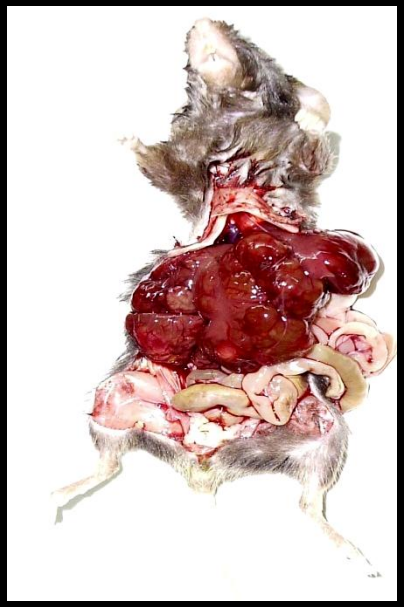
Molecular Medicine

Butea monosperma in Cancer Treatment

Dr.G.Mathan
Assistant Professor
Department of Biomedical Science
Bharathidasan University
Tiruchirappalli -620 024
Tamil Nadu, India

X 15- myc Transgenic Mouse Model for HCC



X15- myc Transgenic Mouse

Normal Liver X15- myc Tg Liver

- Recombinant bicistronic construct (Singh *et al*, 2003) (Kumar *et al*, 2001) US patent no: 6274788 B1.

- X-15 myc transgenic mice appeared to be an ideal model to study the disease process and also screening drugs.

Transgene	expressed Promoters	used Mouse strain	Pathology	Reference
HBx and c-myc	Xp and Cp	C57BL/6 x SJL	HCC in the first half of animals' life span (3 to 5 months)	1. Singh M,et.al., 1998 2. Kumar V,et.al., 2001(us patent)

