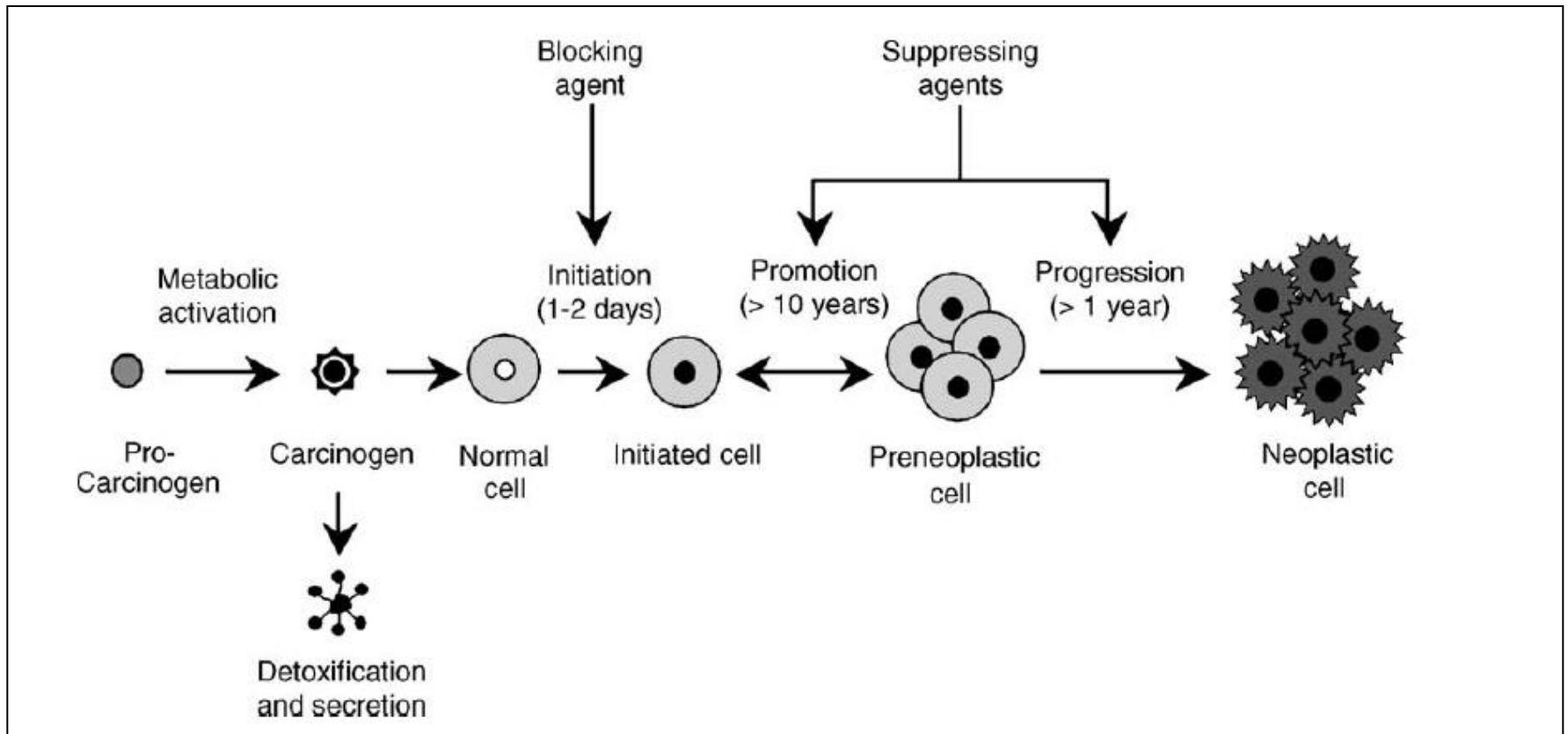


Mechanism of Polymeric Black Tea Polyphenols in Chemoprevention

Prepared By:

**Khushboo A. Gandhi,
Anand Pharmacy College**

Schematic representation of multistep-carcinogenesis



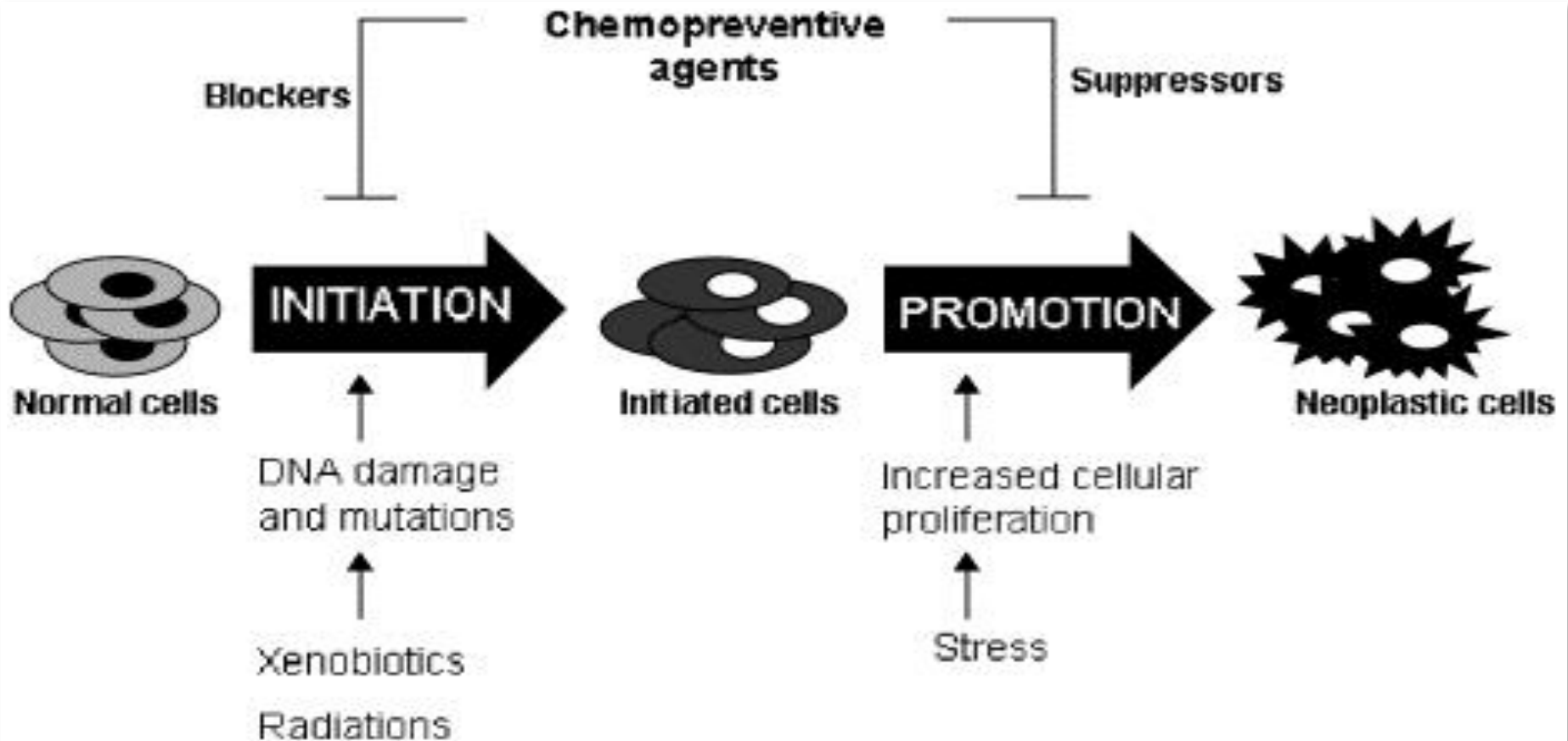
(Adopted from Surh et al., 1999)

Cancer - Uncontrolled proliferation of cell with potential to spread to other organs

Carcinogenesis - Complex, multistep and multifactorial process
Consist of initiation, promotion and progression

CHEMOPREVENTION

Use of natural or synthetic compounds to prevent, suppress or delay the process of carcinogenesis.



List of Herbal anti-oxidants Studied for chemoprevention

- Aegle Marmelos (Singh et al., 2000)
- Garlic (Yang et al., 2001)
- Neem (Dasgupta et al., 2004)
- Onion (Belman, 1983)
- **Black Tea**/ Green Tea (Lambert & Yang, 2003)
- Turmeric (Aziune et al., 1992)
- Amla (Jose et al., 2001)
- Clove (Zheng et al., 1992)
- Capsicum (Surh, 2002)
- Grapes (Aziz et al., 2003)

Polymeric Black Tea Polyphenols

Polyphenols - Most significant group of components in tea

Green tea (20%)

Tea leaves $\xrightarrow[\text{inactivated}]{\text{PPO}}$ Dried



Black tea (78%)

Tea leaves $\xrightarrow[\text{Oxidation}]{\text{PPO mediated}}$ Dried



Polyphenol Content of Green and Black tea

		g % of dry solid extracted *	g % of dry total polyphenols content
BLACK TEA	Catechins (Monomers)	3-10	30
	Theaflavins (Oligomers)	3-6	13
	Thearubigins/PBPs (Polymers)	12-18	47
GREEN TEA	Catechins (Monomers)	30-42	90
	Theaflavins	-	-
	Thearubigins/PBPs	-	-

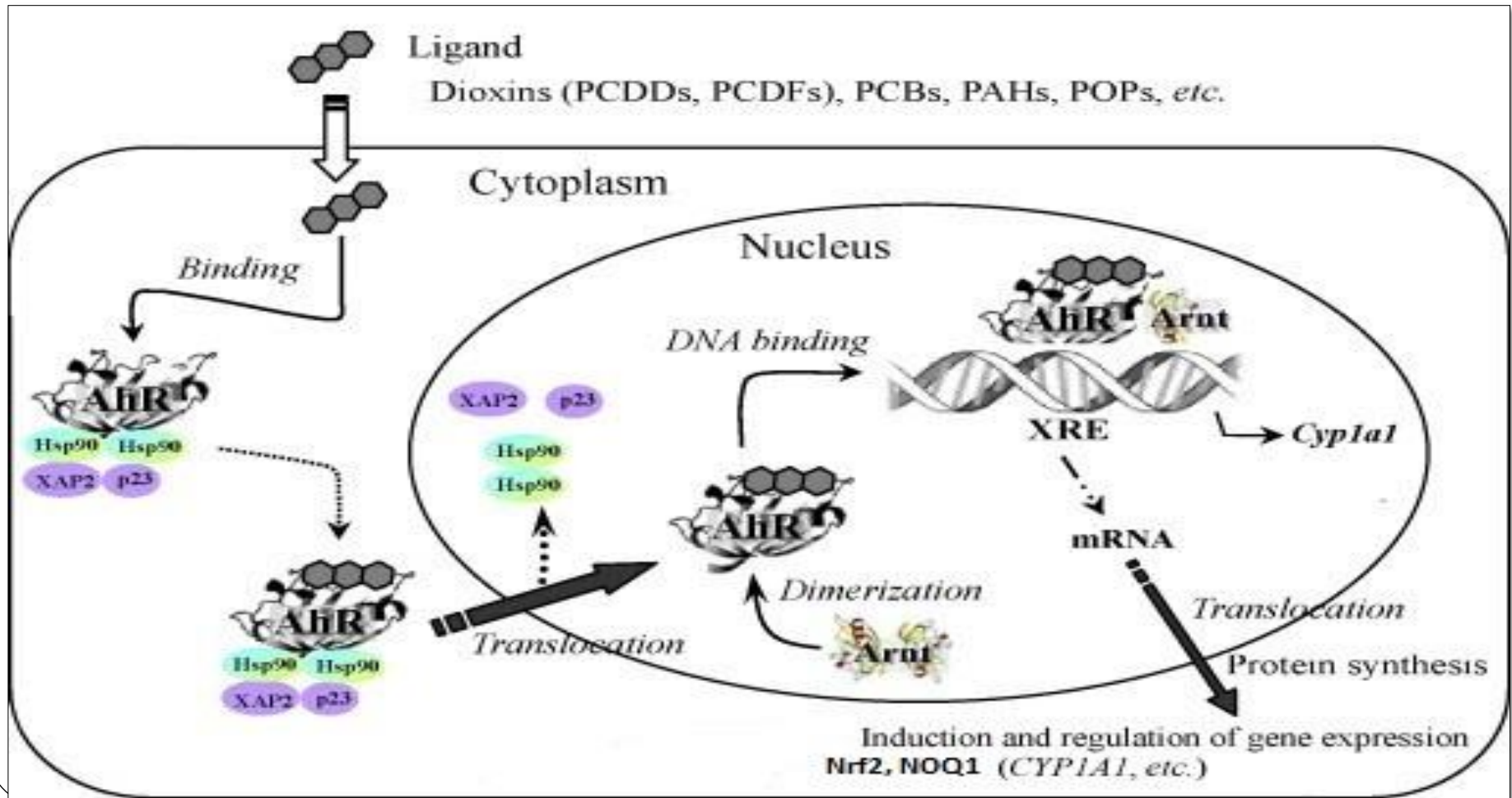
* % of solid extracted from **black tea** = 25-35%

