabolites produced by the Solanaceae that at certain levels may be toxic to bacteria, fungi, viruses, insects, animals, and humans.
- The potential human toxicity of glycoalkaloids has led to the establishment of guidelines limiting the glycoalkaloid content of new cultivars of potatoes before they can be released for commercial use.
- Although glycoalkaloids are toxic, studies during the past 10 years suggest that they may also possess beneficial effects, depending on dose and conditions of use.

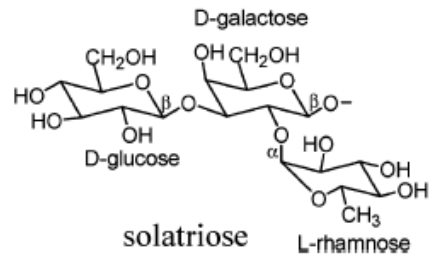
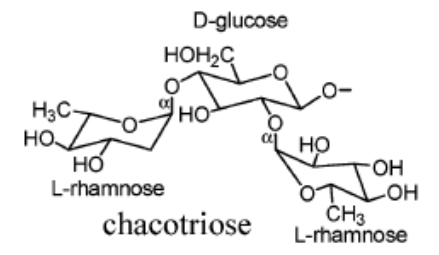
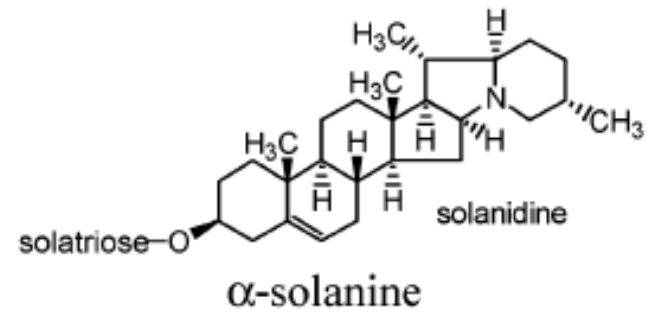
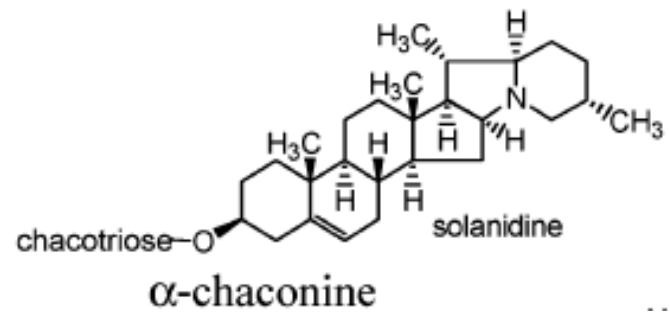
Potato Glycoalkaloids

The two major glycoalkaloids in domestic potatoes (*Solanum tuberosum*) are α -chaconine and α -solanine.

α -Chaconine has a branched α -chacotriose carbohydrate side chain attached to the 3-OH group of the aglycone solanidine.

α -Solanine has a branched α -solatriose side chain also attached to the 3-OH group of the same aglycone.

The trisaccharide chains of both glycoalkaloids can be sequentially cleaved by acid or enzyme hydrolysis to form the aglycone solanidine.



Distribution of Glycoalkaloids in Whole Tubers

- The majority of glycoalkaloids in the potato tuber are located within the first 1 mm from the outside surface and decrease toward the centre of the tuber.
- Peeling of the tissue 3-4 mm from the outside before cooking removes nearly all of the glycoalkaloids.
- Genotype has a strong influence on rates and patterns of accumulation, and ratio of α -chaconine to α -solanine during tuber growth and development.

Glycoalkaloid Content of Extracts of Potato Flesh, Peel, and Whole Potatoes

From: Friedman et al. (2003) *J. Agric. Food Chem.* 51, 2964-2973

sample (dehydrated powder)	$\mu\text{g/g}$			ratio (A/B)
	α -chaconine (A)	α -solanine (B)	total (A + B)	
Atlantic potato peel	59.4	24.4	83.8	2.43
Atlantic potato flesh	22.6	13.9	36.5	1.63
Russet Narkota potato peel	288	138	425	2.09
Russet Norkota potato flesh	3.7	2.7	6.4	1.37
Dark Red Norland potato peel	859	405	1264	2.12
Dark Red Norland potato flesh	16.0	6.1	22.1	2.62
Snowden potato peel	2414	1112	3526	2.17
Snowden potato flesh	366	226	591	1.62
Russet whole potatoes	65.1	35.0	100	1.86
White whole potatoes	28.2	15.3	43.5	1.84
Benji whole potatoes	70.7	27.6	98.3	2.56
Lenape whole potatoes	413	216	629	1.91

**Anti-carcinogenic potential of potato
glycoalkaloids ?**

Previous Studies on Anticancer Activities of Glycoalkaloids

- Glycoalkaloids from potato showed anticancer activity against human colon and liver cancer cells.

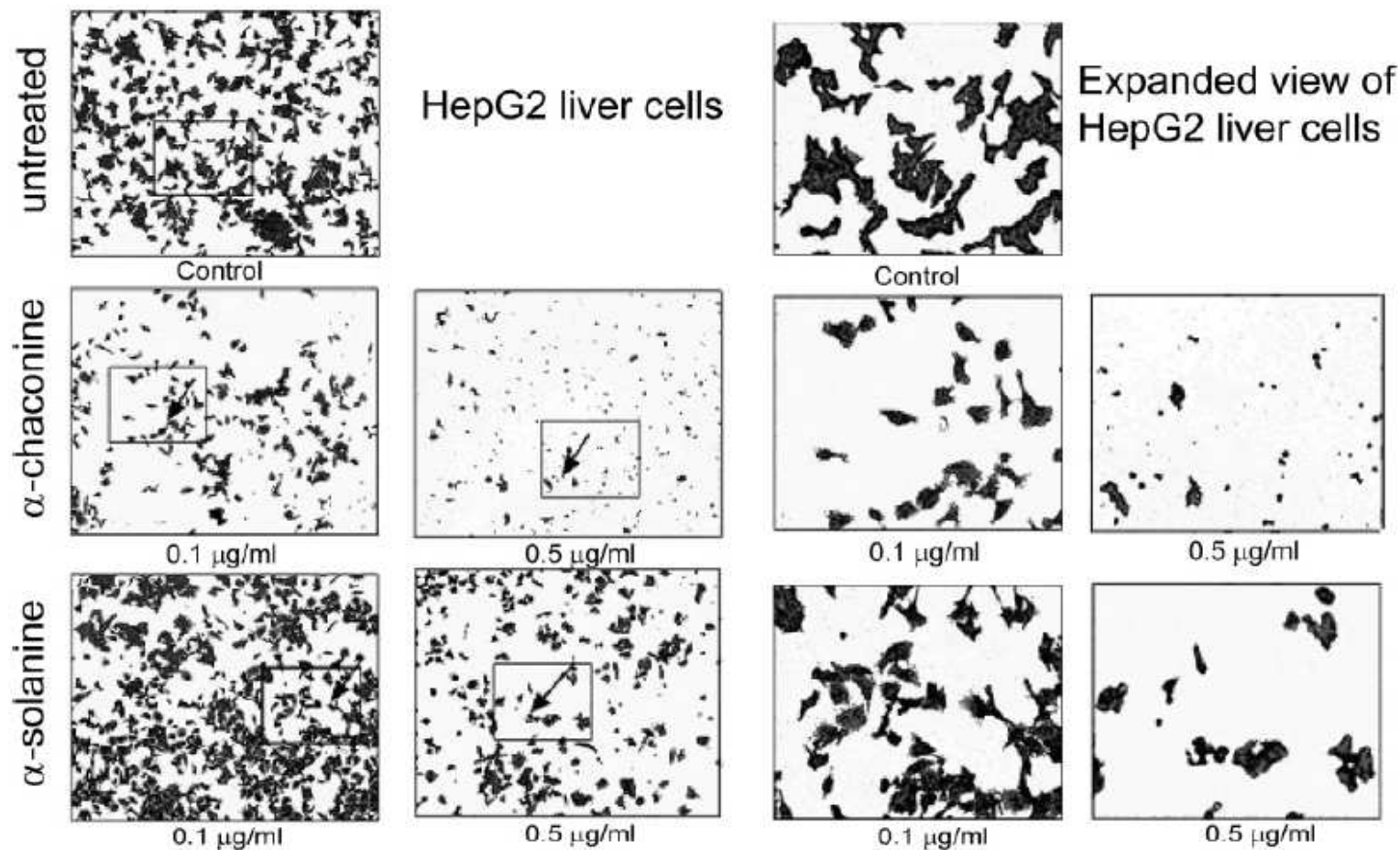
Lee KR, Kozukue N, Han JS, Park JH, Chang EY, et al.: Glycoalkaloids and metabolites inhibit the growth of human colon (HT29) and liver (HepG2) cancer cells. *J Agric Food Chem* **52**, 2832–2839, 2004.