X15-myc transgenic mouse model

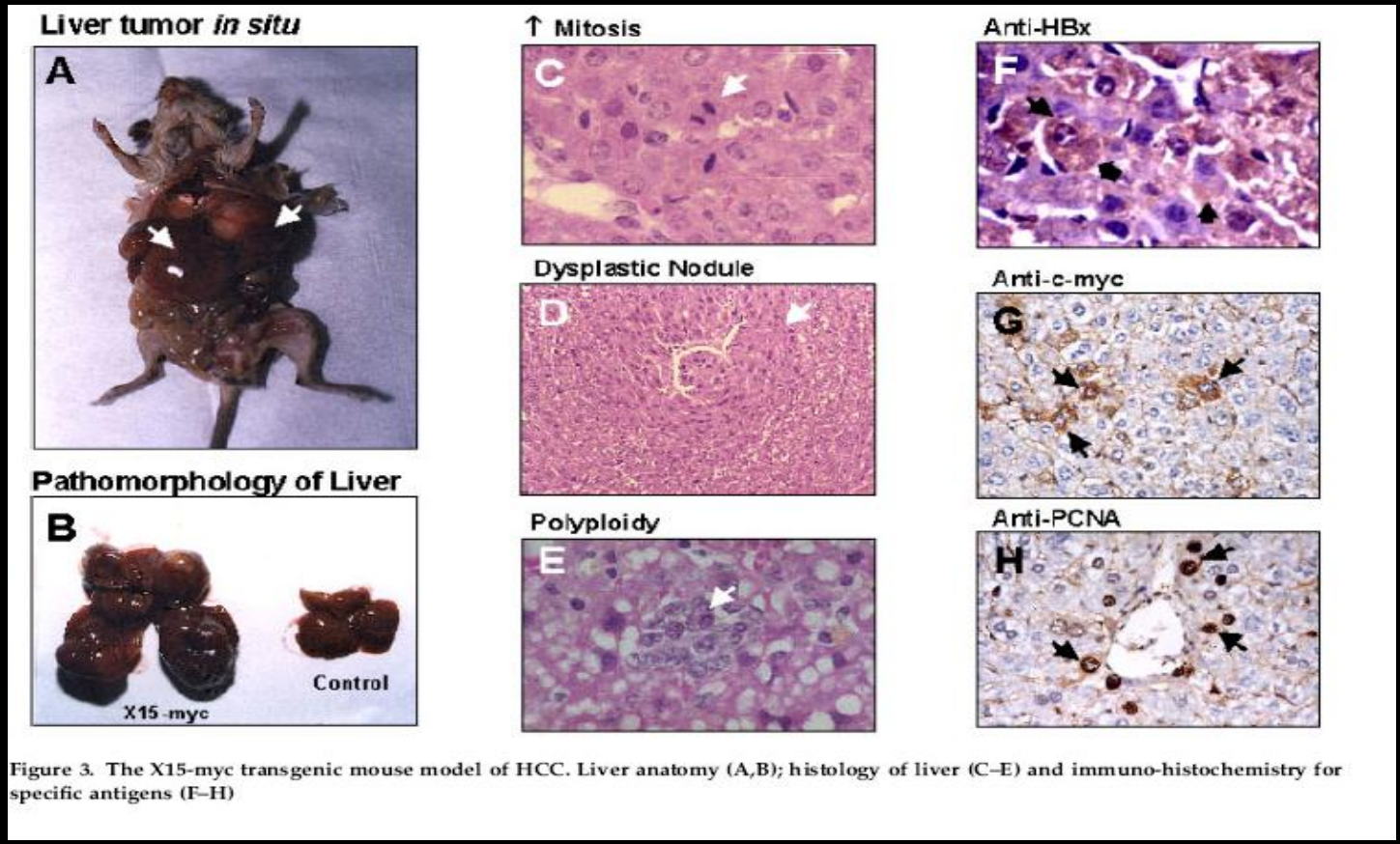
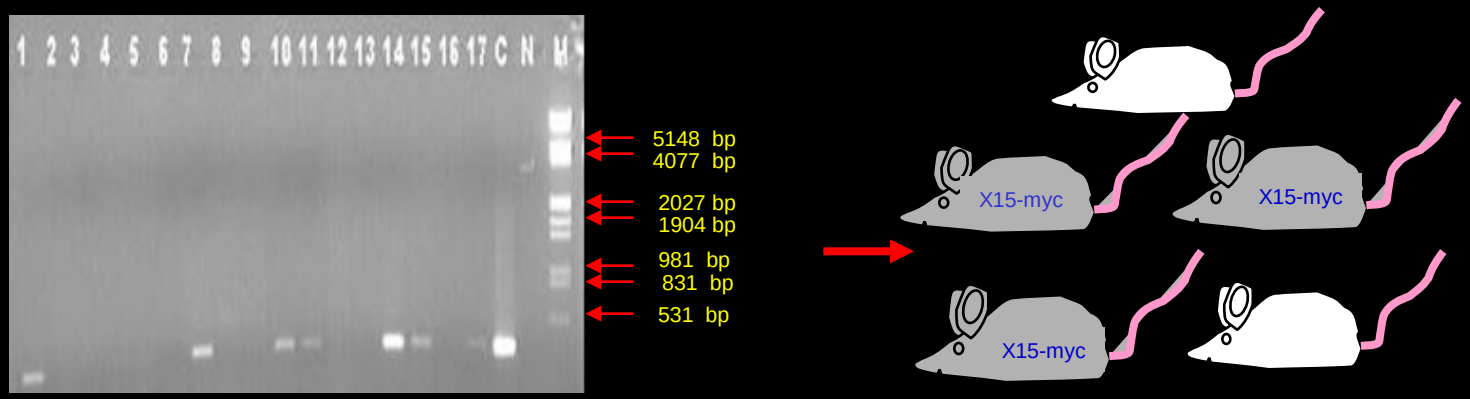


Figure 3. The X15-myc transgenic mouse model of HCC. Liver anatomy (A,B); histology of liver (C–E) and immuno-histochemistry for specific antigens (F–H)

- Most of the pathomorphological and microscopic changes were similar to those observed with the HCC patients (Lakhtakia *et al*, 2003)