(Adopted from Kumar et al., 2010, MRMC, 10: 492-505)

□ PBP's content of an average cup of tea is 65mg/100 ml (150mg per 235 ml cup)

Previous studies shown that PBPs possess anti-initiating and anti-promoting activity *in vivo* in different animal models

(Patel et al., 2008; Kumar et al., 2012)

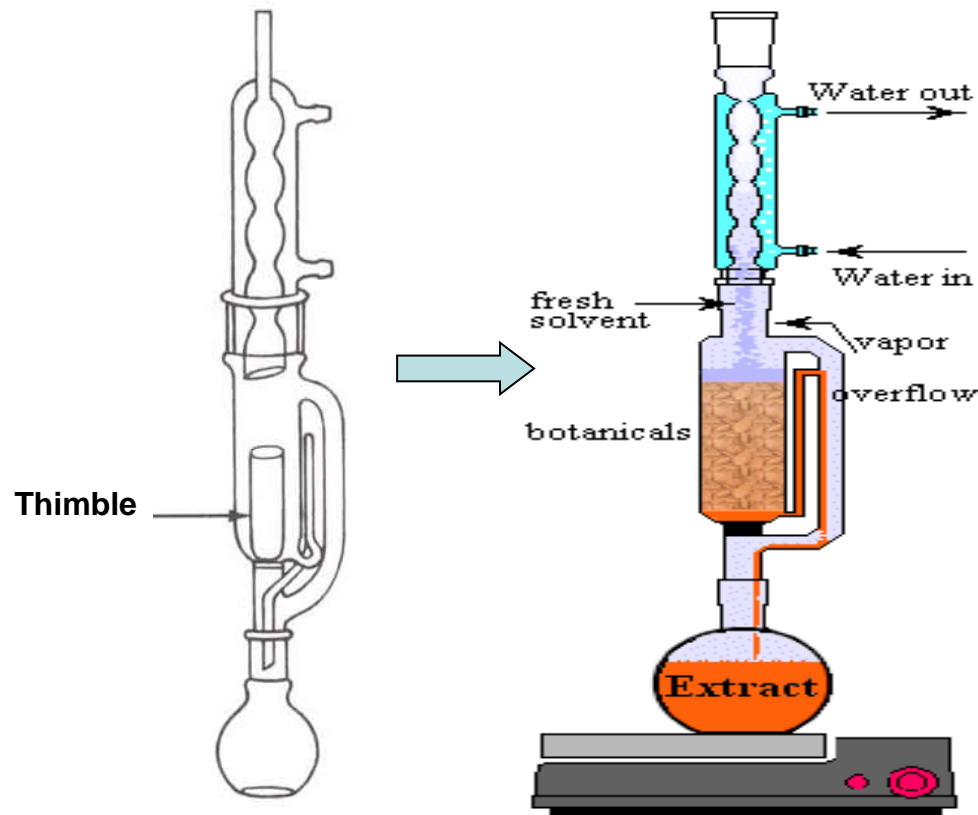


OBJECTIVE

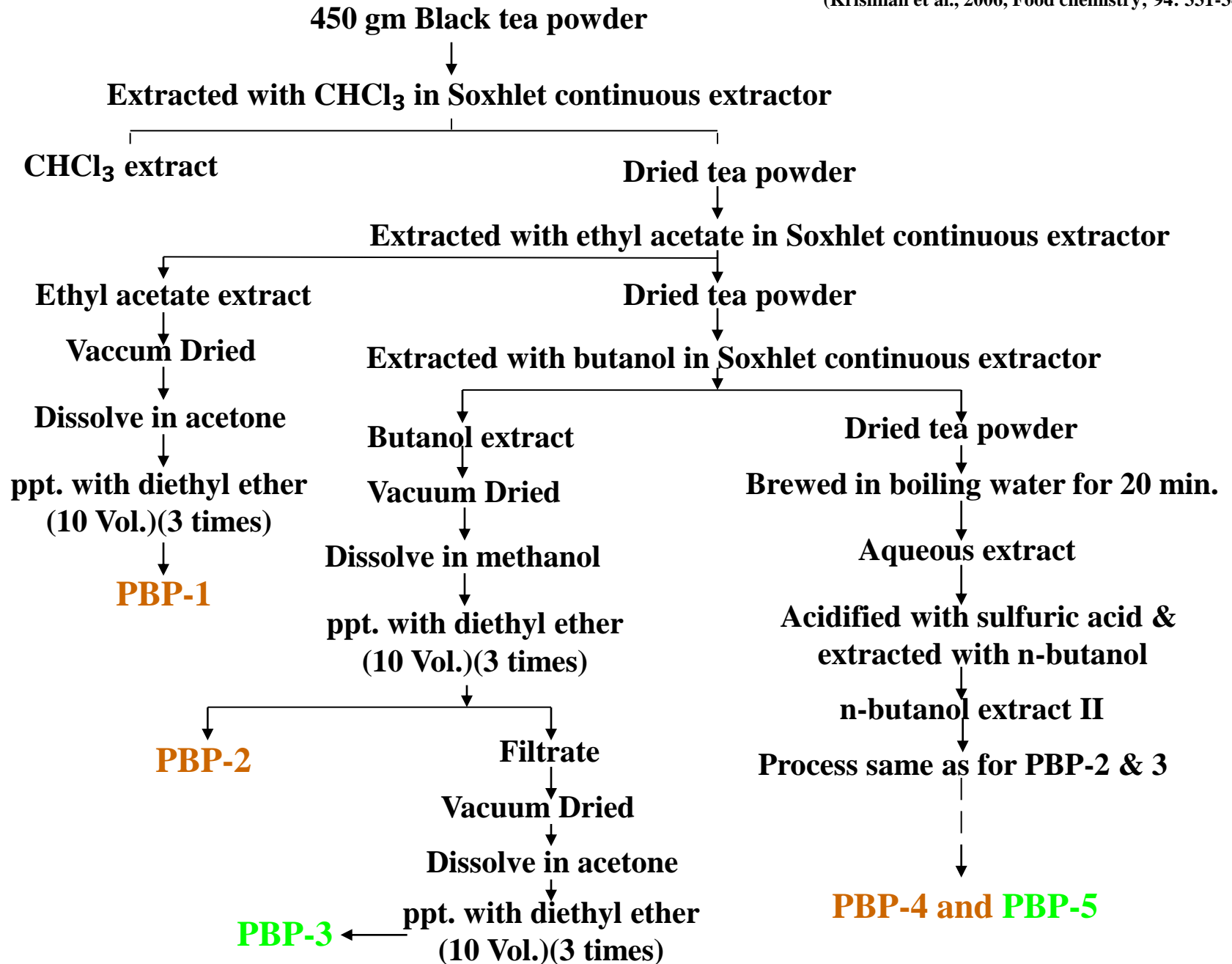
Evaluation of Chemopreventive Efficacy and Mechanisms of anti-initiating activities of Polymeric Black Tea Polyphenols (PBPs) / Thearubigins (TRs) in B(a)P-induced skin epidermis

Extraction of Black Tea Polyphenols (PBPs) (By Soxhlet Based Solid Liquid Extraction Method)

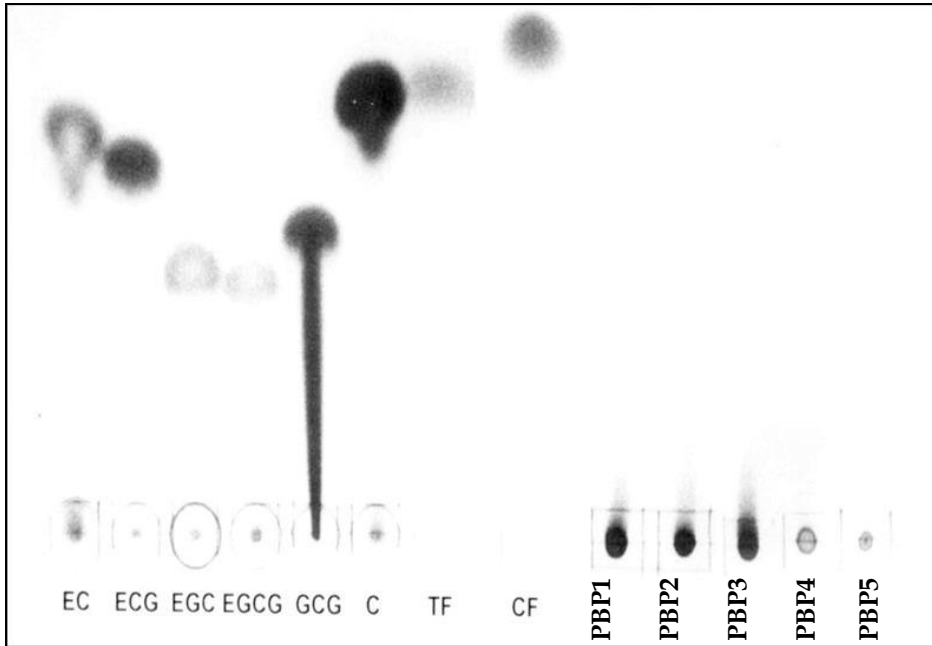
(Krishnan et al., 2006, Food chemistry; 94: 331-340)



Soxhlet Continuous Extractor



Evaluation of contamination of catechins and TFs in PBPs



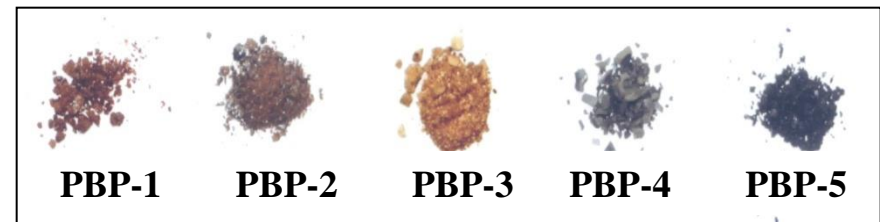
EC = Epicatechin, ECG = Epicatechin gallate, EGC = Epigallocatechin, EGCG = Epigallocatechingallate, GCG = Gallocatechin gallate, C = Catechin, TF = Theaflavin, CF = Caffeine, TR = Thearubigin (PBPs)

Demonstrates the absence of known biologically active black tea-derived contaminants (Caffeine, C, EC, ECG, EGC, EGCG and TFs)

Yield of different PBPs

PBPs	Wt.(gm)/450 gm of dry tea	% of dry tea
PBP-1	12.06	2.68
PBP-2	17	3.79
PBP-3	6.03	1.34
PBP-4	9.9	2.2
PBP-5	1.44	0.32

Different fractions of PBPs obtained from Black tea



Physicochemical Properties of Polymeric Black Tea Polyphenol Fractions

property	PBP-1	PBP-2	PBP-3	PBP-4	PBP-5
Color	Brown	Light Brown	Light Yellow	Dark Brown	Brownish Black
pH of 1% aq. Solution	5.53	5.55	5.40	4.26	4.09
λ max1 (nm)	211	219	217	211	210
λ max2 (nm)	272	272	268	263	270
λ max1/ λ max2	2.78	1.68	1.78	3.21	6.28
FeCl ₃ reactivity	Weakly Positive	Weakly Positive	Weakly Positive	Weakly Positive	Weakly Positive

Animal Study

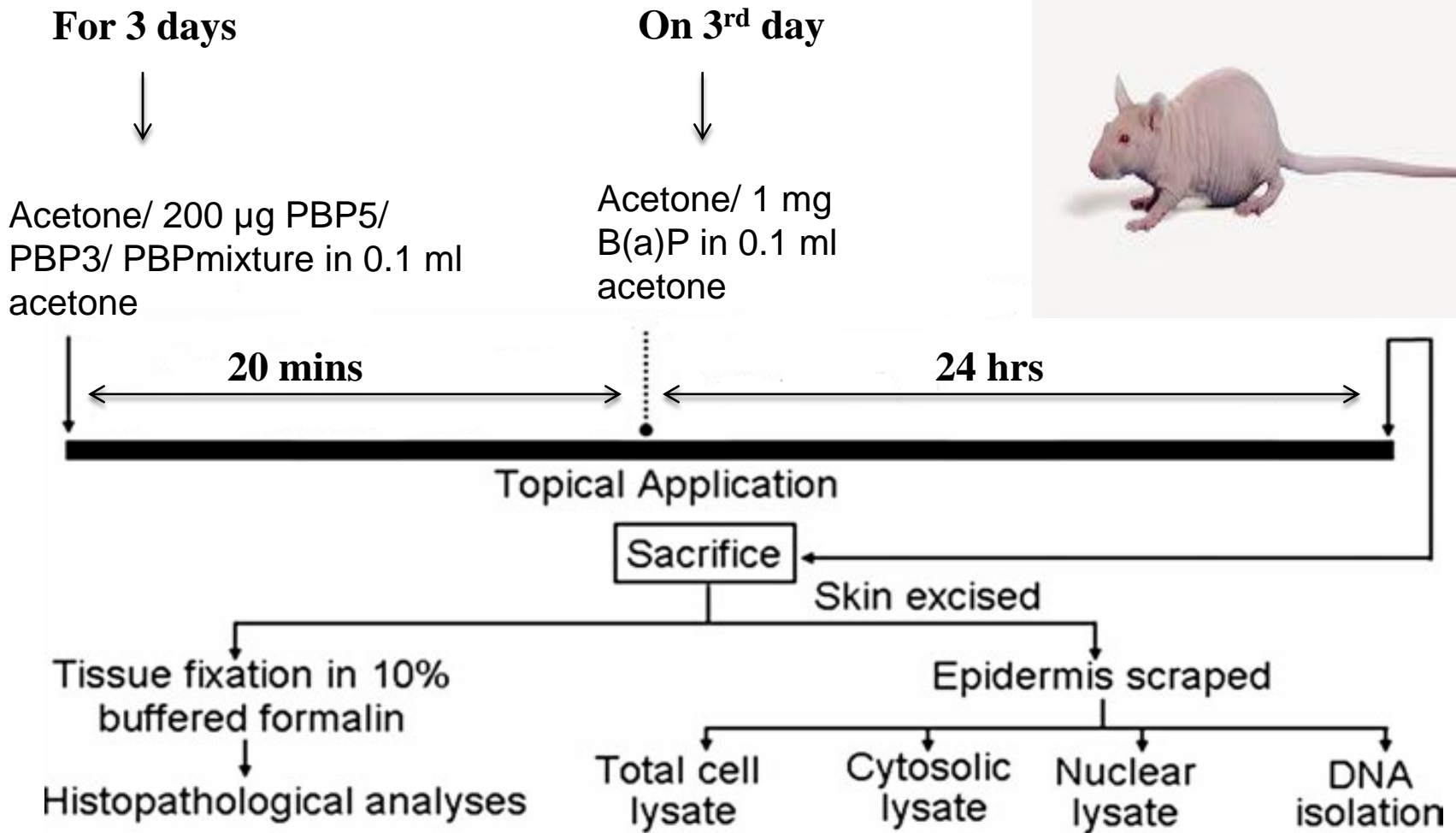
All animal studies were conducted after approval from the Institutional Animal Ethics Committee (ACTREC, Mumbai) as per the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India