- Glycoalkaloids from aubergine inhibited basal cell carcinoma growth in vivo (humans).

Punjabi S, Cook LJ, Kersey P, Marks R, and Cerio R: Solasodine glycoalkaloids: a novel topical therapy for basal cell carcinoma. A double-blind, randomized, placebo-controlled, parallel group, multicenter study. *Int J Dermatol* **47**, 78–82, 2008.

Photomicrographs Showing the Concentration-Dependent Destruction of Human Liver Cancer Cells by α -Chaconine and α -Solanine

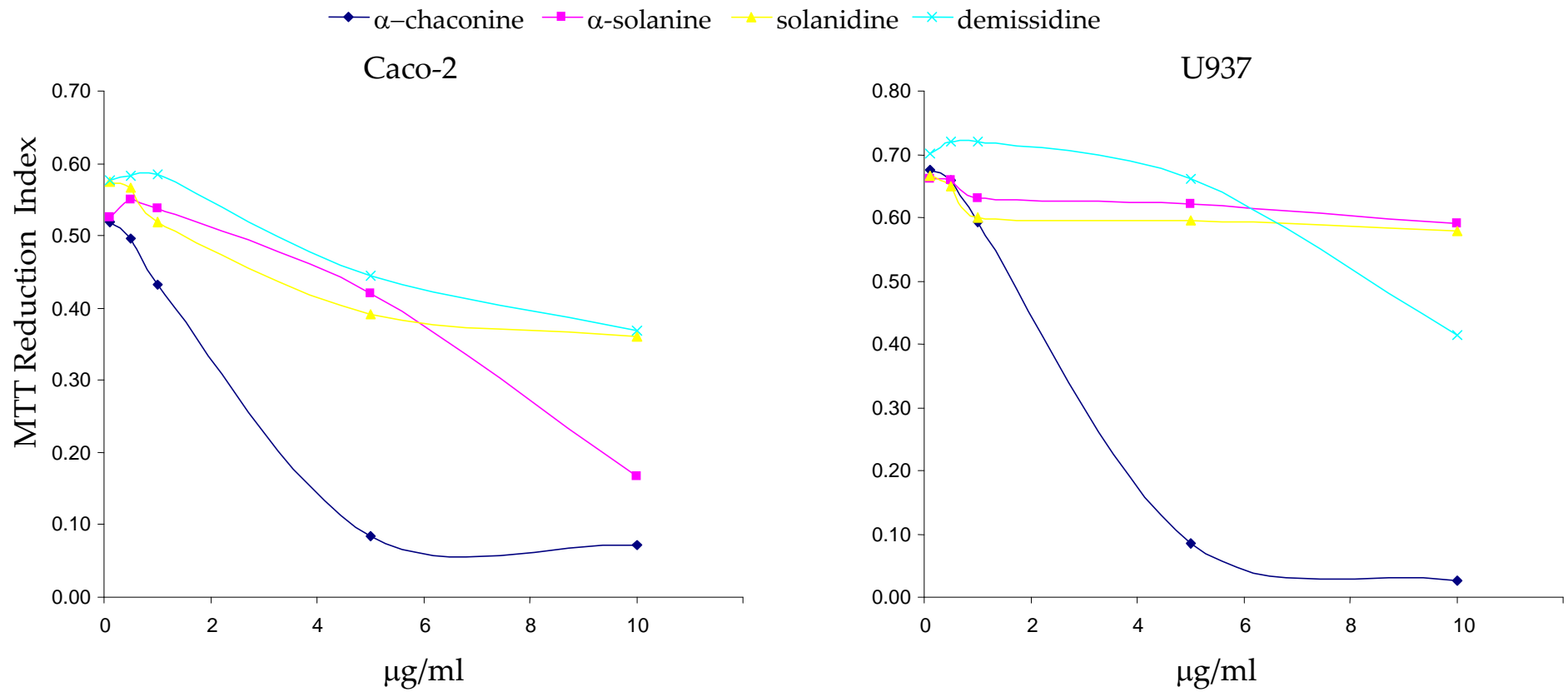
From: Lee et al (2004). *J Agric Food Chem* 52, 2832–2839, 2004.



Assess Anticancer Potential of Potato Glycoalkaloids

1. Inhibition of cancer cell growth (anti-proliferative activities) in a range of human cancer cell lines: Caco-2 (colon), HepG2 (liver), Jurkat (T cell), MCF-7 (breast) and FFF (skin fibroblasts).
2. Establish IC50 values for test compounds.
3. Ability to induce programmed cell death (apoptosis).
4. Effects on COX-2 enzyme activity in human colon cells.
5. Effects on Phase 2 metabolising enzymes in human liver cells.

Antiproliferative Activities of Potato Glycoalkaloids in Human Cancer Cells



IC50 Values for Potato Glycoalkaloid in a Range of Human Cancer Cells

Glycoalkaloids

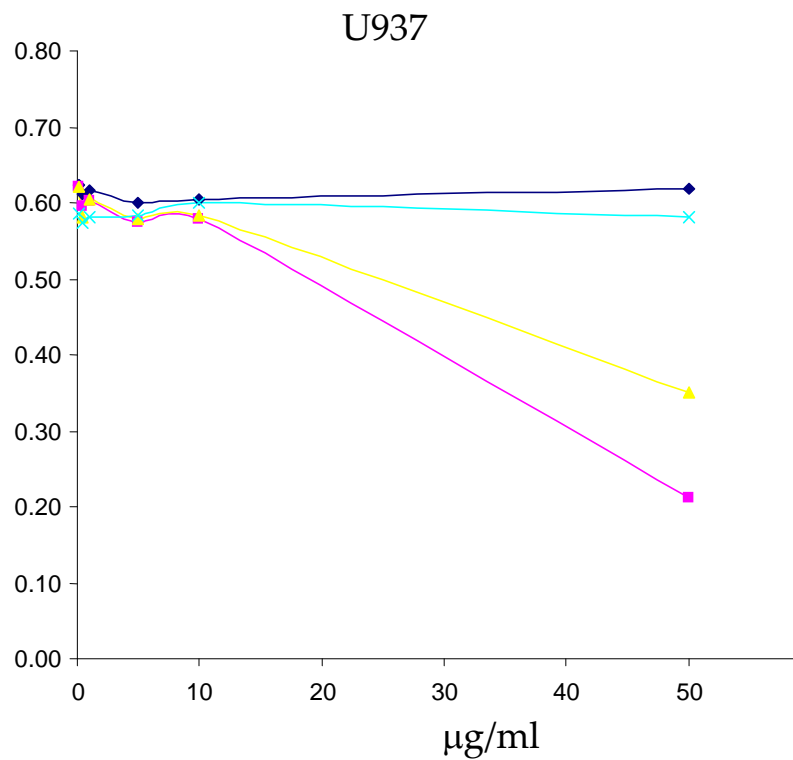
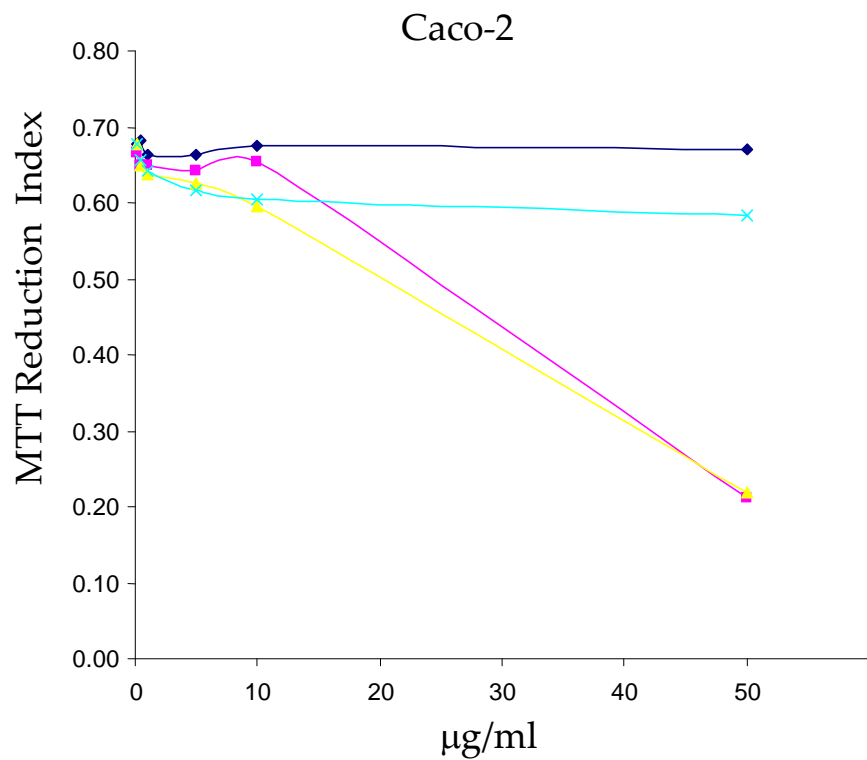
	IC50 ($\mu\text{g/ml}$)						
	<u>Normal</u> (colon)	<u>Jurkat</u> (T cell)	<u>U937</u> (monocyte)	<u>HepG2</u> (liver)	<u>Caco-2</u> (colon)	<u>MCF-7</u> (breast)	<u>HFFF2</u> (skin)
α -Chaconine	2	3	2	2	4	2	3
α -Solanine	8	15	>10 (ND)	6	8	10	7
Solanidine	5	40	>10 (ND)	34	25	30	14
Demissidine	---	11	13	16	14	---	7