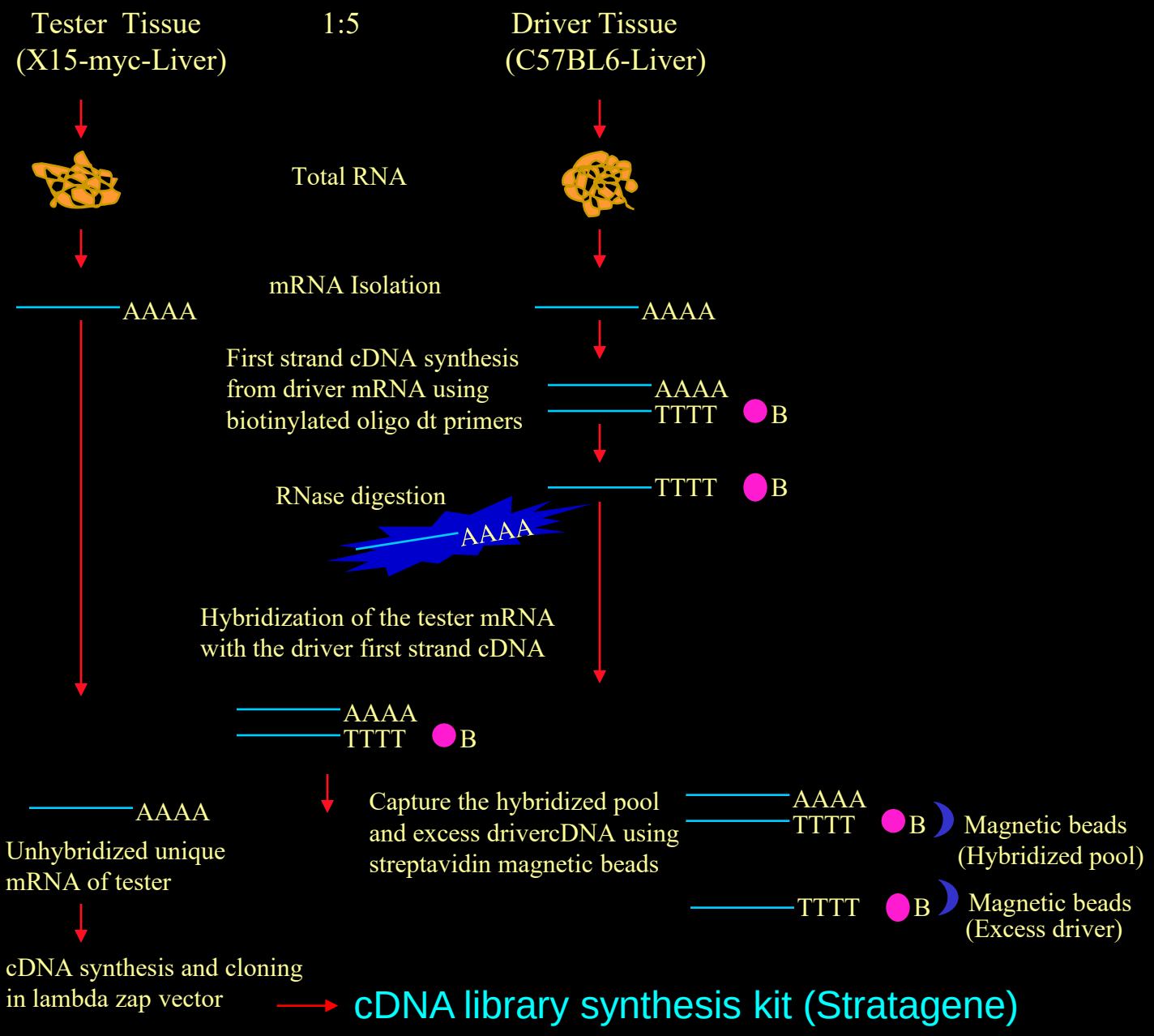
Analysis of differential gene expression in the tumors of X15-myc oncomouse.



PCR analysis for X15-myc Transgenic positive animals

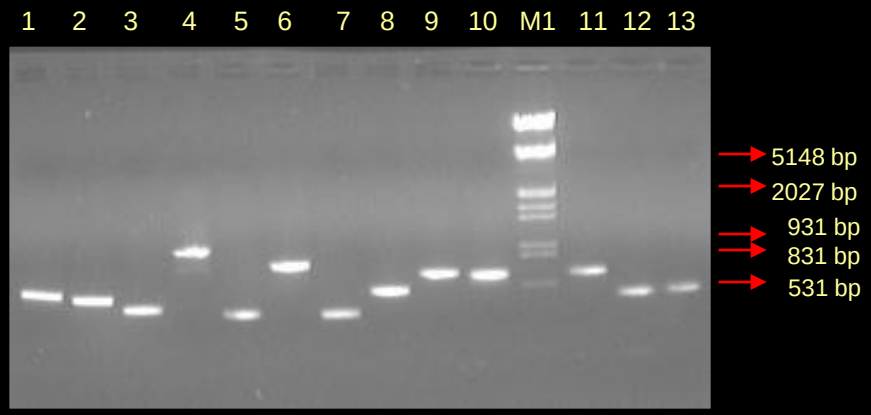
" C "- Positive control; " N" – Negative control; " M "- λ DNA Marker

cDNA subtraction

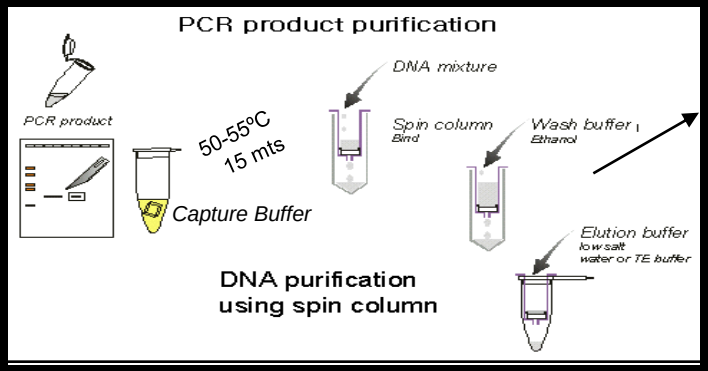


Primers M13 F: 5'-CGTTGTAAAACGACGGCCAGTG -3'
M13 R: 5'-CACAGGAAACAGCTATGACCATG-3'