**Animal Model: Swiss
bare mice (6-8 weeks)**

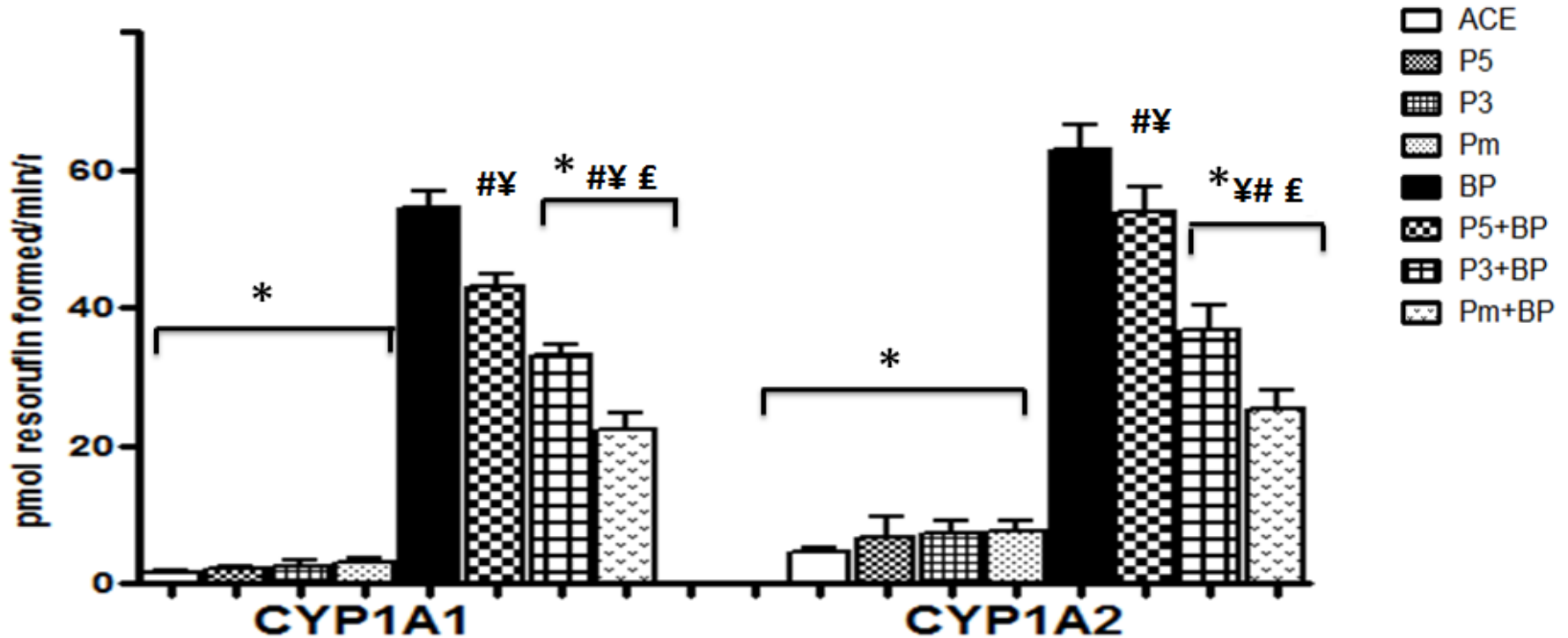


	Groups	Pre-treatment	Treatment after 20 mins of pre- treatment
1	Vehicle control	Acetone	Acetone
2	PBP-5 control	PBP-5	Acetone
3	PBP-3 control	PBP-3	Acetone
4	PBP-mix control	PBP-mix	Acetone
5	B(a)P control	Acetone	B(a)P
6	PBP-5 + B(a)P	PBP-5	B(a)P
7	PBP-3 + B(a)P	PBP-3	B(a)P
8	PBP-mix + B(a)P	PBP-mix	B(a)P

Experimental Plan

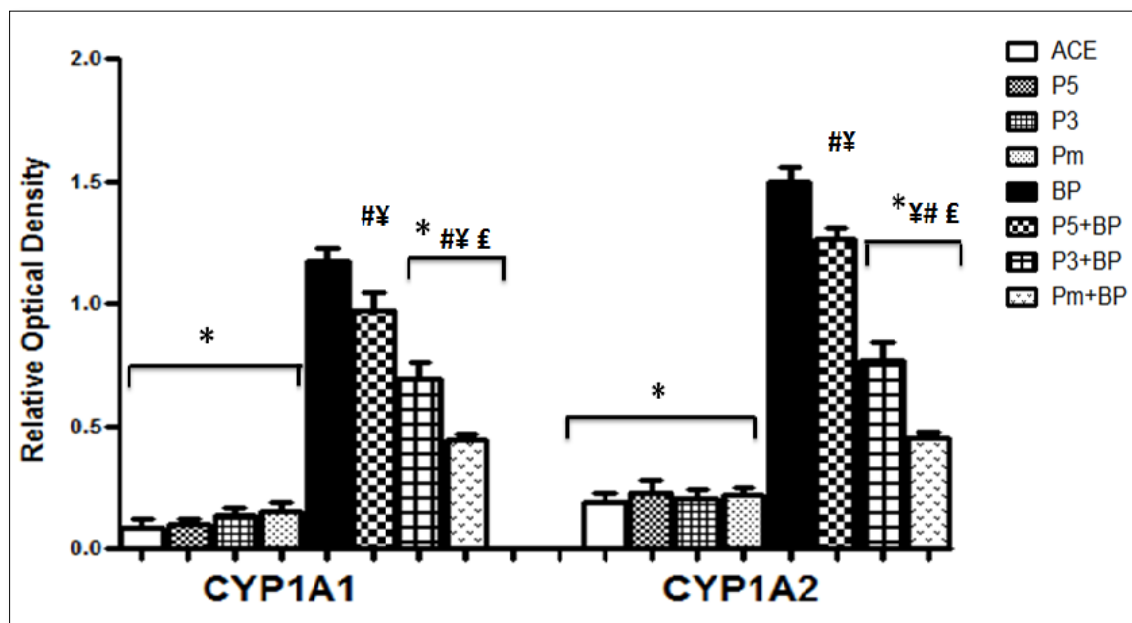
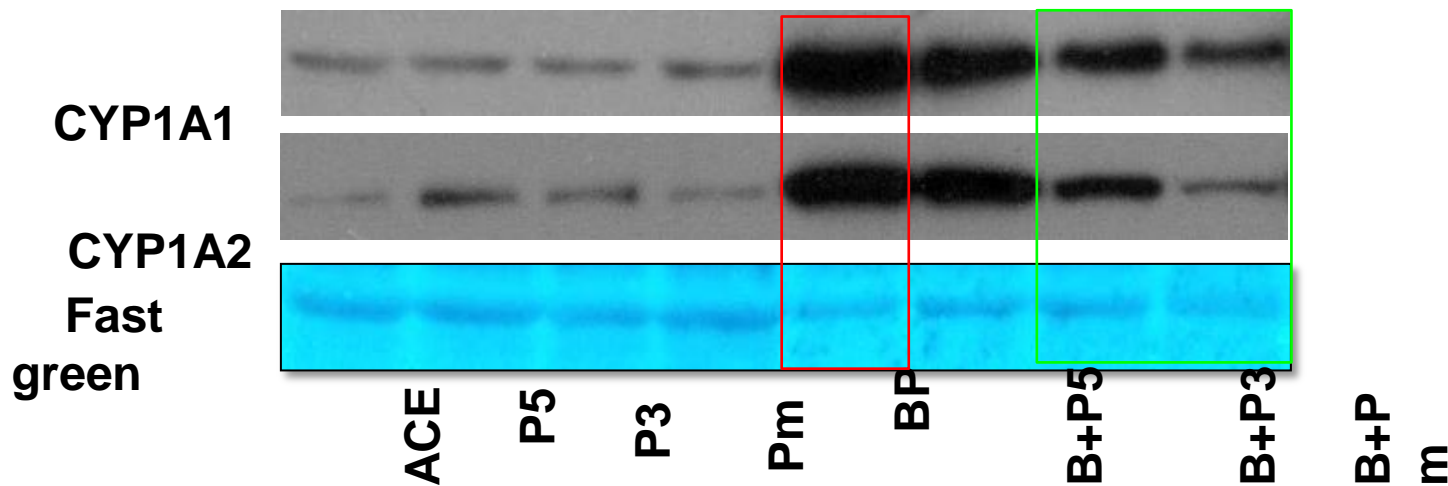


Effect of PBPs on CYP1A1 and CYP1A2 activity



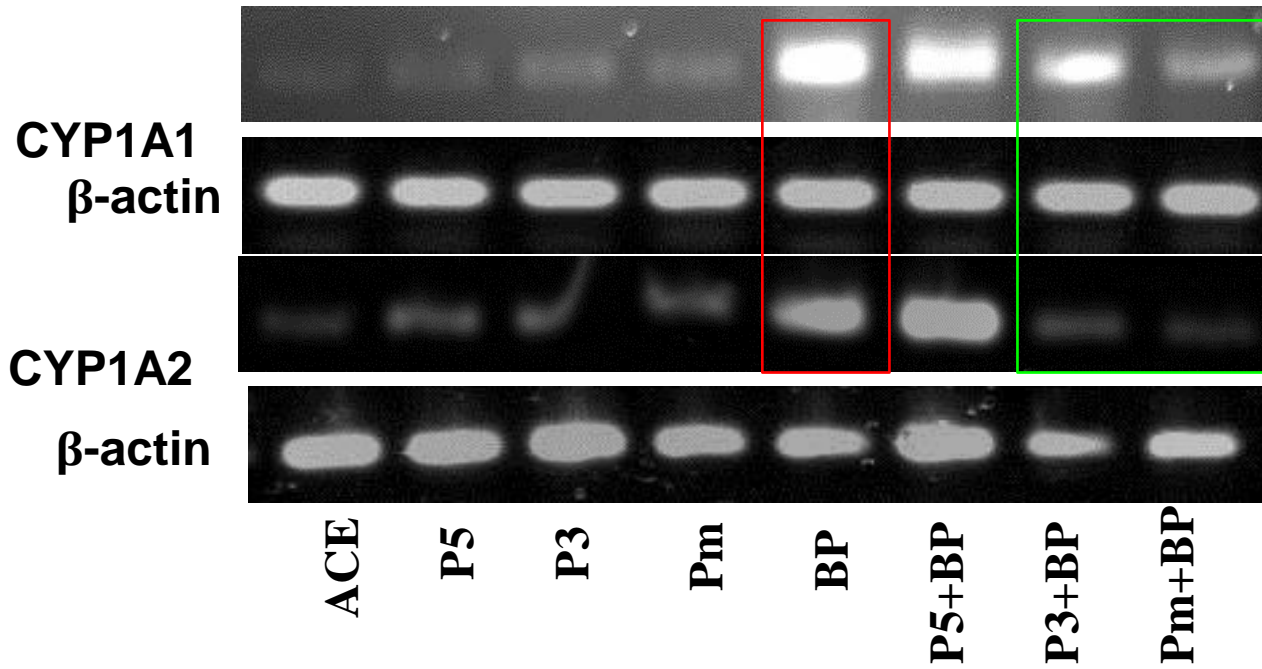
Data represent mean \pm standard error of four observations (three pooled epidermis for one sample). Differences among groups were determined by one-way ANOVA followed by Bonferroni's test, $p < 0.05$. '*' significant when compared with B(a)P; '#' significant when compared with Acetone; '¥' significant when compared with respective controls; '£' significant when compared with respective P5+BP