Concentrations of Glycoalkaloids in Crude and Semi-purified Potato Peel Extracts

	<u>Crude Extract</u> <u>($\mu\text{g}/\text{mg}$)</u>	<u>Semi Purified</u> <u>Extract (4)</u> <u>($\mu\text{g}/\text{mg}$)</u>	<u>Semi Purified</u> <u>Extract (5)</u> <u>($\mu\text{g}/\text{mg}$)</u>	<u>Semi Purified</u> <u>Extract (6)</u> <u>($\mu\text{g}/\text{mg}$)</u>
α -Chaconine	5.5	18.6	12.9	0.3
Solanidine	1.7	18.0	---	---
Demissidine	0.6	1.3	---	---
α -Solanine	3.9	---	---	22

Antiproliferative Activities of Potato Peel in Human Cancer Cells

—◆— Crude Extract —■— SPE Fraction 4 —▲— SPE Fraction 5 —×— SPE Fraction 6



IC50 Values for Potato Peel Extracts in Range of Human Cancer Cells

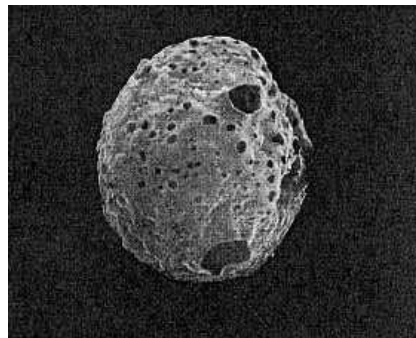
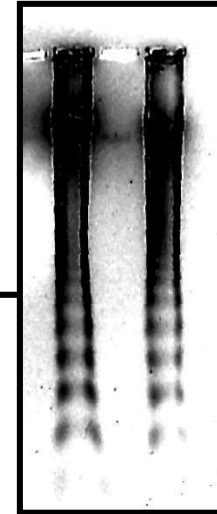
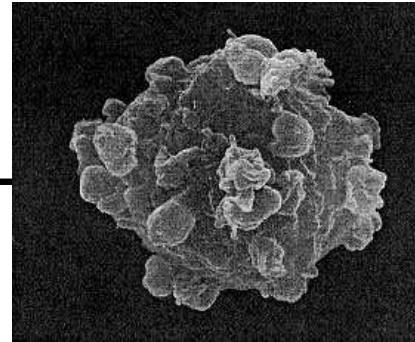
Potato Peel Extracts

	<u>IC50 range (μg extract/ml)</u>
Crude Extract	71 (Caco-2) – 117 (U937)
Semi- Purified Extracts	
Fraction 4	19 (Jurkat) – 40 (Caco-2)
Fraction 5	34 (Caco-2) – 140 (U937)
Fraction 6	> 50 (all)

Apoptosis vs Necrosis

Apoptosis

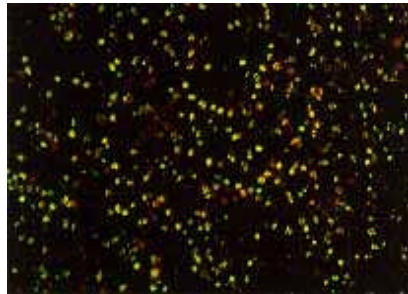
- “Active” process
- Requires gene expression
- No tissue inflammation
- Cells shrink in size
- Membrane integrity maintained until fairly late
- DNA cleaved into fragments



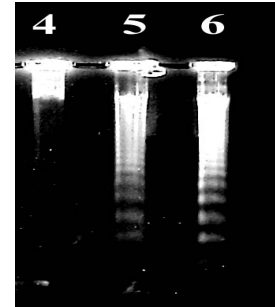
Necrosis

- “Passive” process, lysis
- Inflammation
- Cells swell in size
- Membrane integrity lost early

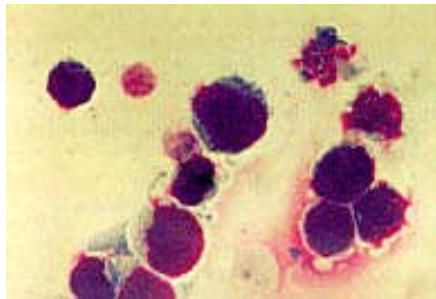
Measurement of Apoptosis



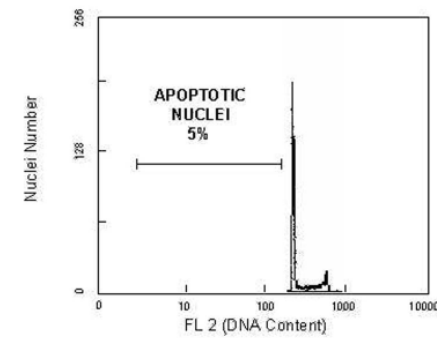
Cell Membrane Integrity



DNA Fragmentation Assays



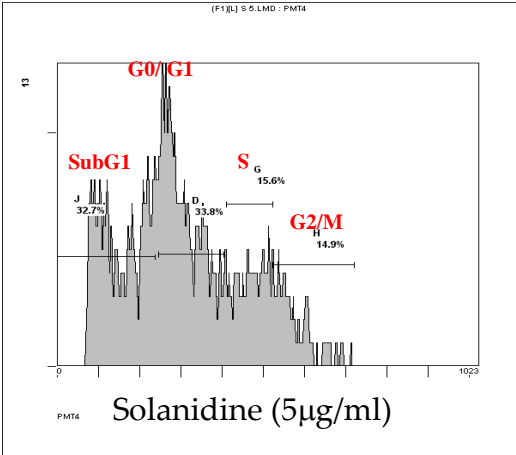
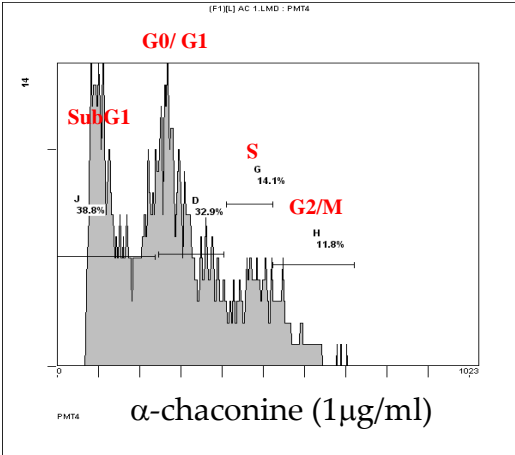
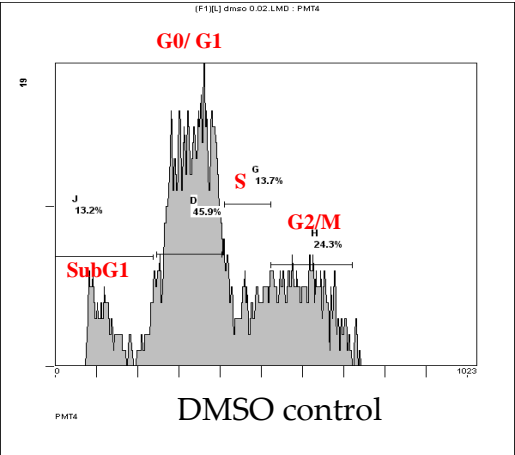
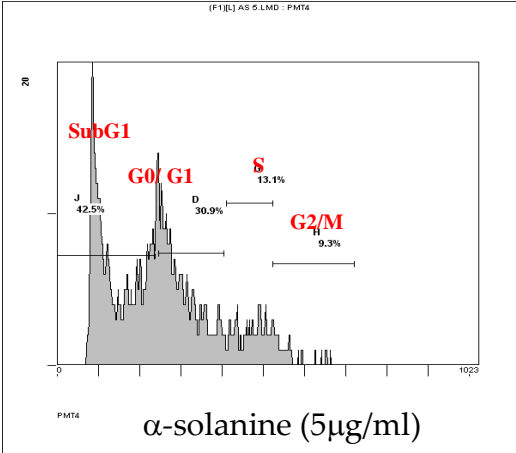
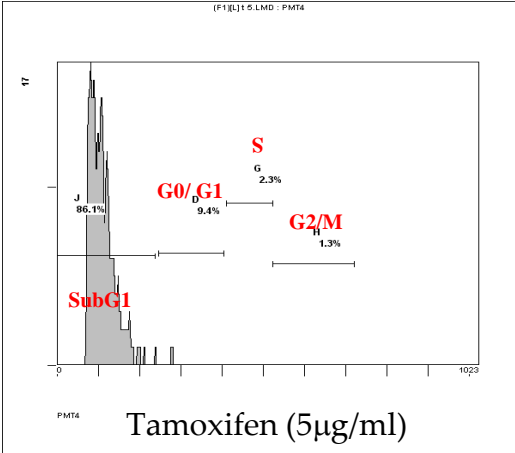
Morphological Analysis



Flow Cytometry

Potato Glycoalkaloids and Apoptosis

Flow Cytometry



COX-2 and Phase 2 Enzyme Activities

- Inhibition of cyclooxygenase-2 (COX-2) is a potential mechanism by which compounds reduce colorectal cancer risk. COX-2 activity is elevated in disease (cancer) states.
- One of the major mechanisms of protection against carcinogenesis, mutagenesis, and other forms of toxicity mediated by carcinogens is the induction of enzymes involved in their metabolism, particularly phase 2 enzymes such as glutathione S-transferases (GSTs).