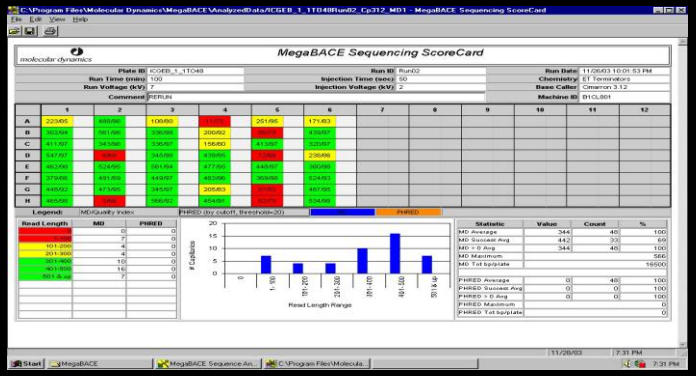
A.



B.



C.



A. Agarose gel analysis of PCR amplified cDNA inserts from phagemids. M1, DNA Marker, B. DNA band purification steps by GFX PCR, C. Chromotogram of MegaBACE sequencing score card.

Vector Screened & Changed in to FASTA format sequences

↓

BLAST Analysis

http://www.ncbi.nlm.nih.gov/blast/blast.cgi

List of differentially expressed genes.

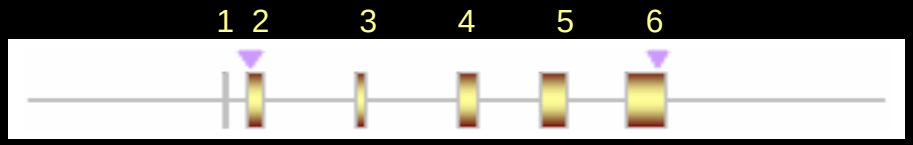
Sequence analysis of transcripts revealed 19 discreet categories

Protein biosynthesis genes	100
Electron transport genes	81
Metabolism related genes	75
Transport molecule genes	36
Ubiquitin proteasome pathway genes	25
Protein metabolism genes	23
Signal transduction genes	22
RNA processing genes	18
Calcium/sugar/carbohydrate/copper binding genes	14
Cytoskeletal prganisation/ cell adhesion genes	17
Endocytosis /protein transport genes	13
Oxidative stress genes	13
Transcription genes	13
Cell cycle and growth differentiation genes	12
Immune response genes	11
Apoptosis and anti apoptosis genes	9
Blood cogulation genes	9
Molecular chaperone genes	8
Replication gene	1

List of top five differentially expressed genes obtained from X15-myc Transgenic mouse liver cDNA subtraction library.

SYMBOL	GENE NAME	FREQUENCY
Rps27a	Ribosomal Protein s27a	20
COX3	Cytochrome C Oxidase, Subunit III	16
ATP6	ATP Synthase F0 Subunit 6	14
COX2	Cytochrome C Oxidase, Subunit II	11
ND1	NADH dehydragenase, Subunit, type 1	11