Effect of PBPs on CYP1A1 and CYP1A2 expression

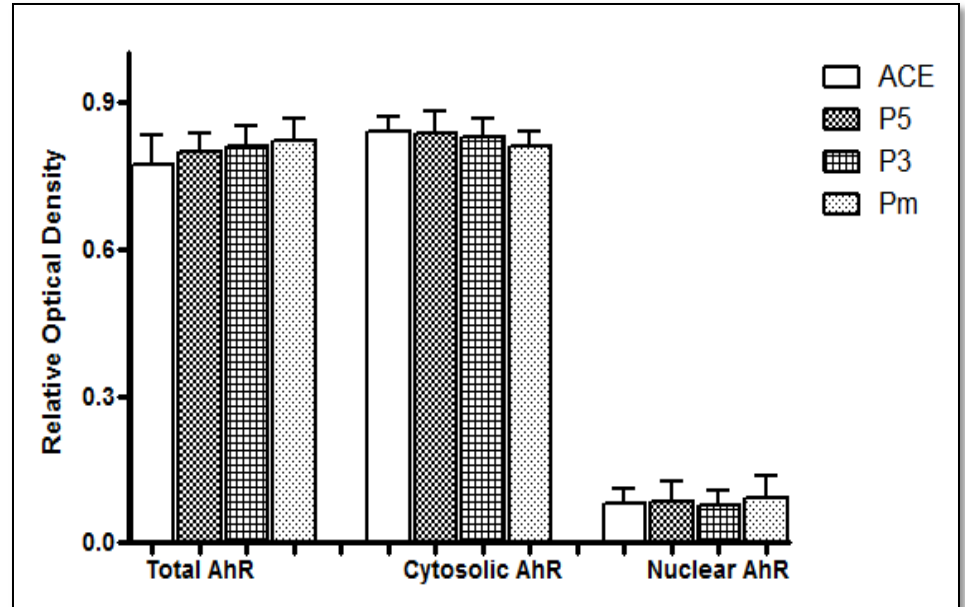
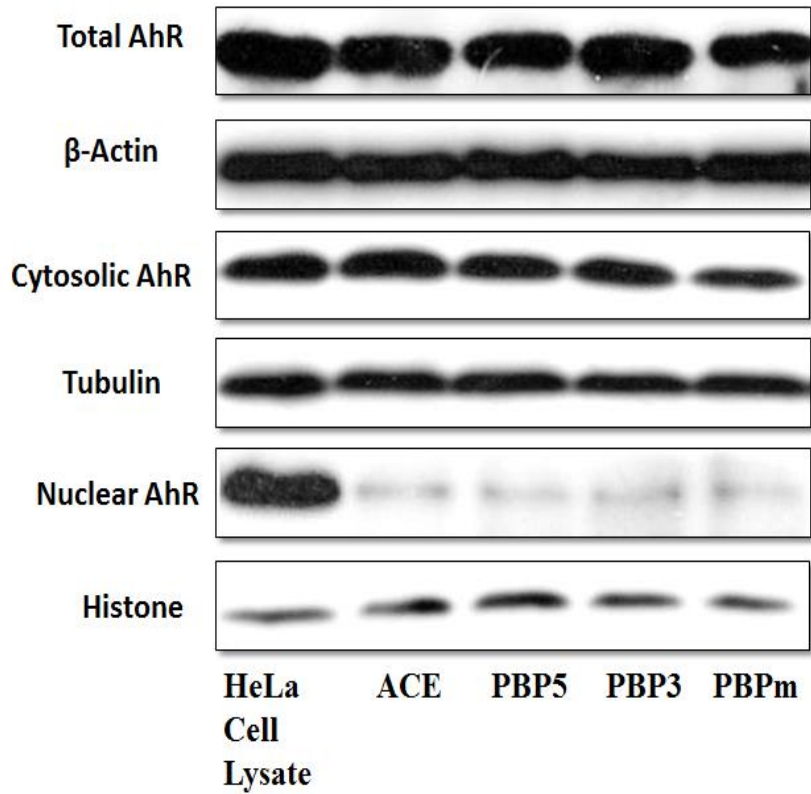


Data represent mean \pm standard error of five observations. Differences among groups were determined by one-way ANOVA followed by Bonferroni's test, $p \leq 0.05$. '*' significant when compared with B(a)P; '#' significant when compared with Acetone; '¥' significant when compared with respective controls; '£' significant when compared with respective B+P5.

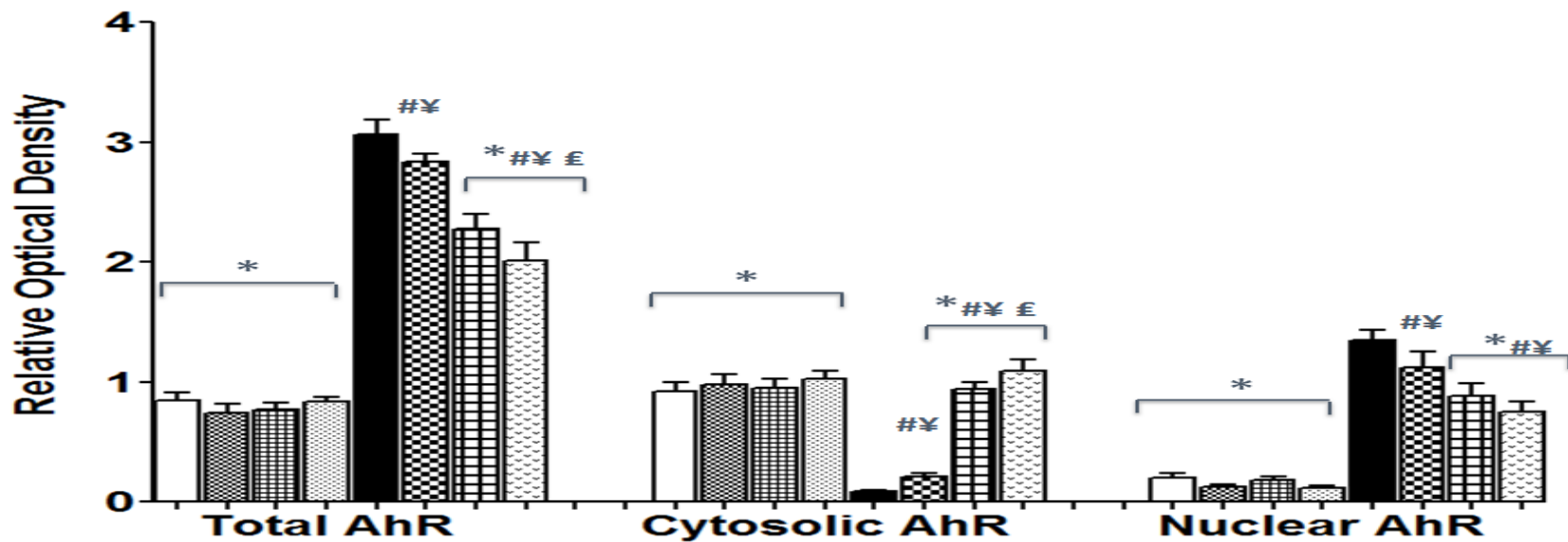
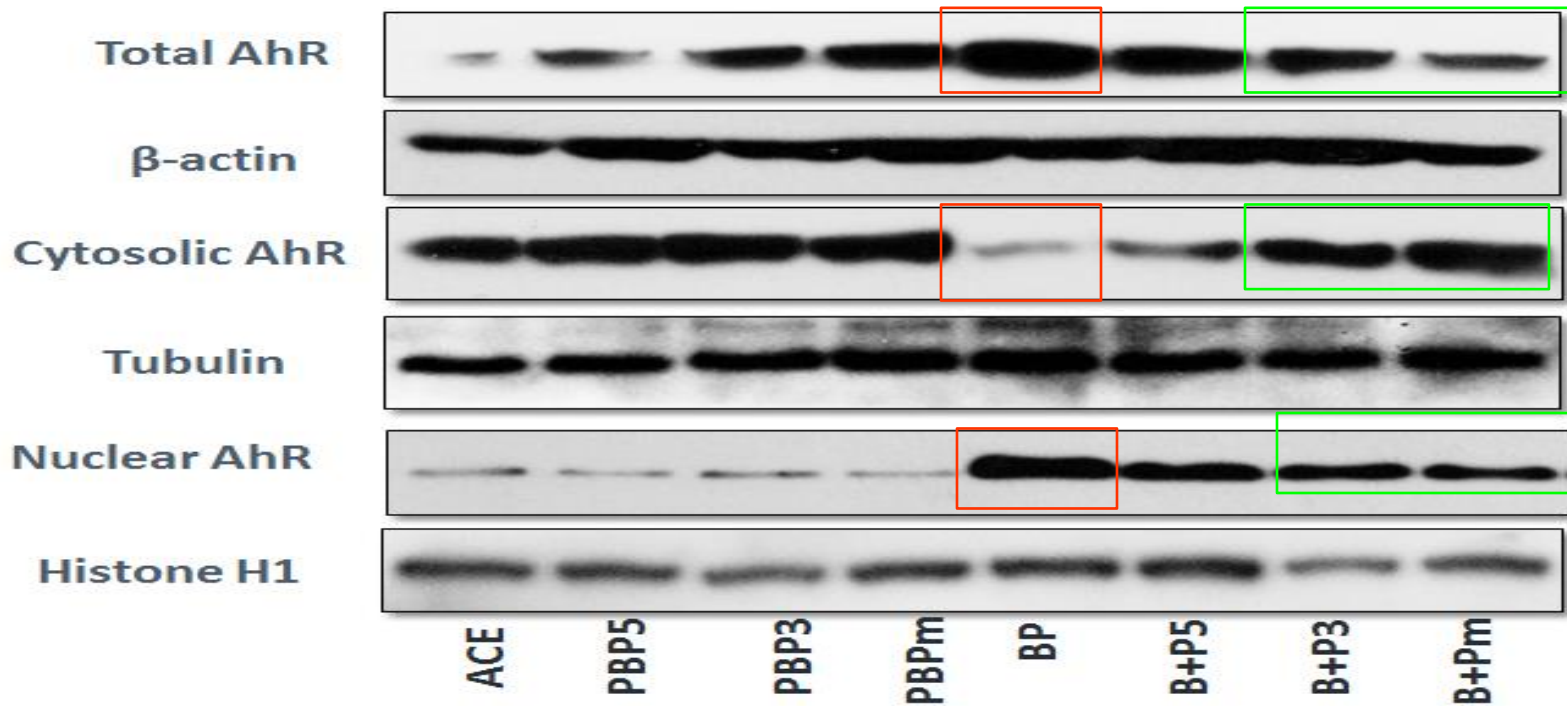
Effect of PBPs on mRNA levels of CYP1A1 and CYP1A2