The Potential of Potato Glycoalkaloids to Affect COX-2 Activity and Phase 2 Enzymes

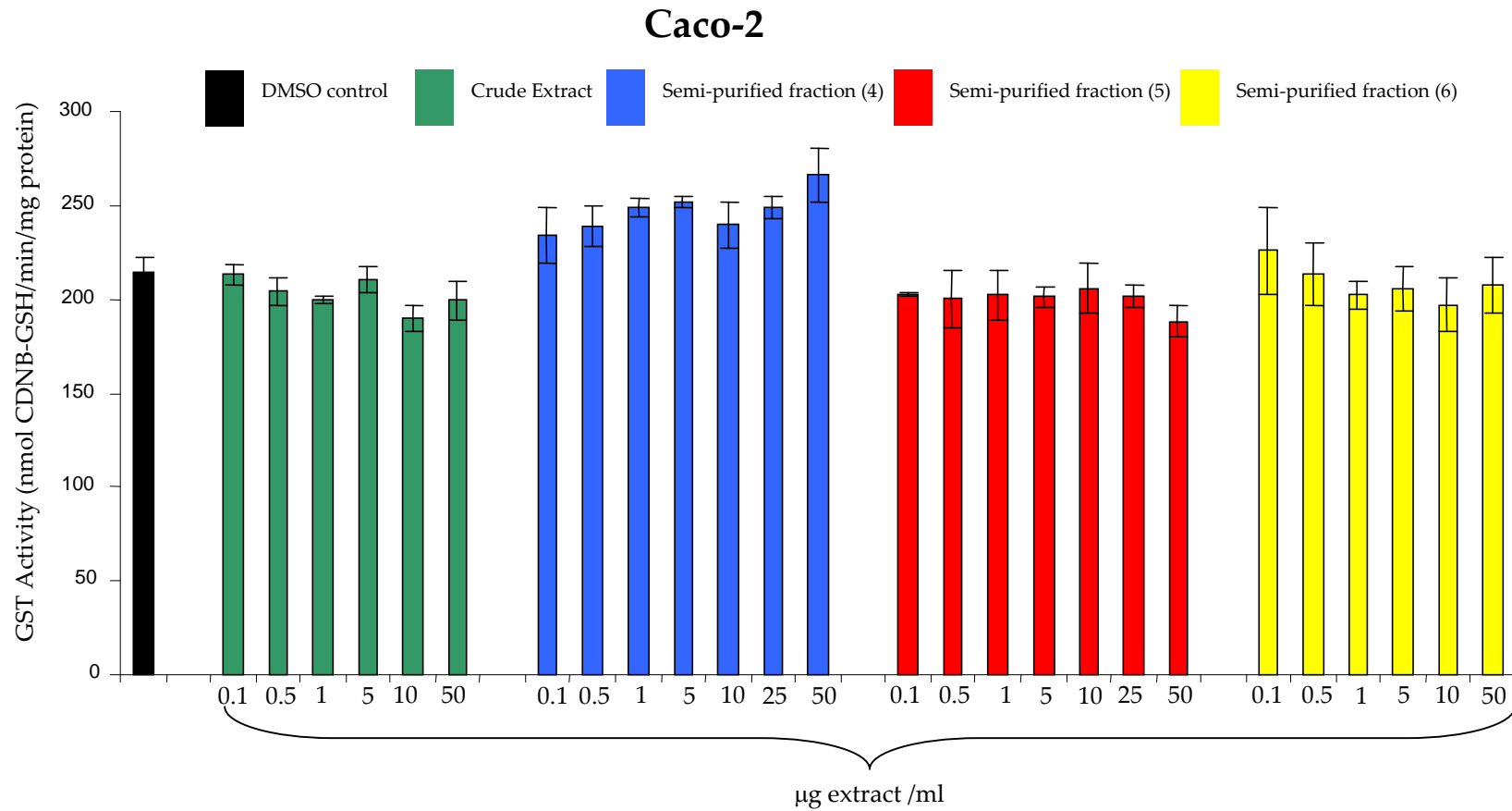
Cox-2 activity

- Caco-2 cell COX-2 activity was not significantly affected by the addition of glycoalkaloids.

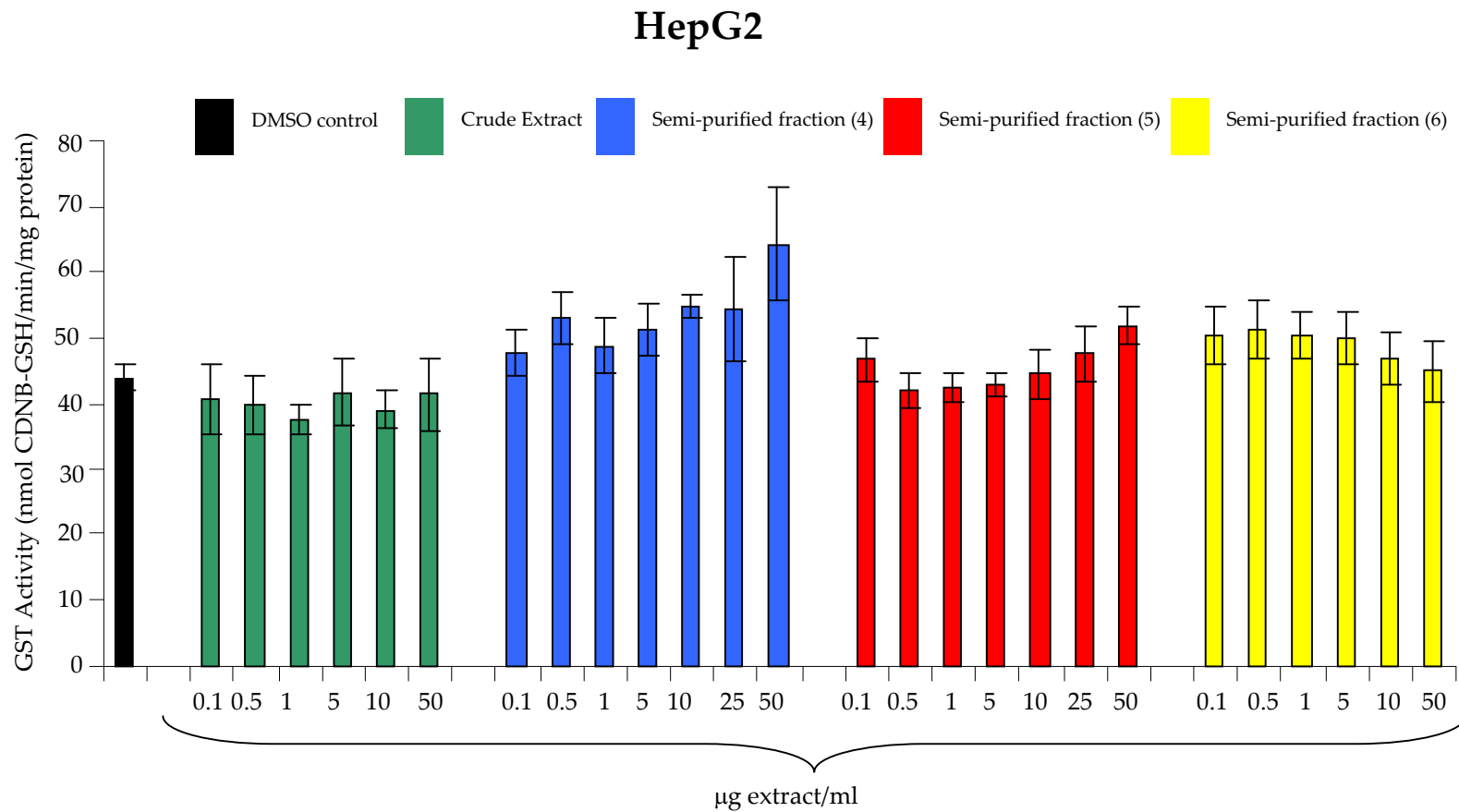
Phase 2 enzymes

- Glutathione levels in Caco-2 & HepG2 cells are unaffected by the addition of glycoalkaloids, crude extract and semi-purified potato peel extracts.
- Glutathione-S-transferase: activity in Caco-2 & HepG2 cells was not significantly affected by the addition of glycoalkaloids or extracts.