A



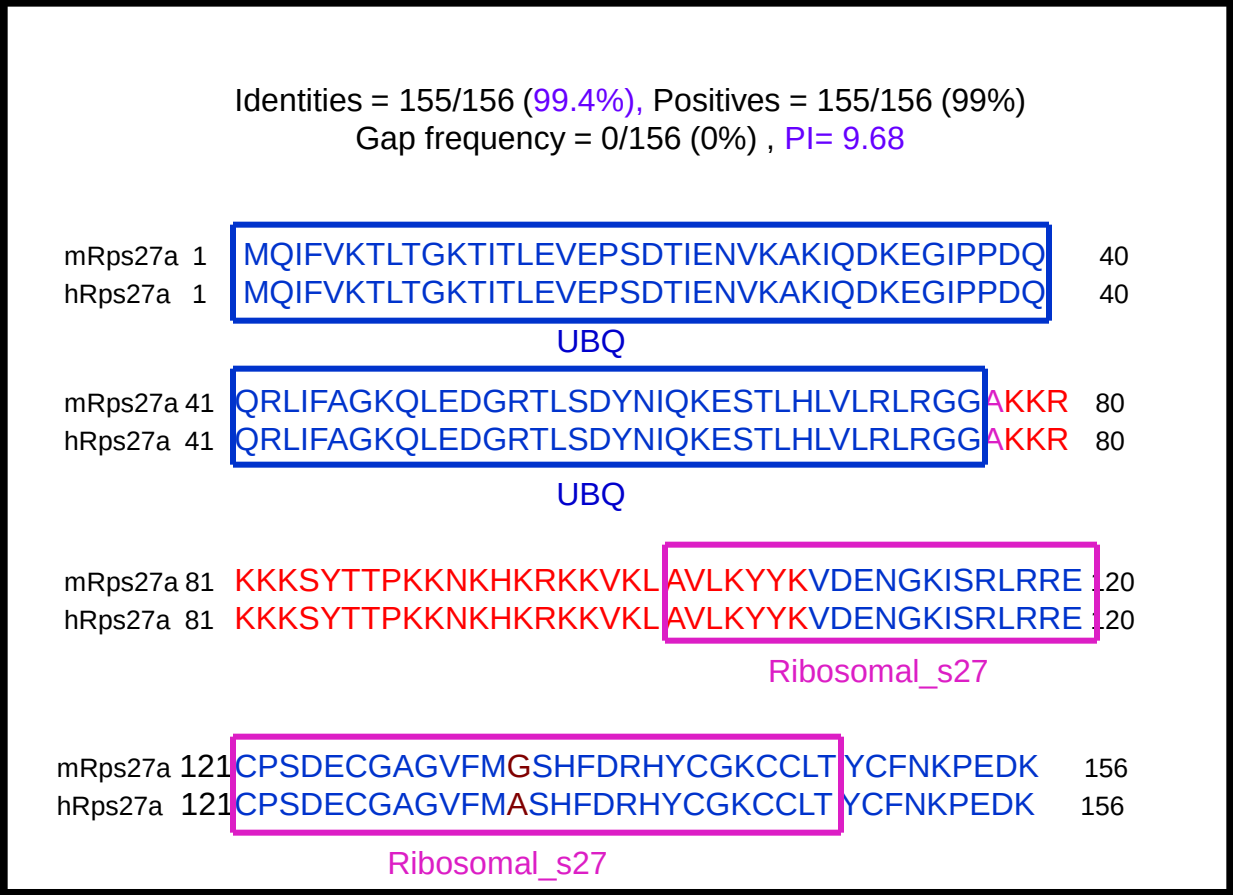
Exons

B

Chromosome: 11, Location 11 A3.3
Filename : *Mus musculus* Rps27a-CDS
Sequence Size : 471
Translation Position : 1 - 471; Genetic Code : Universal

```
         10        20        30        40        50        60
ATGCAGATCTTTGTGAAGACCCTTACGGGGAAAACCATCACGCTCGAGGTTGAACCCTCG
M  Q  I  F  V  K  T  L  T  G  K  T  I  T  L  E  V  E  P  S  20
         70        80        90       100       110       120
GACACTATAGAAAATGTAAAGGCCAAGATCCAGGATAAGGAAGGAATTCCTCCTGATCAG
D  T  I  E  N  V  K  A  K  I  Q  D  K  E  G  I  P  P  D  Q  40
        130       140       150       160       170       180
CAGAGGCTGATCTTTGCTGGTAAGCAGCTGGAAGATGGCCGGACTTTGTCTGACTACAAC
Q  R  L  I  F  A  G  K  Q  L  E  D  G  R  T  L  S  D  Y  N  60
        190       200       210       220       230       240
ATTCAAAAGGAGTCCACCCTTCATCTGGTGTTGAGACTTCGGGGTGGTGCTAAGAAAAGG
I  Q  K  E  S  T  L  H  L  V  L  R  L  R  G  G  A  K  K  R  80
        250       260       270       280       290       300
AAGAAGAAGTCTTACACCACTCCCAAGAAGAACAAGCATAAGAGGAAGAAGGTTAAGTTG
K  K  K  S  Y  T  T  P  K  K  N  K  H  K  R  K  K  V  K  L  100
        310       320       330       340       350       360
GCTGTGCTGAAATACTATAAGGTGGATGAAAATGGCAAAATTAGCCGACTTCGTCGAGAG
A  V  L  K  Y  Y  K  V  D  E  N  G  K  I  S  R  L  R  R  E  120
        370       380       390       400       410       420
TGTCCTTCTGATGAATGTGGTGCTGGAGTTTTCATGGGAAGCCACTTTGACAGGCATTAC
C  P  S  D  E  C  G  A  G  V  F  M  G  S  H  F  D  R  H  Y  140
        430       440       450       460       471
TGTGGCAAGTGTTGTCTGACTTACTGCTTCAACAAACCAGAAGACAAGTAG
C  G  K  C  C  L  T  Y  C  F  N  K  P  E  D  K  *         156-AA
```

Structure of the Rps27a gene of *Mus musculus* and cDNAs. A, Organization and chromosomal location. In total, 6 exons are shown in boxes. Closed boxes designate the coding sequences. B, ORF of the Rps27a gene showing 156 amino acids in single letter code (An red aerrowmark indicates the site of proteolytic cleavage that would be required to generate mature ubiquitn from the primary translation product).