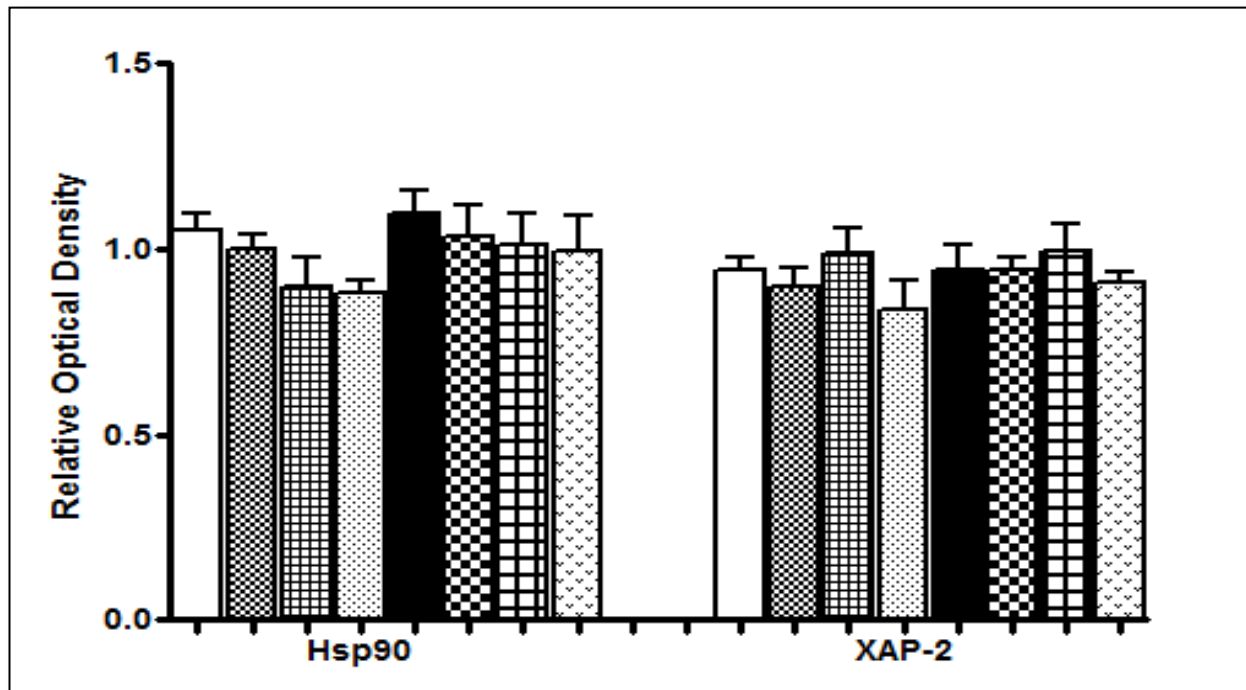
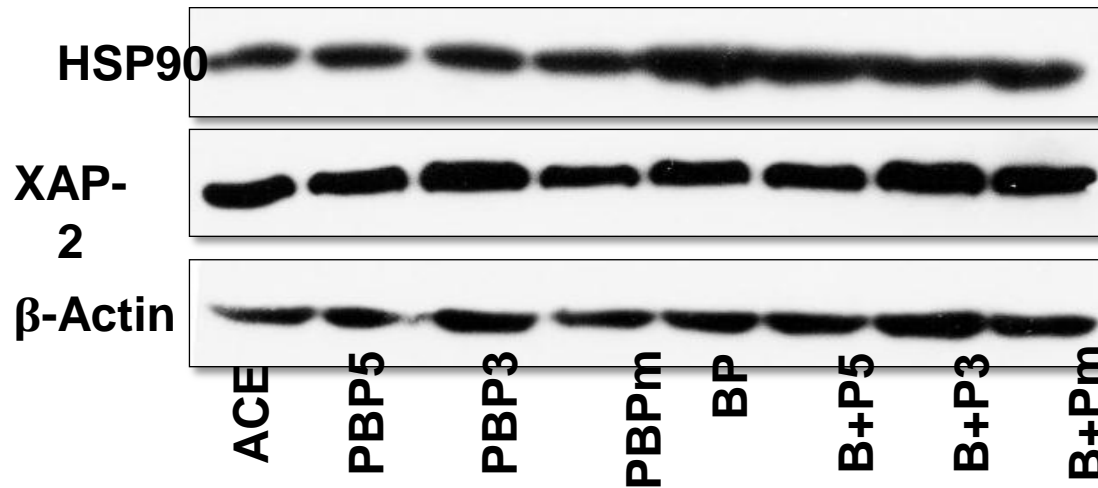
Effect of PBPs on Basal levels of AhR



Effect of PBPs on AhR levels



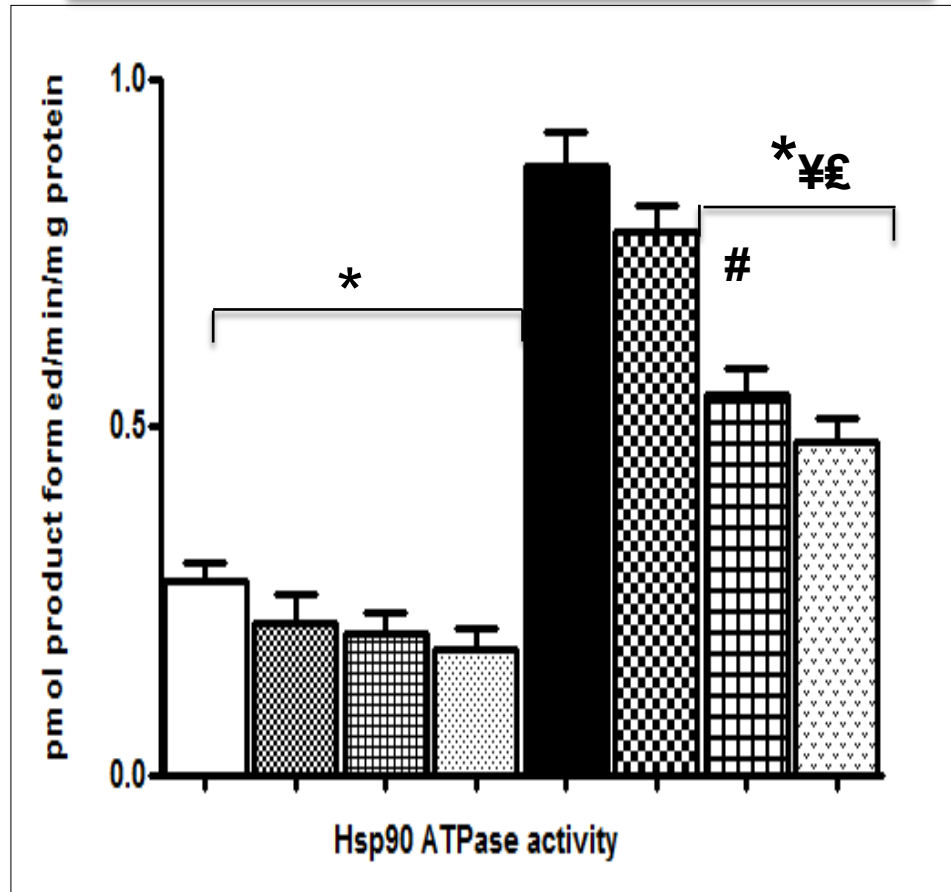
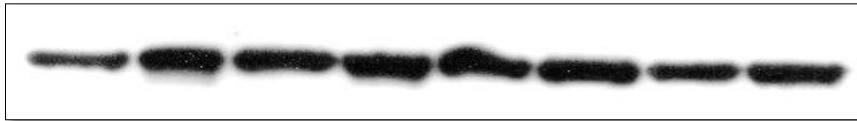
Effect of PBPs on AhR related Proteins (WB)



- **PBPs did not alter levels of Hsp90 and XAP-2**

Effect of PBPs on Hsp90 ATPase activity

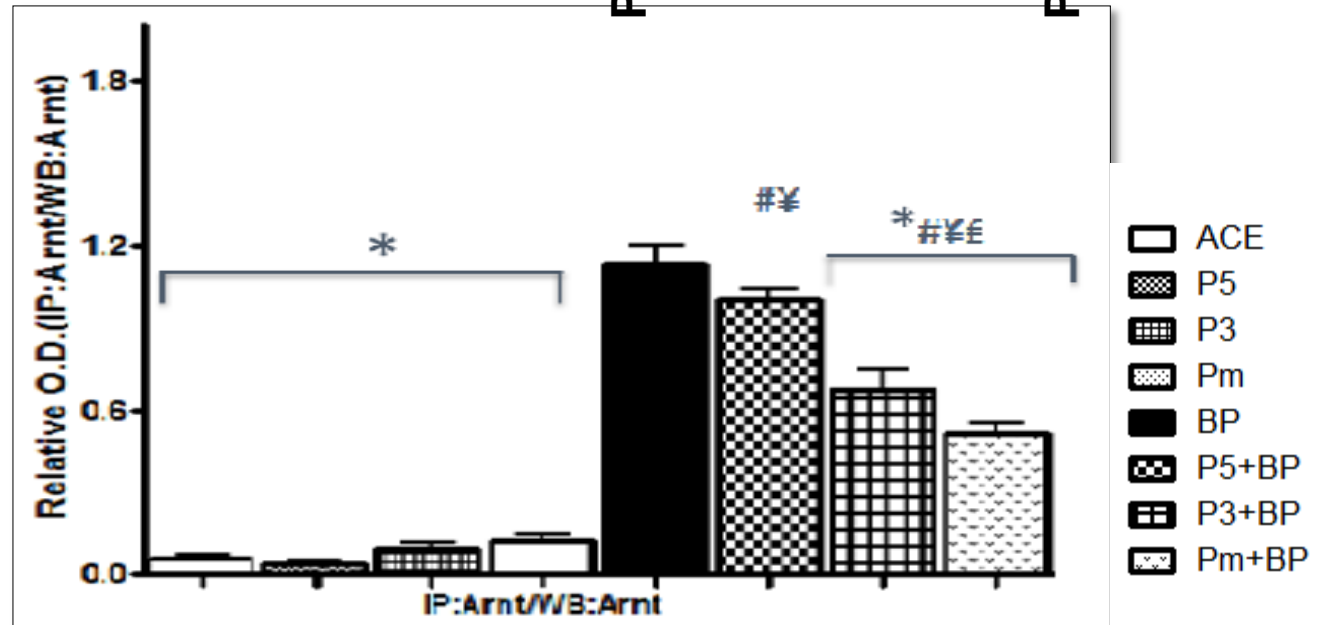
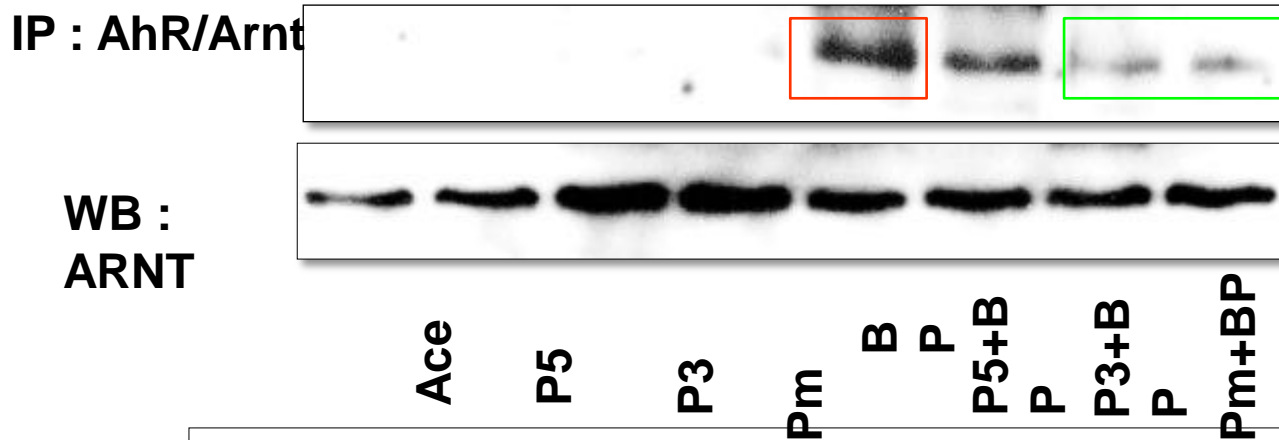
IP : Hsp90