The Potential of Potato Peel Extracts to Affect GST Activity in Human Colon Cancer cells



The Potential of Potato Peel Extracts to Affect GST Activity in Human Liver Cancer Cells



Growth Inhibition of Normal Human Colon Cells by Potato Glycoalkaloids

- Examined the effect of exposing a normal colon cell line to α -chaconine and α -solanine.
- The data show that these compounds also inhibited the growth of the normal cells. The lack of an apparent differential effect on carcinoma and normal liver cells implies that in addition to efficacy, safety considerations should govern possible therapeutic uses of the plant compounds.

IC50 Values for Potato Glycoalkaloid in a Range of Human Cancer Cells

Glycoalkaloids

	IC50 ($\mu\text{g/ml}$)						
	<u>Normal</u> (colon)	<u>Jurkat</u> (T cell)	<u>U937</u> (monocyte)	<u>HepG2</u> (liver)	<u>Caco-2</u> (colon)	<u>MCF-7</u> (breast)	<u>HFFF2</u> (skin)
α -Chaconine	2	3	2	2	4	2	3
α -Solanine	8	15	>10 (ND)	6	8	10	7
Solanidine	5	40	>10 (ND)	34	25	30	14
Demissidine	---	11	13	16	14	---	7

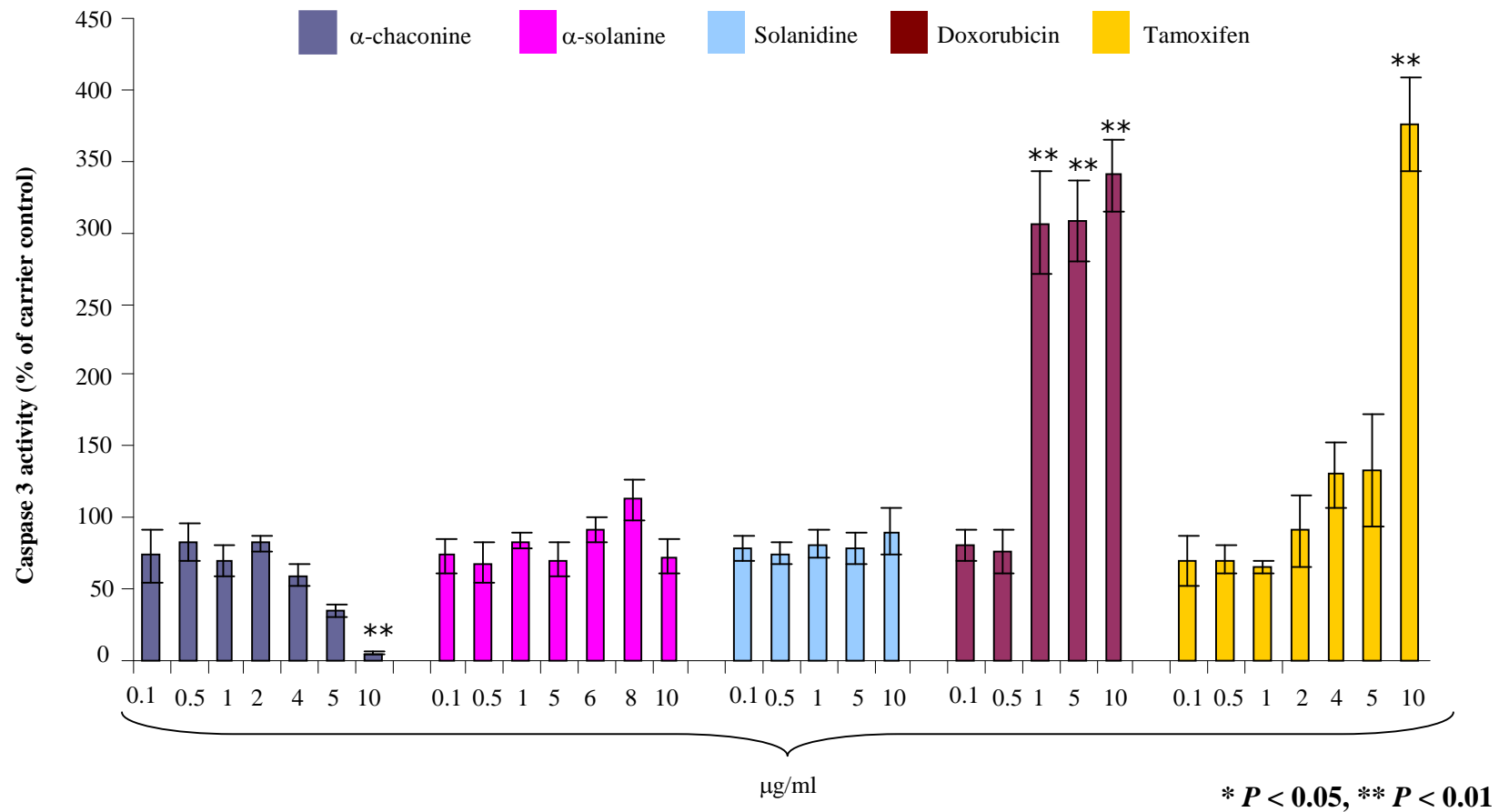
Comparison with Tamoxifen and Doxorubicin

- The drugs doxorubicin and tamoxifen are currently used to treat cancer patients.
- We compared the potencies of these two drugs to the plant-derived compounds.
- The inhibitory effects of tamoxifen on growth of the liver and colon cancer cells were similar to α -chaconine and α -solanine.
- However, the drugs did induce apoptotic cell death.

IC50 Values ($\mu\text{g/ml}$)

	<u>Caco-2</u> Colon cancer cells	<u>HepG2</u> Liver cancer cells
α -Chaconine	4	2
α -Solanine	8	6
Solanidine	25	34
Demissidine	14	16
Therapeutic drug Tamoxifen	4	3

Caspase 3/7 activity in Human Liver Cancer Cells (HepG2)



Summary

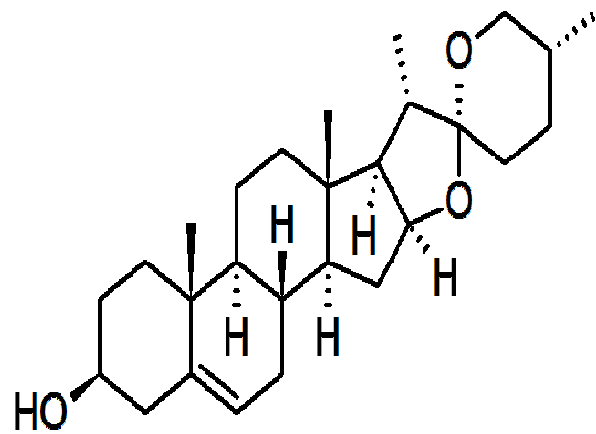
- ❖ Potato glycoalkaloids (especially α -chaconine) are as toxic as the cancer drug tamoxifen.
- ❖ Potato glycoalkaloids are non-apoptotic.
- ❖ Potato glycoalkaloids – low anticarcinogenic potential.

Anti-inflammatory potential of potato glycoalkaloids

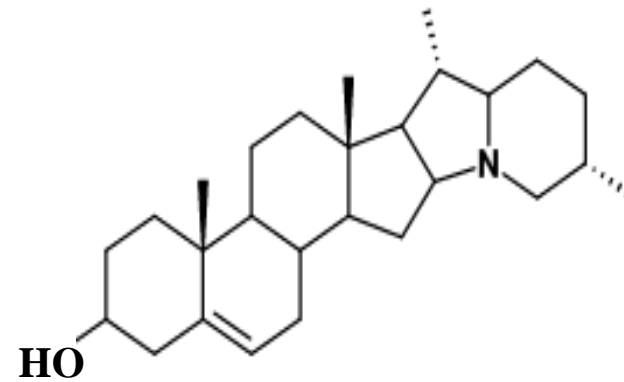
Aim of study

Rationale:

- The potato aglycone, solanidine shares structural similarities to diosgenin, a precursor of steroidal hormones and anti-inflammatory steroids.
- Therefore, α -chaconine, α -solanine and solanidine, along with potato peel extracts were investigated for potential anti-inflammatory effects.
- Their potential to reduce two biomarkers of inflammation:
 - pro-inflammatory cytokines (IL2 and IL8)
 - nitric oxide (NO) productionwere assessed in the stimulated T cells and macrophages, respectively.