Alignment of the primary sequences of human and mouse Rps27a Identical residues are shown in same color, while the non-identical regions are marked in different colors. Blue and rose boxes denote the regions of ubiquitin and Ribosomal_s27 respectively.

- The Rps27a Highly conserved very basic protein due to presence of 80 basic AA in the Carboxyl terminal (CEP) of Ubiquitin.

- UBQ-76 AA conserved eukaryotic protein- Diverse cellular functions

- UBQ-Poly ubiquitin chain (UBb, UBc) or Fused to unrelated protein (UBA)

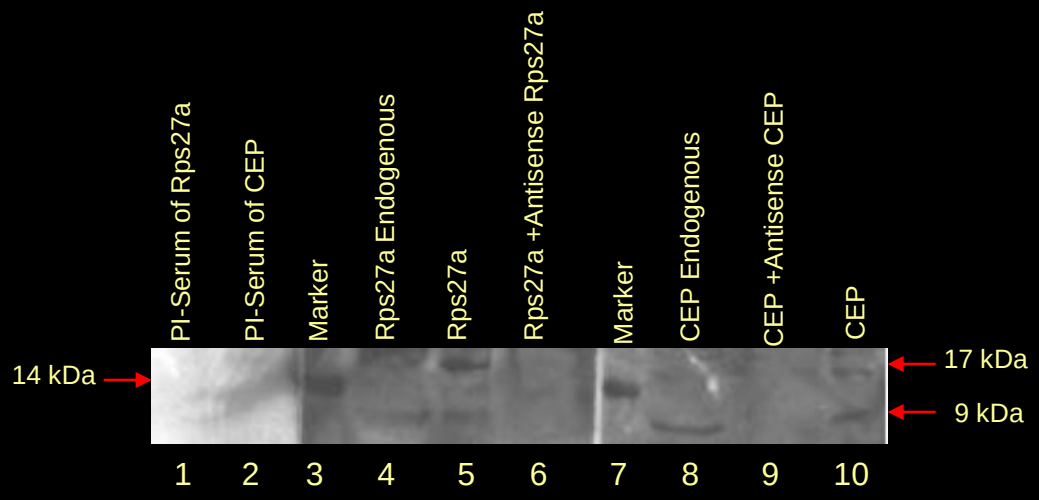
- UBA → UBA80 (Rps27a)or UBA52 (RpL40). Conserved in man, yeast and plants

[Salvensan et al, Nucleic Acid Research (1987), Ozkaynak et al, EMBO J (1987), Lund et al, J. Bio. Chem (1985)]