- ACE
- ▣ P5
- ▤ P3
- ▥ Pm
- BP
- ▧ P5+BP
- ▨ P3+BP
- ▩ Pm+BP

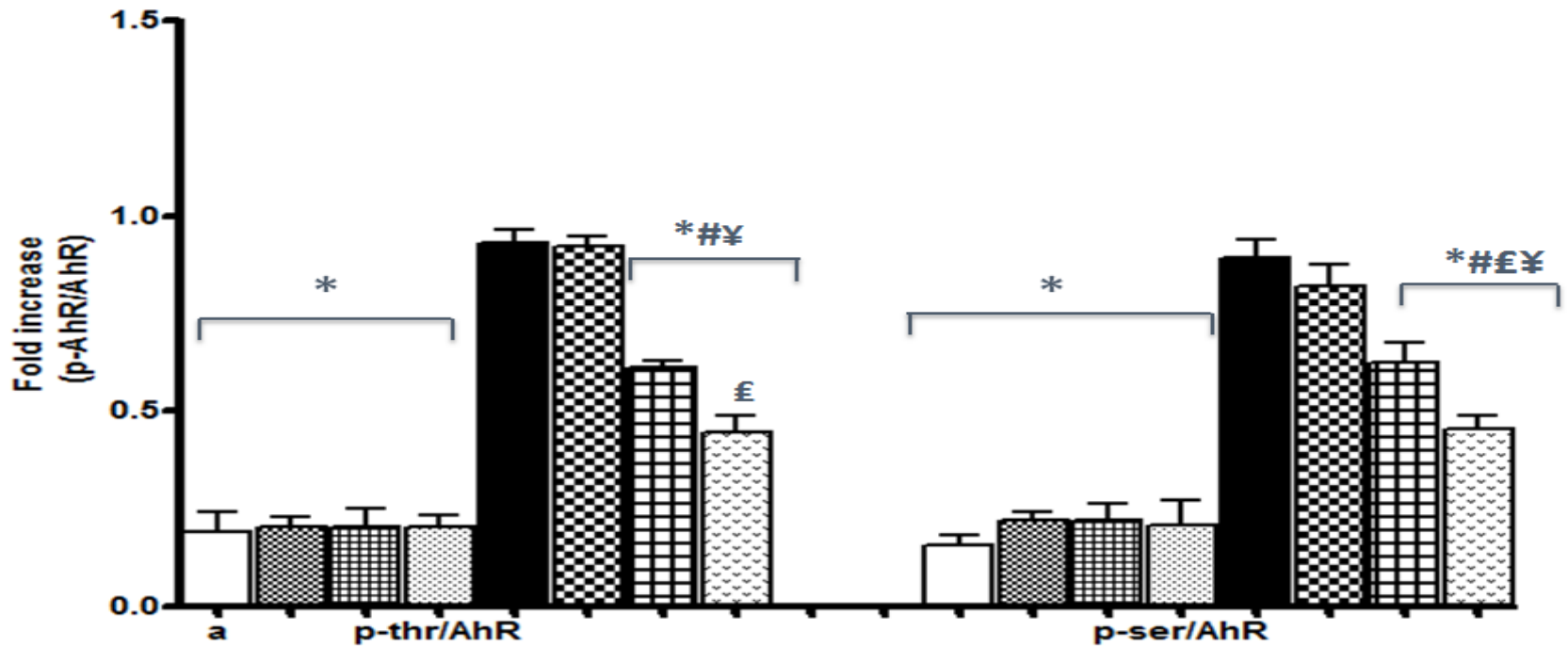
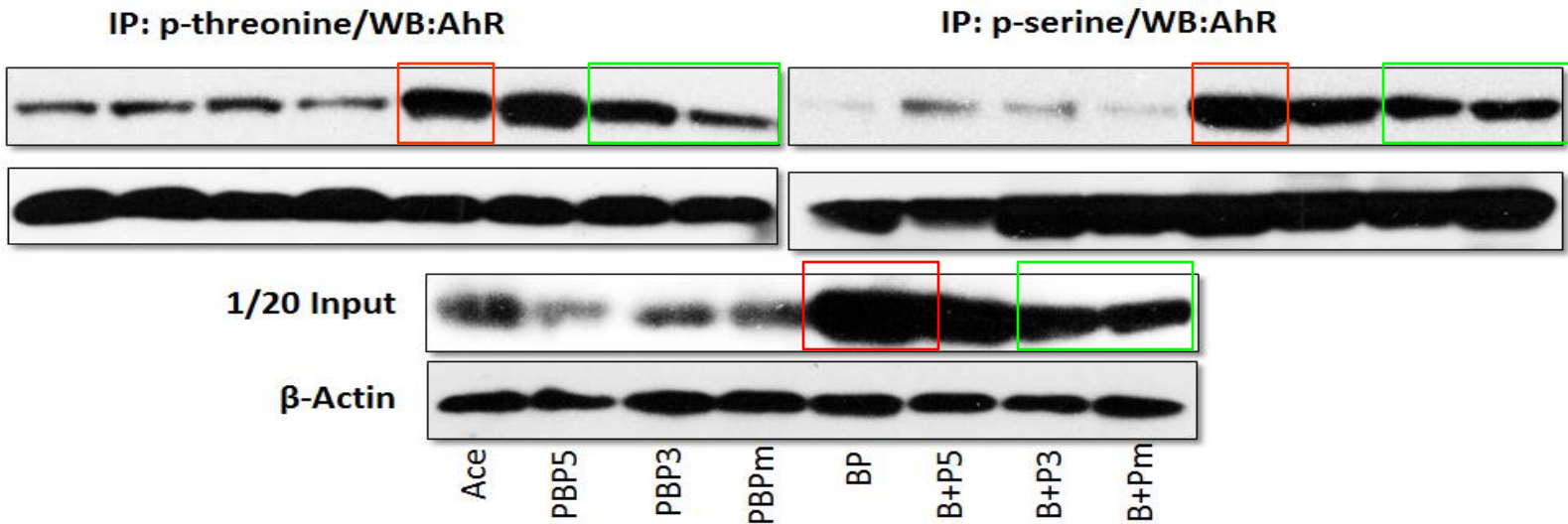
Data represent mean \pm standard error of three observations. Differences among groups were determined by one-way ANOVA followed by Bonferroni's test, $p \leq 0.05$. '*' significant when compared with B(a)P; '#' significant when compared with Acetone; '¥' significant when compared with respective controls; '£' significant when compared with respective P5 + BP.

Effect of PBPs on binding of ligand:AhR complex to Arnt

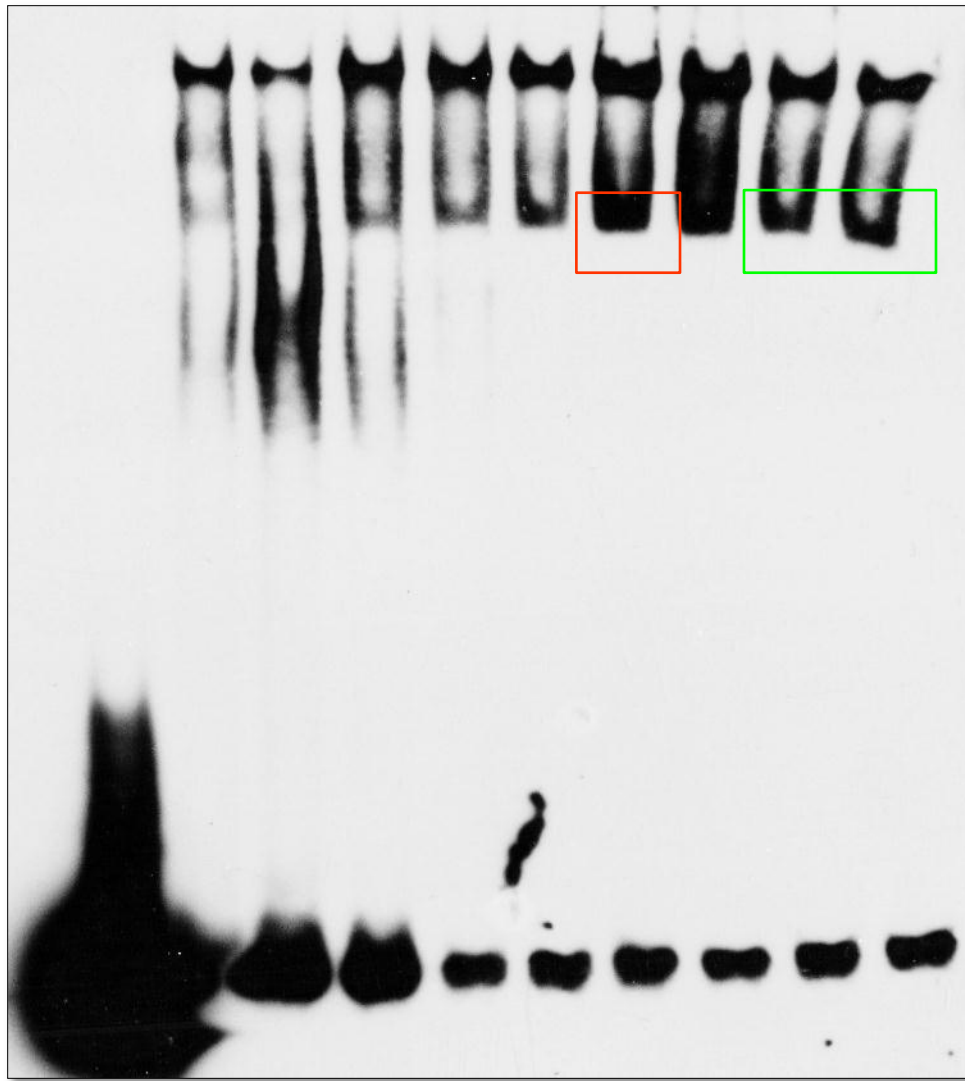


Data represent mean \pm standard error of three observations . Band density of IP Arnt was normalized with band density of Arnt. Differences among groups were determined by one-way ANOVA followed by Bonferroni's test, $p \leq 0.05$. '*' significant when compared with B(a)P; '#' significant when compared with Acetone; '¥' significant when compared with respective controls; '£' significant when compared with respective P5 + BP

Effect of PBPs on phosphorylation of AhR



Effect of PBP on AhR: DNA binding (EMSA)