Diosgenin



Solanidine

Structural similarities of the potato aglycone solanidine to the saponin diosgenin

Cell lines

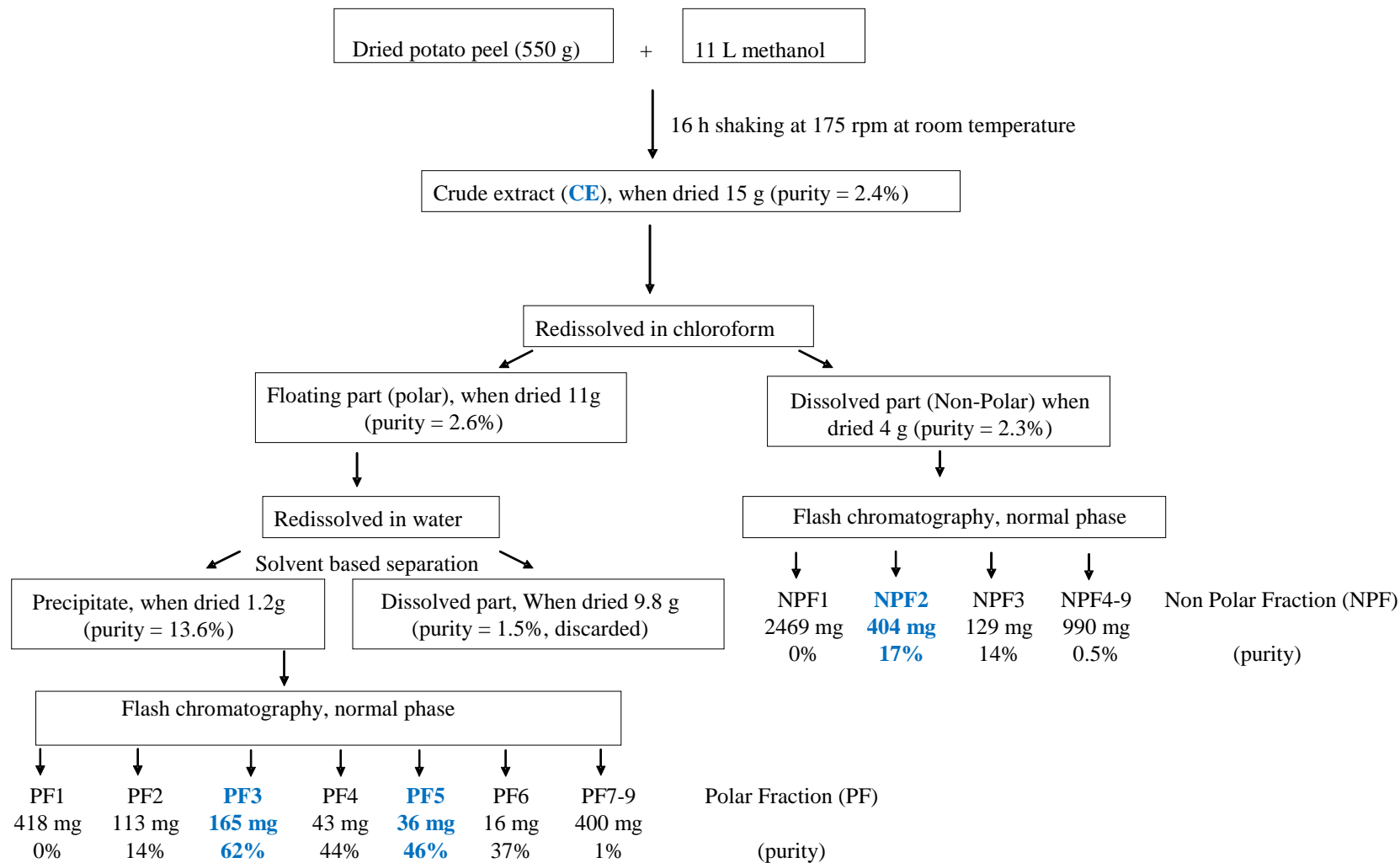
- **Human Jurkat T cells** –lymphocyte cell line stimulated to produce cytokines on exposure to concanavalin A (Con A).
- **RAW 264.7 mouse macrophages** – stimulated to produce NO on exposure to bacterial lipopolysaccharide (LPS).

Cytokines

- Cytokines are involved in almost every disease and manipulation of cytokine levels through diet is an exciting area of research.
- IL-2 is secreted by activated T helper cells and acts as a growth factor/activator for T cells, NK cells and B cells.
- IL-8 is a member of the chemokine superfamily and acts as a chemotactic cytokine responsible for the migration and activation of cells such as neutrophils, monocytes and lymphocytes to the site of inflammation.

Nitric oxide (NO)

- NO is an activated macrophage secretory product.
- Nitric oxide is produced by NO synthase (iNOS, EC 1.14.13.39).
- iNOS is induced in macrophages and several other cell types by cytokines and/or products derived from microorganisms.
- iNOS-derived NO is involved in suppression of several immune responses.
- NO production is assessed by measuring nitrite accumulation in culture supernatants. This is carried out using the Griess reaction.



Schematic diagram for the partial purification of glycoalkaloids from potato peel. Crude extract (CE) was generated from potato peel and subjected to solvent based separation producing semi-purified extracts (SPEs) containing Polar Fraction (PF) and Non Polar Fraction (NPF). Fractions selected for biological testing included **CE, PF3, PF5 and NPF2**

Concentration of glycoalkaloids present in crude extract (CE) and semi-purified extracts (SPEs) from potato peel

	CE ($\mu\text{g}/\text{mg}$)	SPE PF3 ^a ($\mu\text{g}/\text{mg}$)	SPE PF5 ^a ($\mu\text{g}/\text{mg}$)	SPE NPF2 ^b ($\mu\text{g}/\text{mg}$)
α -Chaconine	10.03	533	22.5	46.1
Solanidine	5.13	70	0.1	107
Demissidine	1.67	11.3	---	16.7
α -Solanine	7.56	1.7	439.5	0.4
Total glycoalkaloid content	24.39	616	460	170.2

^a Polar Fraction (PF), ^b Non Polar Fraction (NPF)

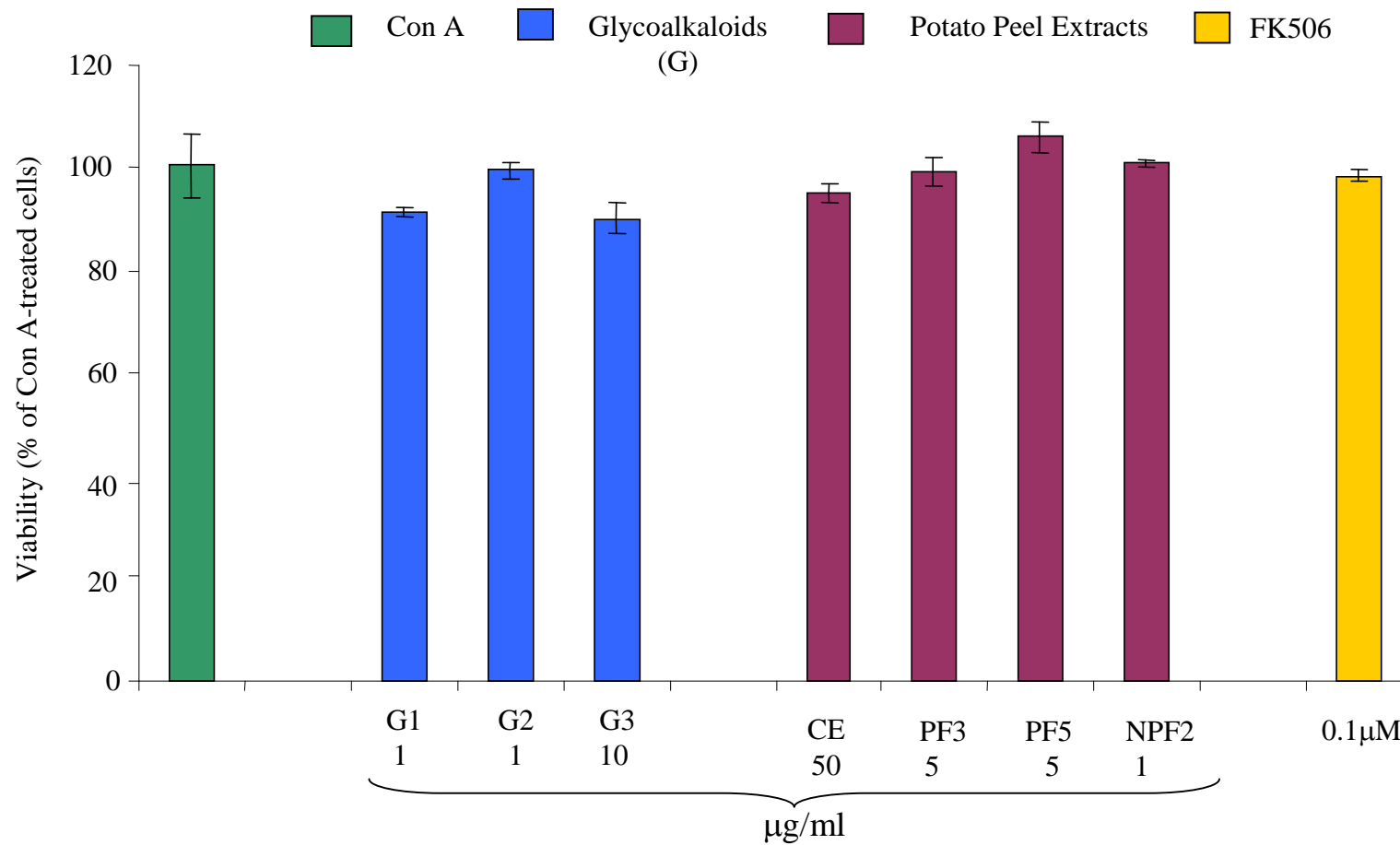
IC50 values for glycoalkaloids and potato peel extracts in Jurkat cells