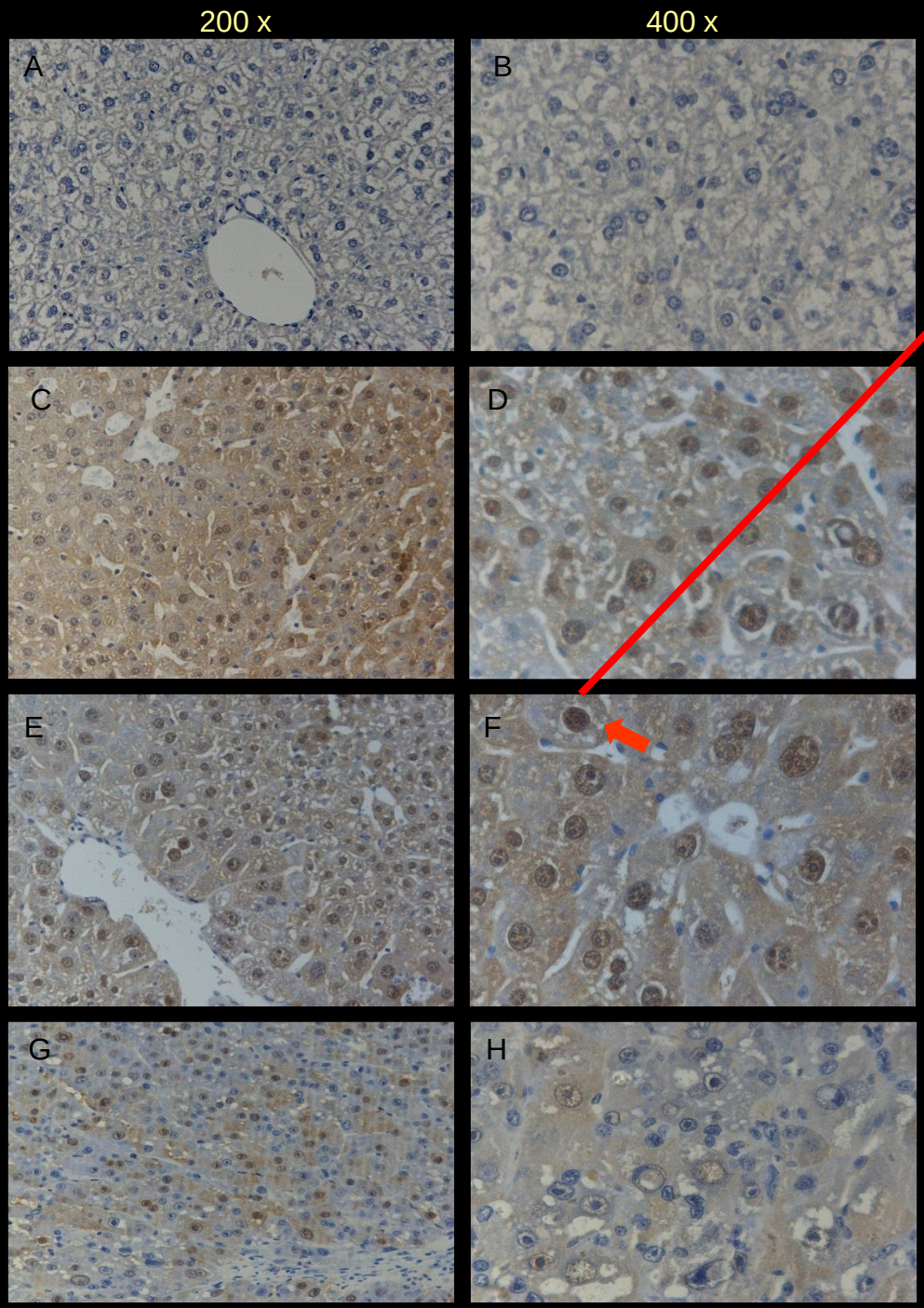
- CEP analysis shows strong homology at the AA level with Zinc finger protein.

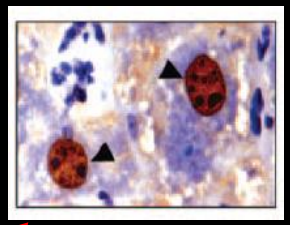
- CEP can associate with ribosome binding specifically ribosome or messenger RNA.

- Function of UBQ-CEP fusion genes has not been fully elucidated

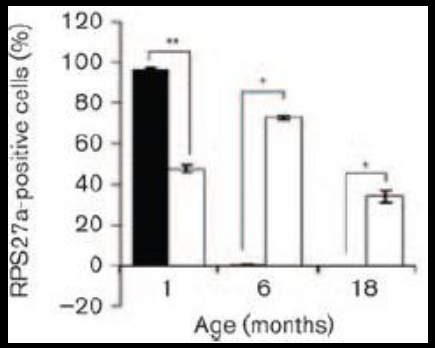


Detection of Rps27a and CEP expression in Huh7 cell lines. Total cells were lysed after 48hr post transfection with Rps27a (lanes 5), anti-Rps27a (lanes 6), CEP (lanes10) and anti-CEP (lanes 9).





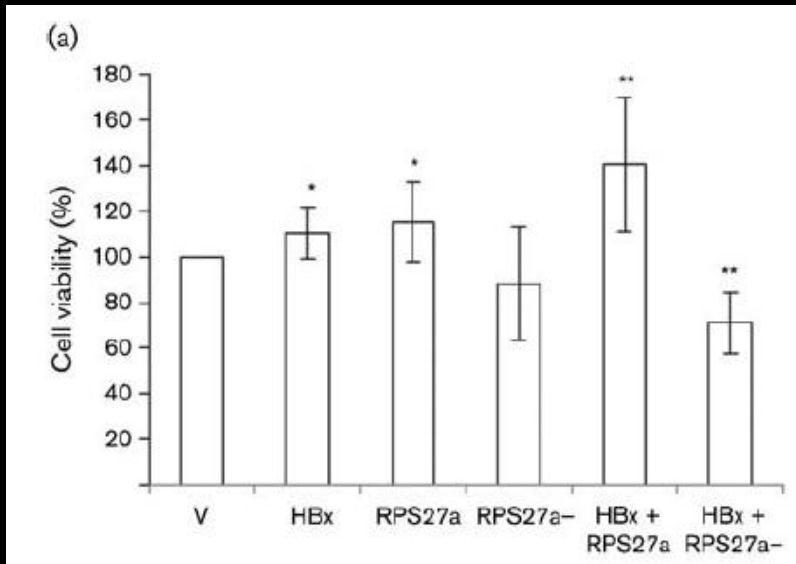
Positive staining is noted more in nuclear and perinuclear (red arrow)