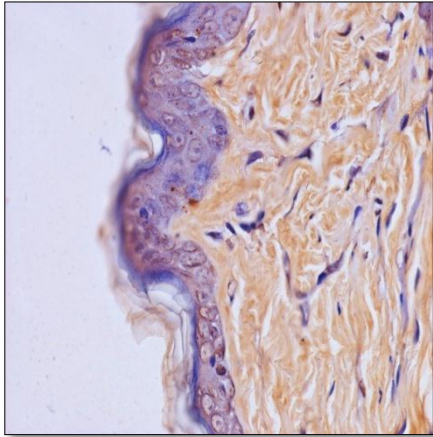
F Co Anti-
AhR



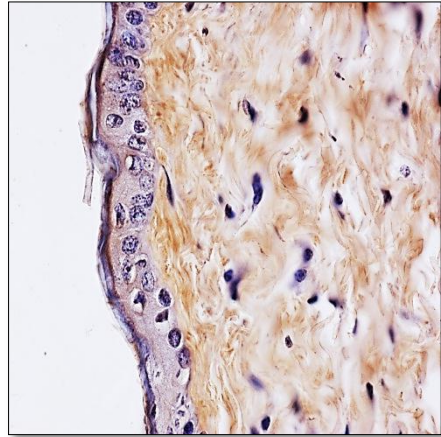
SUPERSHI
ET
SHIFT

F A P5 P3 Pm C BP B+P5 B+P3
B+Pm

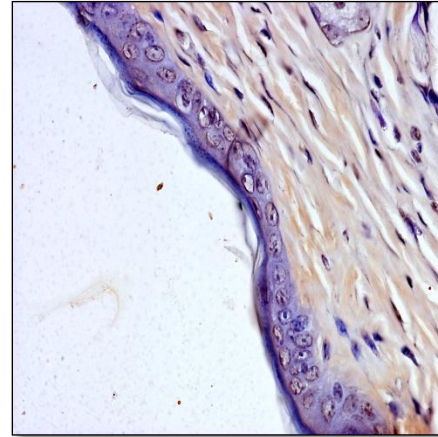
Effect of PBPs on DNA adduct formation



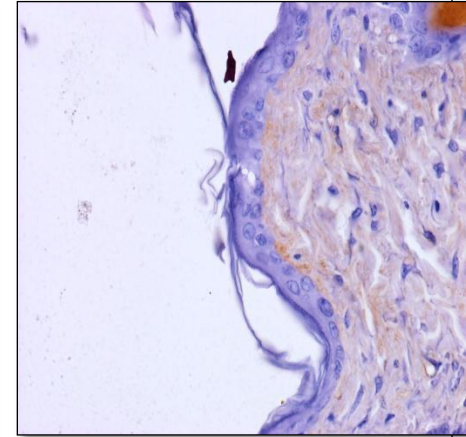
ACE



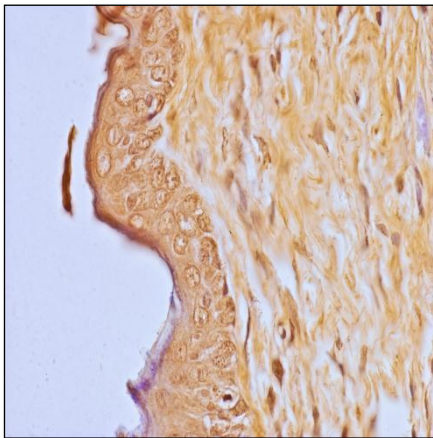
PBP5



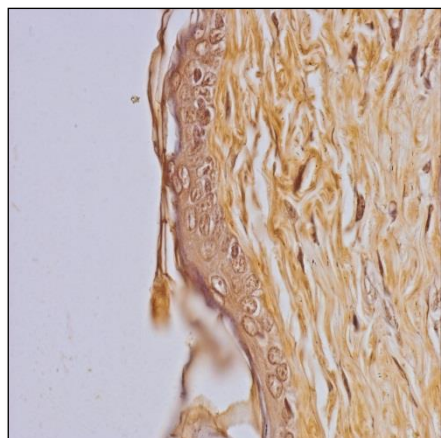
PBP3



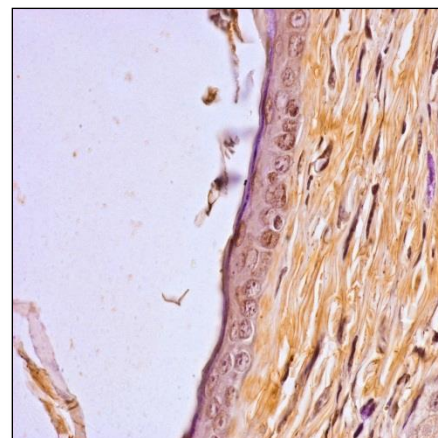
PBPm



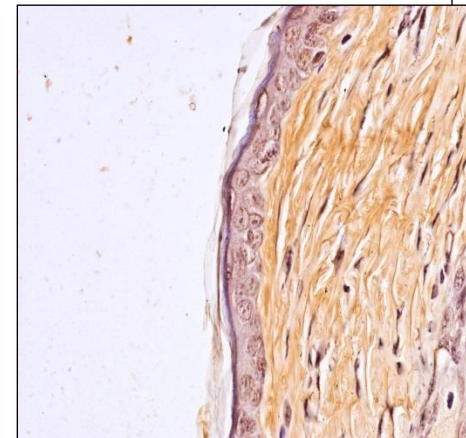
BaP



B+P5

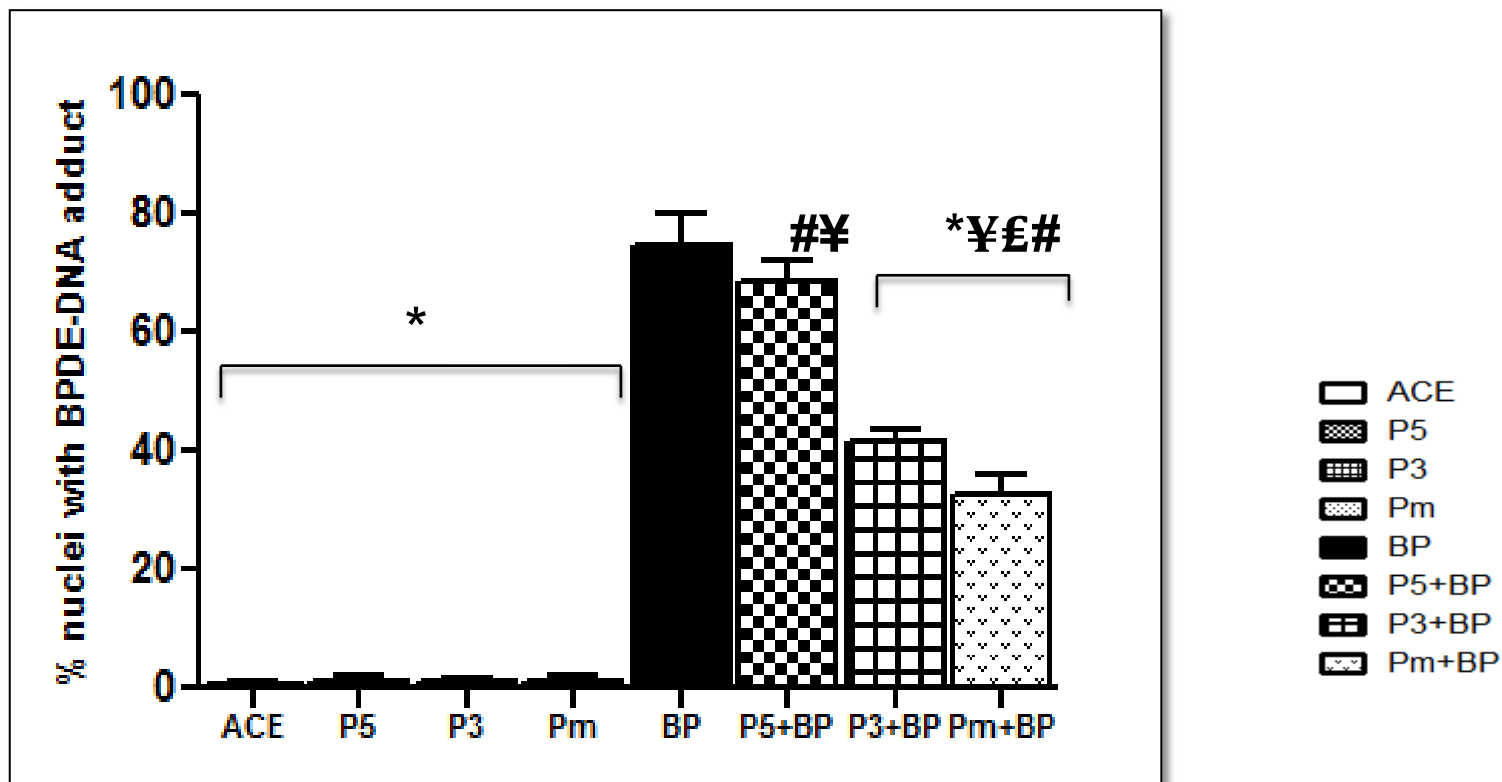


B+P3



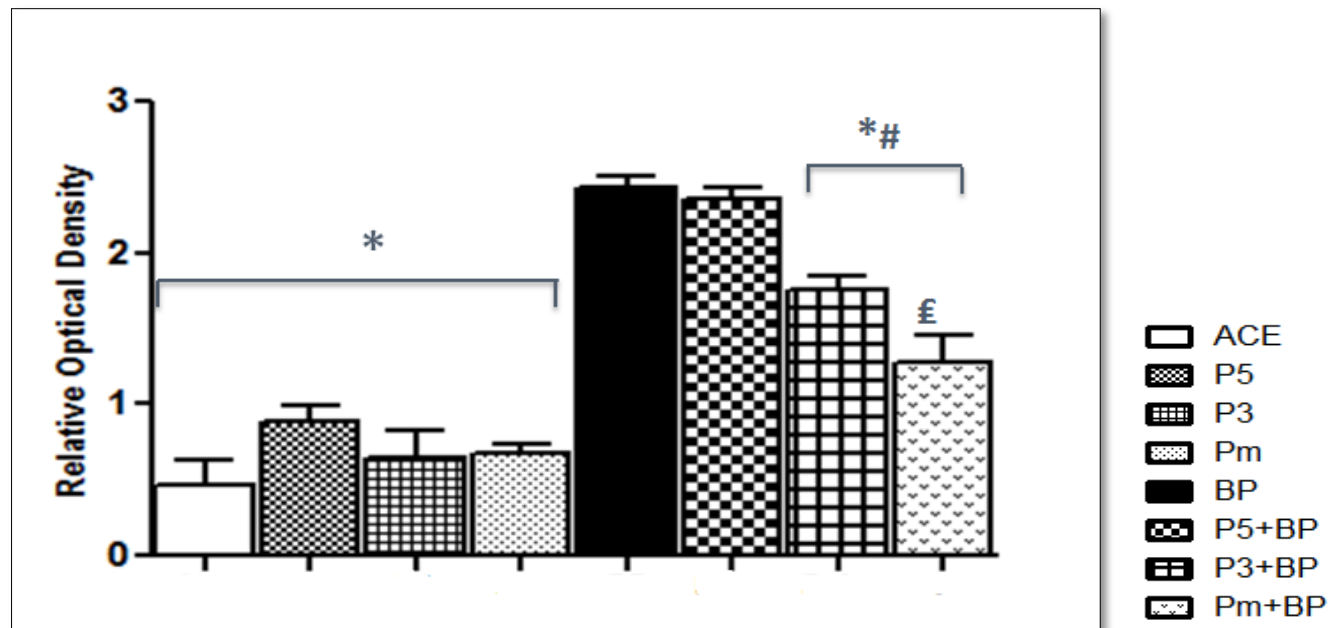
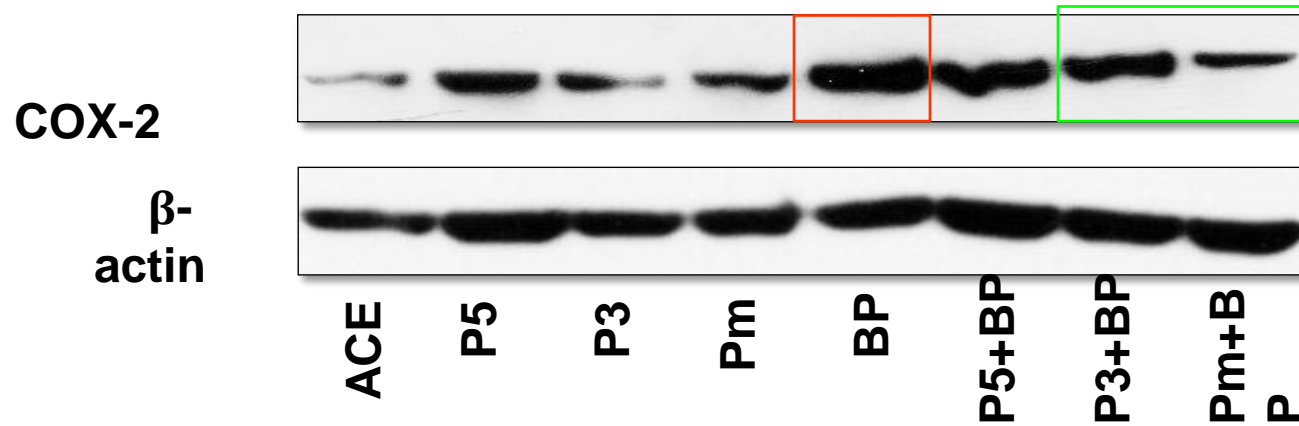
B+Pm

Effect of PBP on DNA adduct formation



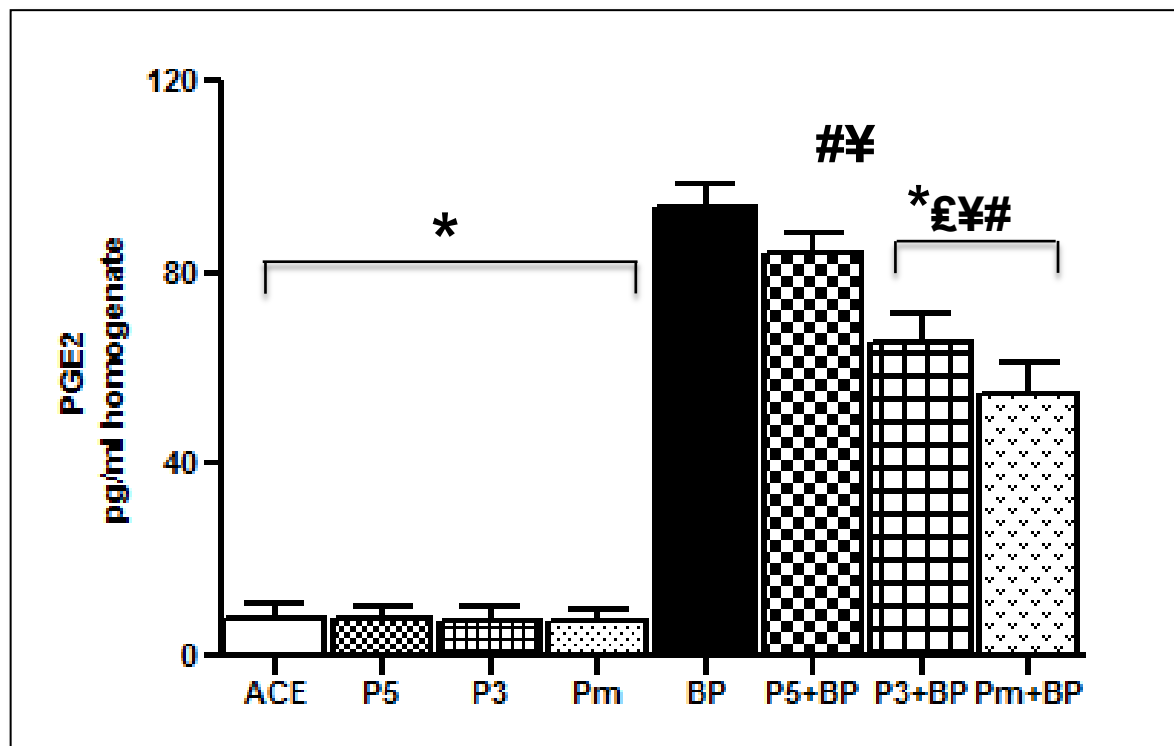
Results are presented as representative photomicrographs at X400 magnification. Quantitative analysis was done by digital image analysis in minimum 10 photomicrographs with at least three mice per group. Whereas semi-quantitative analysis was done by counting percentage of nuclei with BPDE-DNA adducts in only epidermis of 10 randomly selected images with at least three mice per group. Data represent mean \pm SE of three observations. Differences among groups were determined by one-way ANOVA followed by Bonferroni's test, $p \leq 0.05$. '*' significant when compared with B(a)P; '#' significant when compared with acetone; '¥' significant when compared with respective controls ; '£' significant when compared with respective P5+BP.

Effect of PBP on levels of COX-2



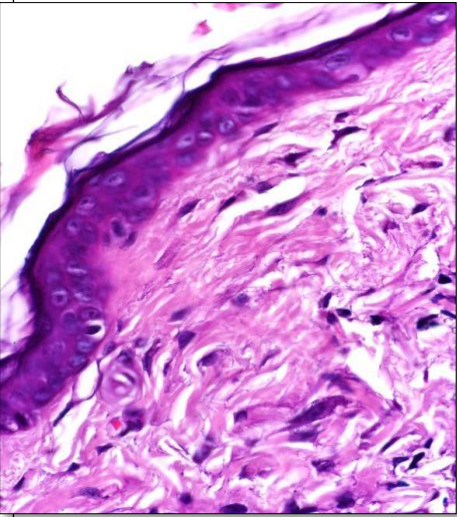
Data represent mean \pm standard error of three observations. Differences among groups were determined by one-way ANOVA followed by Bonferroni's test, $p \leq 0.05$. '*' significant when compared with B(a)P; '#' significant when compared with Acetone; '¥' significant when compared with respective controls; '£' significant when compared with respective B+P5.

Effect of PBPs on levels of PGE2

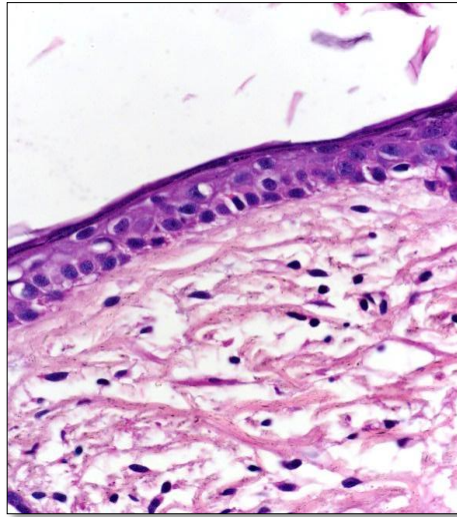


Data represent mean \pm standard error of three observations. Differences among groups were determined by one-way ANOVA followed by Bonferroni's test, $p \leq 0.05$. '*' significant when compared with B(a)P; '#' significant when compared with Acetone; '¥' significant when compared with respective controls; '£' significant when compared with respective B+P5.