IC50 data generated from the MTT assay

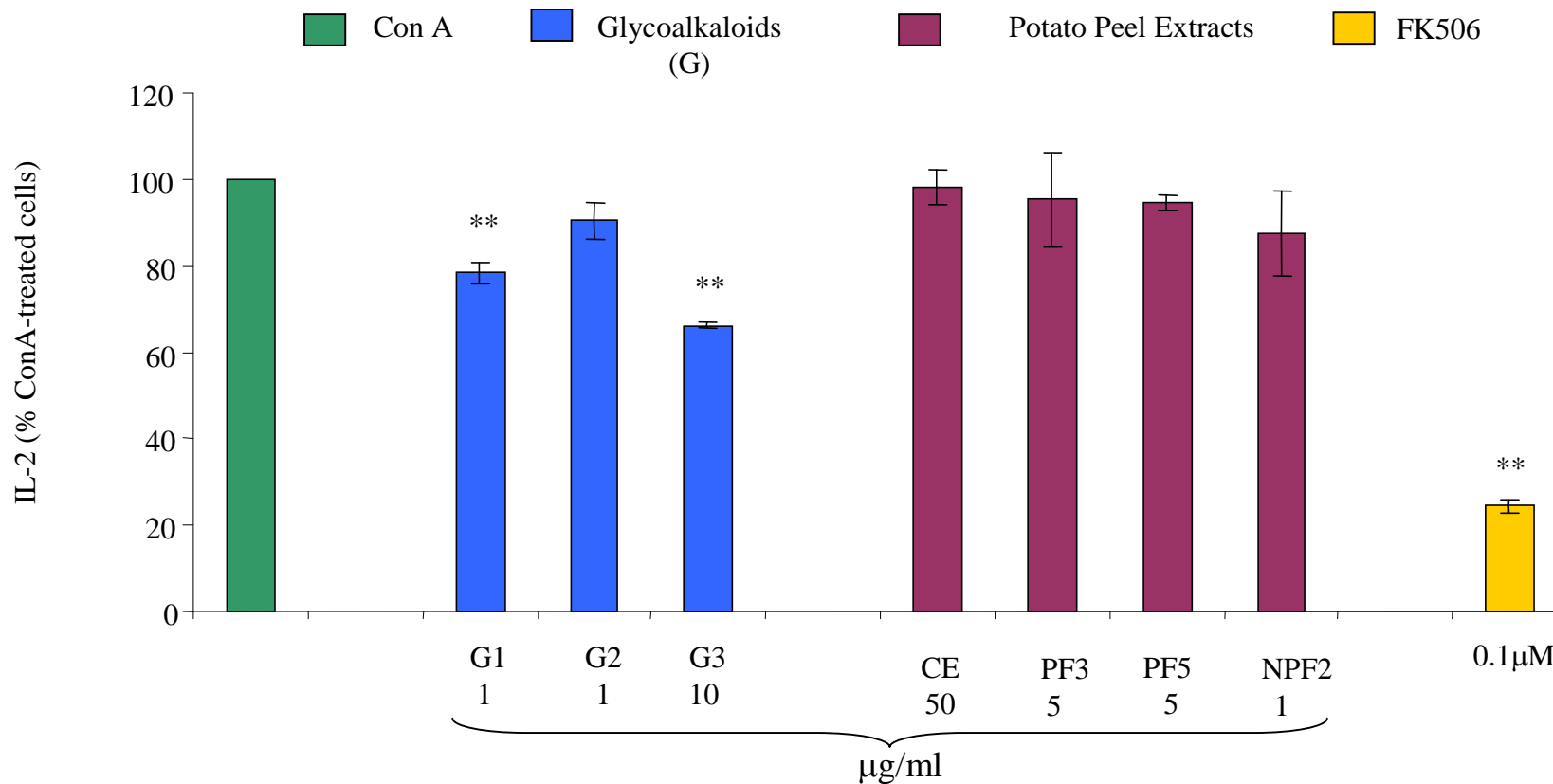
Compound	IC50 * ($\mu\text{g/ml}$)
<i>Glycoalkaloids</i>	
α -chaconine	3
α -solanine	15
solanidine	40
demissidine	11
<i>Potato Peel Extracts (PPE)</i>	
crude extract	89
semi-purified extract polar fraction 3	8
semi-purified extract polar fraction 5	16
semi-purified extract non-polar fraction 2	17

Effect of glycoalkaloids and potato peel extracts on cell viability in Con A-treated Jurkat cells

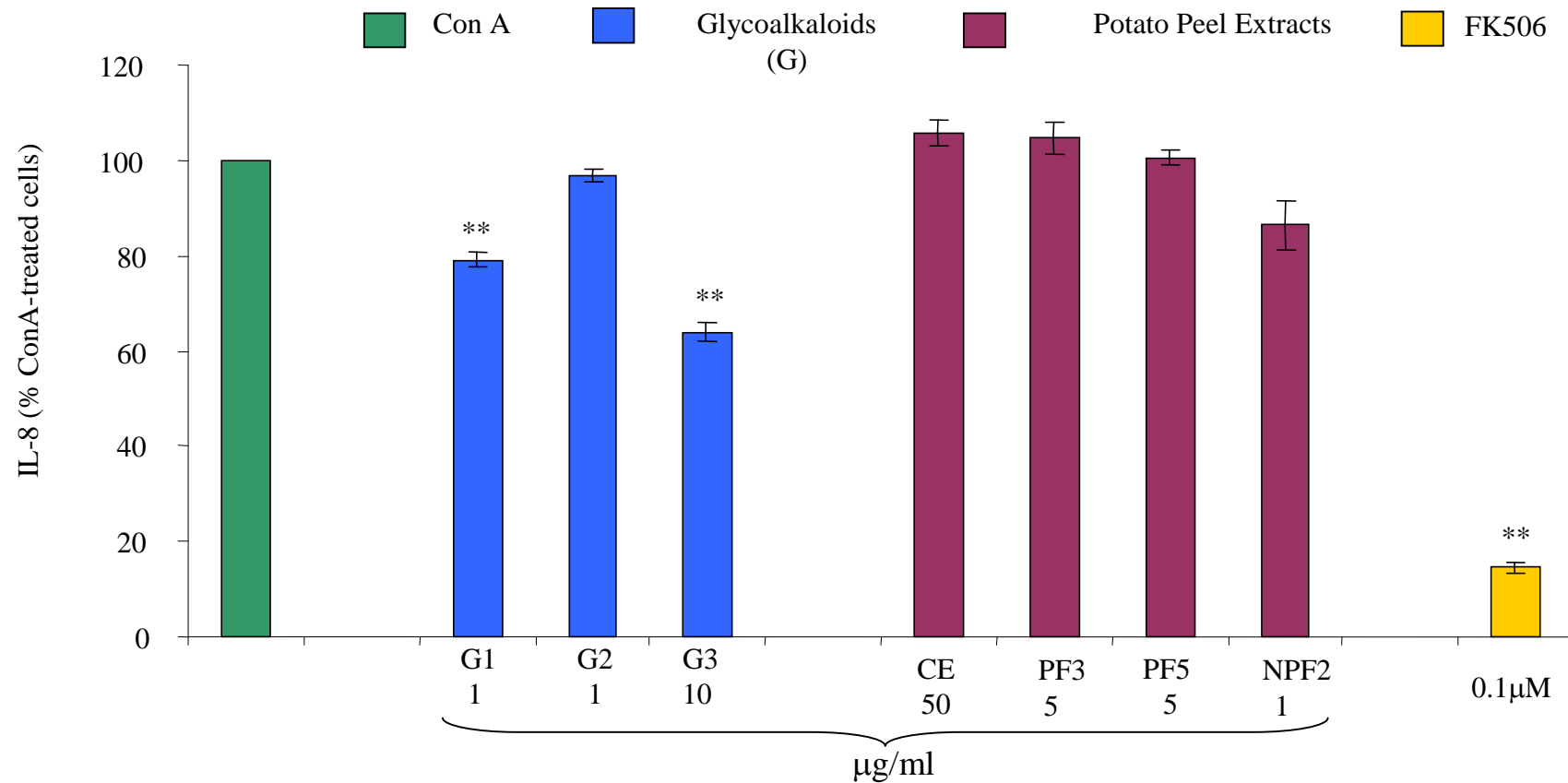
- Sub-cytotoxic concentrations of glycoalkaloids and potato peel extracts were used.
- The immunosuppressant drug FK506 (Tacrolimus) was positive control.