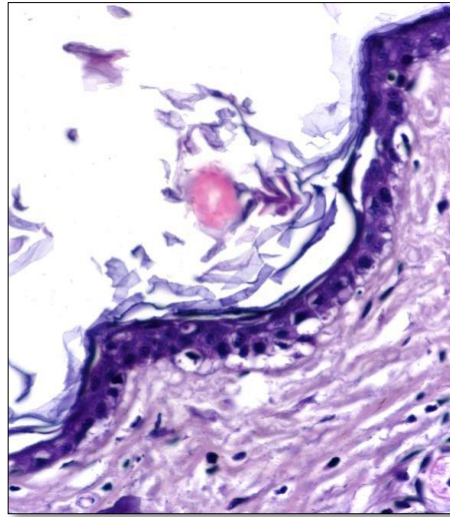
Effect of PBPs on B(a)P induced Hyperplasia



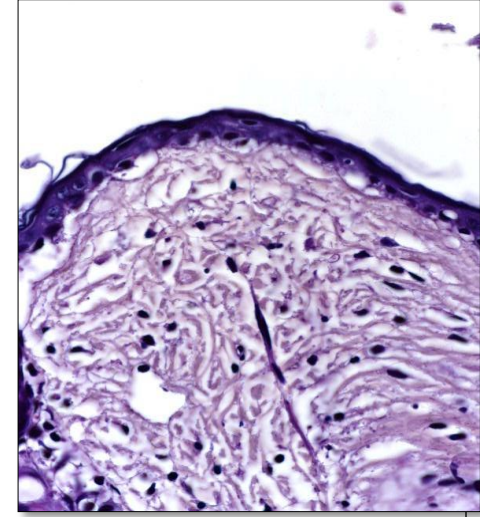
ACE



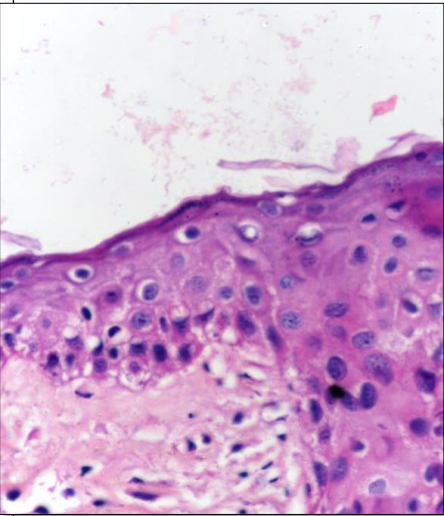
PBP5



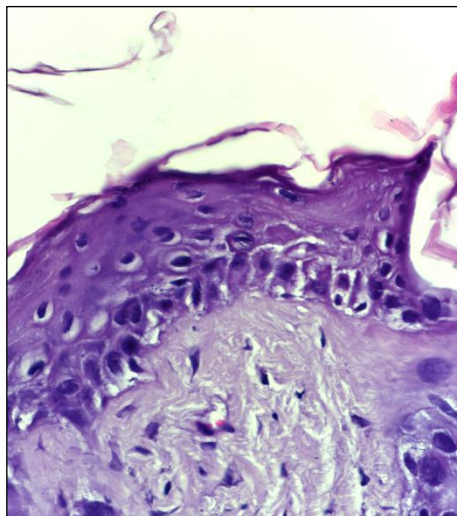
PBP3



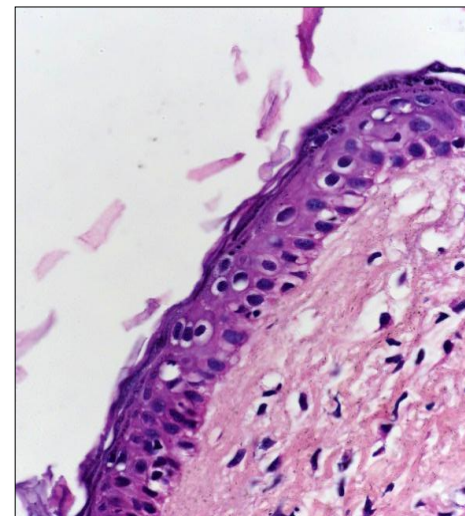
PBPm



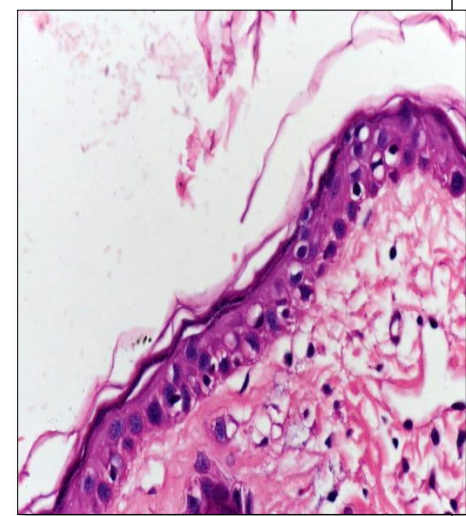
BaP



B+P5

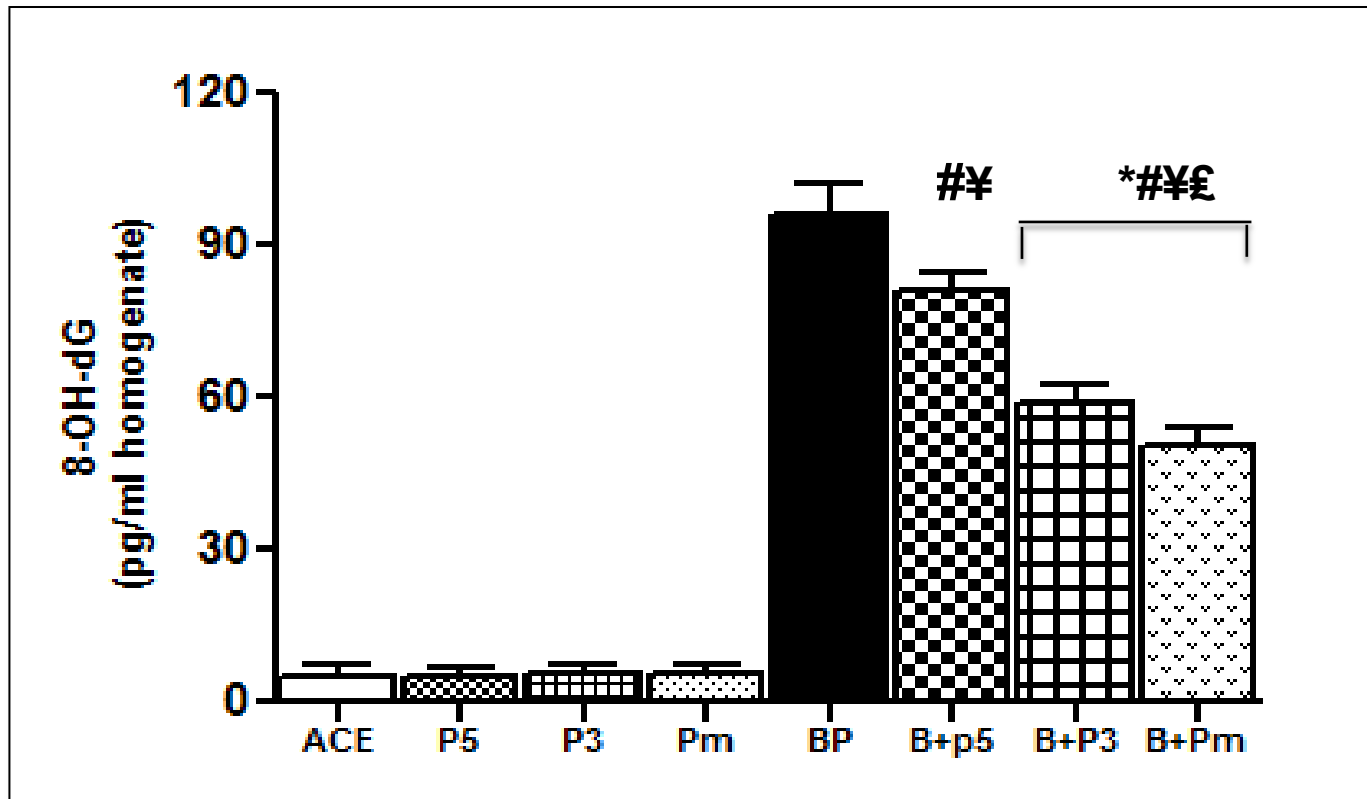


B+P3

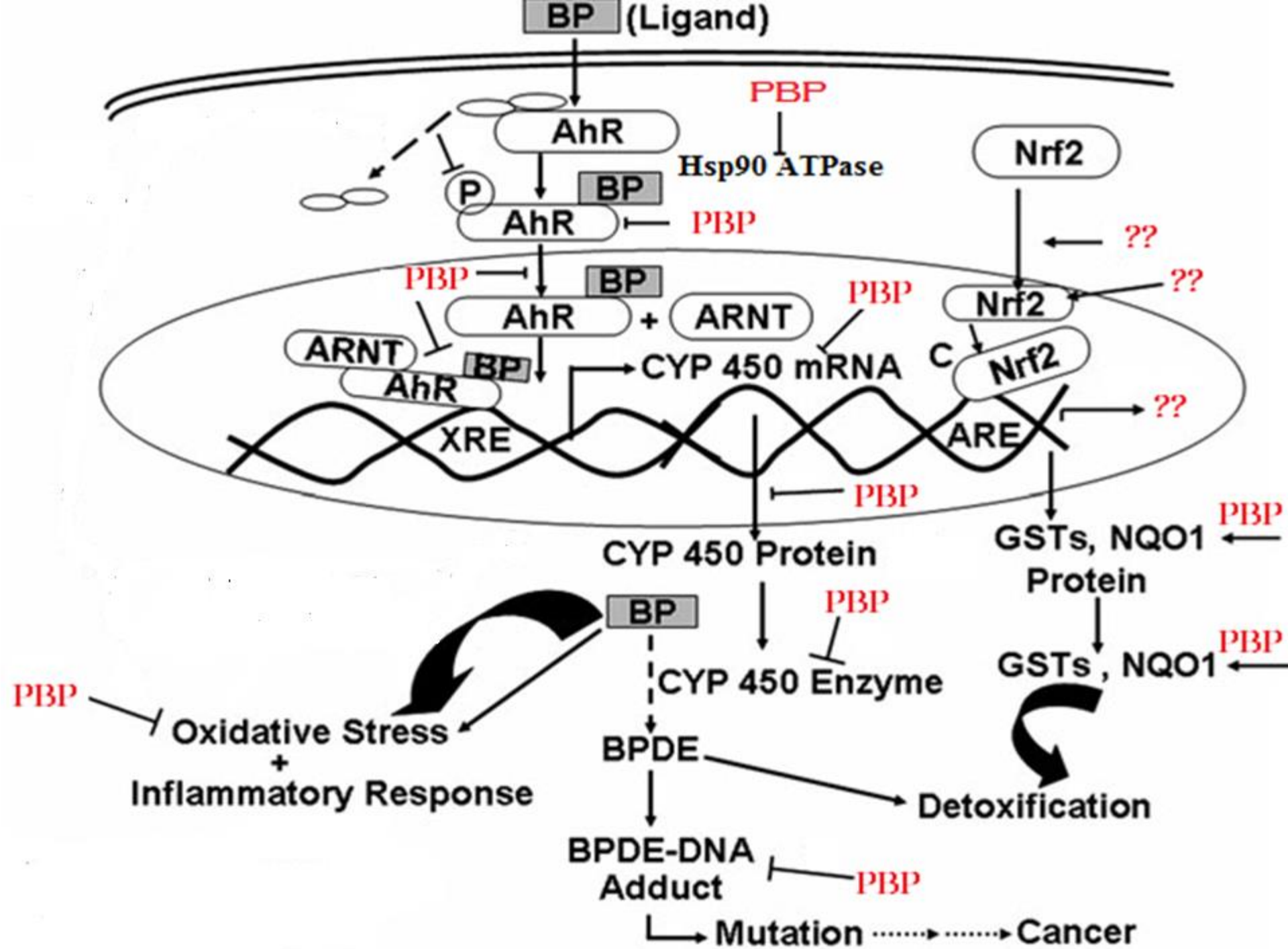


B+Pm

Effect of PBP's on levels of 8-OH-dG



Data represent mean \pm standard error of three pooled sample (3 animals per sample). Differences among groups were determined by one-way ANOVA followed by Bonferroni's test, $p \leq 0.05$. '*' significant when compared with B(a)P; '#' significant when compared with Acetone; '¥' significant when compared with respective controls; '£' significant when compared with respective P5+BP.



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Thank You