Viability of Con A-stimulated Jurkat cells following supplementation with glycoalkaloids and potato peel extracts. : α -chaconine (G1), α -solanine (G2) and solanidine (G3) or the potato peel extracts, crude extract (CE) polar fraction 3 (PF3), polar fraction 5 (PF5) and non-polar fraction 2 (NPF2). * $P < 0.05$ and ** $P < 0.01$ vs Con A.



Effect of glycoalkaloids α -chaconine (G1), α -solanine (G2) and solanidine (G3) or the potato peel extracts, crude extract (CE) polar fraction 3 (PF3), polar fraction 5 (PF5) and non-polar fraction 2 (NPF2). and potato peel extracts on **IL-2** release from Con A-treated Jurkat cells. 100% IL-2 production represents 164 pg/ml, whereas basal levels of IL-2 were approximately 7-9 pg/ml in control untreated cells. * $P < 0.05$ and ** $P < 0.01$ vs Con A.



Effect of glycoalkaloids α -chaconine (G1), α -solanine (G2) and solanidine (G3) or the potato peel extracts, crude extract (CE) polar fraction 3 (PF3), polar fraction 5 (PF5) and non-polar fraction 2 (NPF2). and potato peel extracts **on IL-8** release from Con A-treated Jurkat cells. 100% IL-8 production represents 45 pg/ml, whereas basal levels of IL-8 were approximately 1-2 pg/ml in control untreated cells. * $P < 0.05$ and ** $P < 0.01$ vs Con A.

Results - cytokines

- Findings highlights significant reduction in the production of both cytokines with sub-lethal concentrations of α -chaconine and solanidine.
- NPF2 which predominantly contained α -chaconine and α -solanine did reduce cytokine production more effectively but the levels of reduction were not significant.
- Glycoalkaloids at the concentrations used have potential as immunosuppressants.

NO Production

- Accumulated nitrite (NO_2^-) in culture media, as an indicator of NO production, was determined using the Griess method.
- NO production was induced with LPS and cells were co-treated with specific concentrations of glycoalkaloids and potato peel extracts for 24 h.
- Additionally, LPS-treated macrophages were exposed with or without the positive control, iNOS inhibitor, N^G -monomethyl-L-arginine (L-NMMA) at 50, 100 and 150 μM for 24 h.

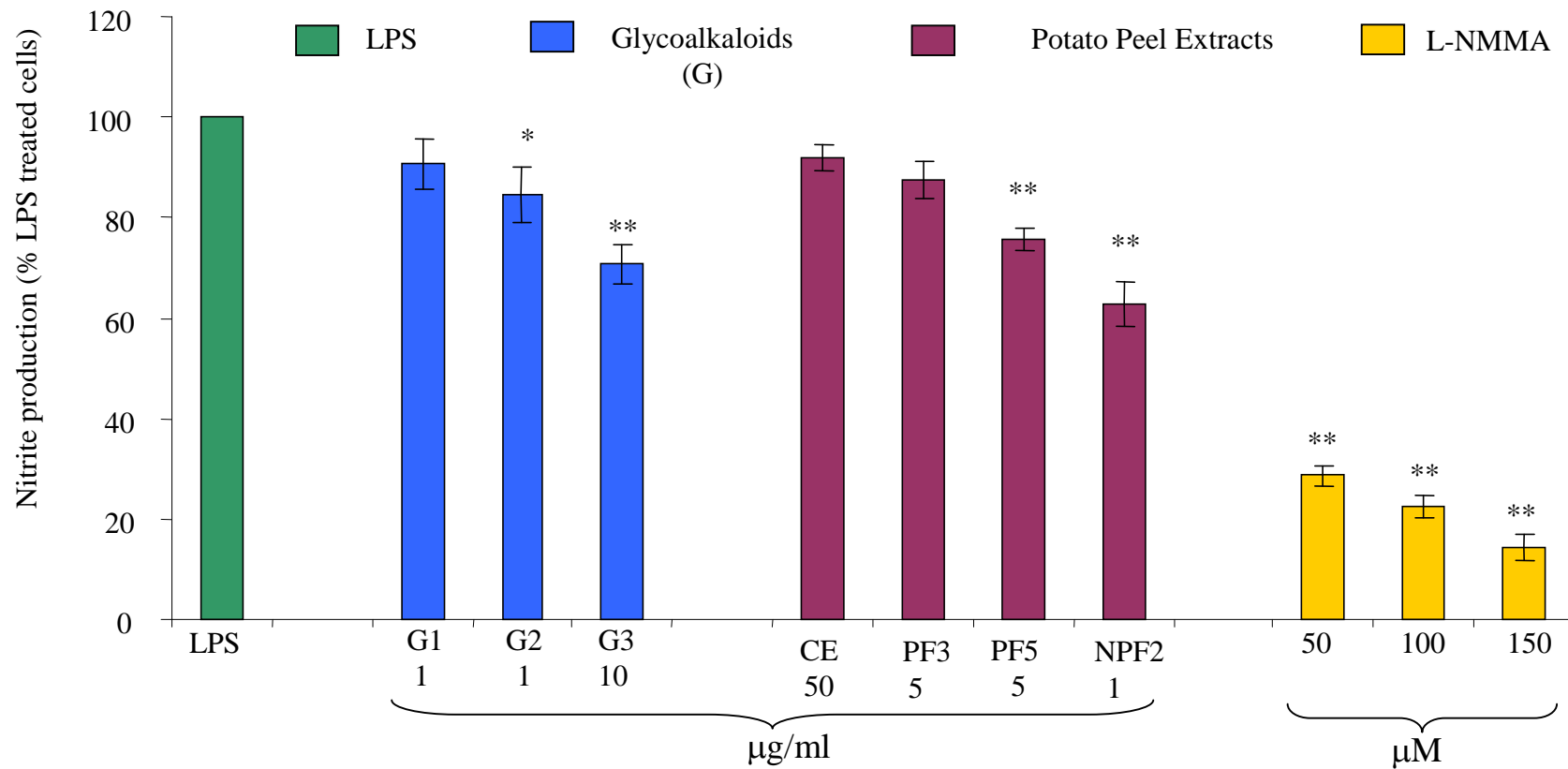
Effects of selective concentrations of glycoalkaloids and potato peel extracts on the viability of LPS-stimulated Raw 264.7 mouse macrophages.

	Viability
α -Chaconine 1 μ g/ml	94.4 \pm 0.8
α -Solanine 1 μ g/ml	99.0 \pm 2.7
Solanidine 10 μ g/ml	85.4 \pm 1.9*
CE 50 μ g/ml	90.5 \pm 1.2
SPE PF3 5 μ g/ml ^a	100.0 \pm 1.6
SPE PF5 5 μ g/ml ^a	103.2 \pm 1.1
SPE NPF2 5 μ g/ml ^b	95.9 \pm 3.1
L-NMMA 50 μ M	94.0 \pm 1.8
L-NMMA 100 μ M	95.2 \pm 1.7
L-NMMA 150 μ M	95.2 \pm 2.6

^a Polar Fraction (PF)

^bNon Polar Fraction (NPF)

* $P < 0.01$ vs LPS



Effect of glycoalkaloids α -chaconine (G1), α -solanine (G2) and solanidine (G3) or the potato peel extracts, crude extract (CE) polar fraction 3 (PF3), polar fraction 5 (PF5) and non-polar fraction 2 (NPF2). and potato peel extracts on LPS-induced nitrite production. 100% nitrite production represents 28 μ M, whereas basal levels of nitrite were approximately 0.5 μ M in control untreated cells. * P <0.05 and ** P <0.01 vs LPS.

Results - nitrite production

- α -Solanine and solanidine significantly reduced LPS-induced nitrite production in Raw mouse macrophages.
- Among the potato peel extracts, PF5 and NPF2 significantly reduced nitrite production.
- Glycoalkaloids and potato peel extracts at the concentrations used show potential for reducing NO production by macrophages.

Conclusion

- ❖ First study to demonstrate potential anti-inflammatory activity of individual potato glycoalkaloids and potato peel extracts enriched in glycoalkaloids.
- ❖ These promising results were induced at sub-toxic concentrations of test compounds.
- ❖ Glycoalkaloids are nitrogen analogues of steroid saponins such as diosgenin which has proven effective in inhibiting inflammatory responses.

Thank you !