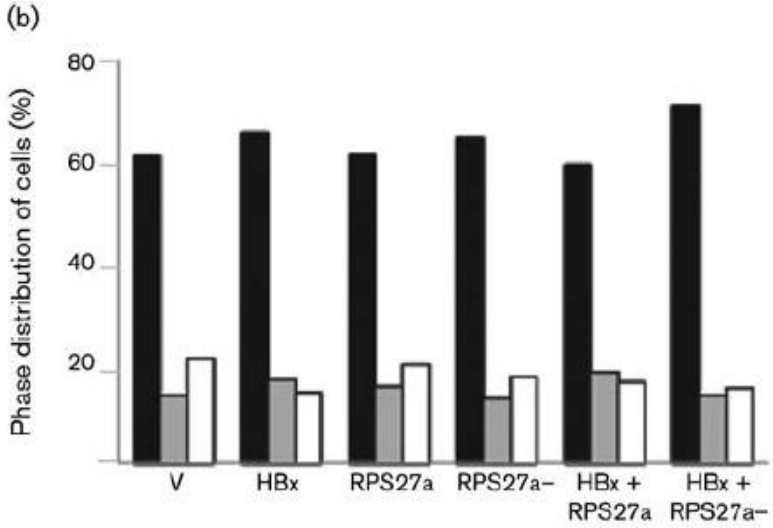


transgenic mice (white bars). Cells (n=500) were counted randomly from 10 different areas. *P<0.01; **P<0.05.

Rps27a expression in X15-myc Tg mice liver tissues.

Co-expression of Rps27a and HBx leads to improved survival and proliferation of cells





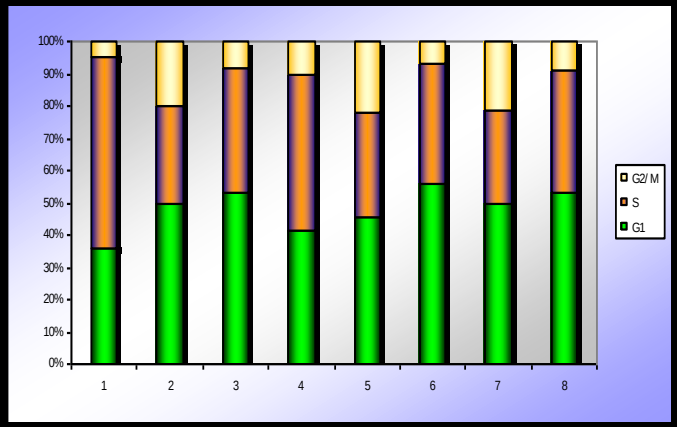
G0/G1	61.0	65.6	61.3	64.4	59.4	71.0
S	13.4	16.7	15.2	12.8	18.1	13.3
G2/M	20.6	14.0	19.7	17.2	16.2	15.0

*$P<0.01$ **$P<0.001$, compared with controls.

Fig. 3. Cooperation between RPS27a and HBx for cell survival, proliferation and size. Huh7 cells were transfected with expression vectors for HBx, RPS27a or RPS27a–, either alone or in combinations as indicated, and their viability, size and cell-phase distribution were monitored. (a) Cell viability by MTT assay. The bar graph shows means ± SD of ten independent observations. (b) Flow cytometry of asynchronous cells to show percentage distribution of cells in different phases of the cell cycle (black bars, G0/G1; grey bars, S; white bars, G2/M). Results are means of three independent observations. (c, d) The

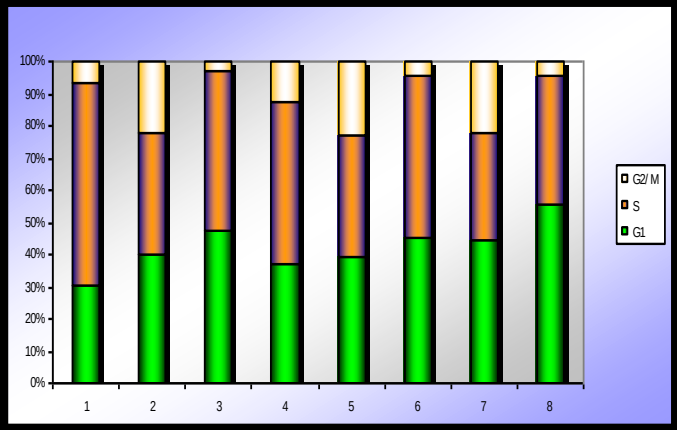
A

12hrs



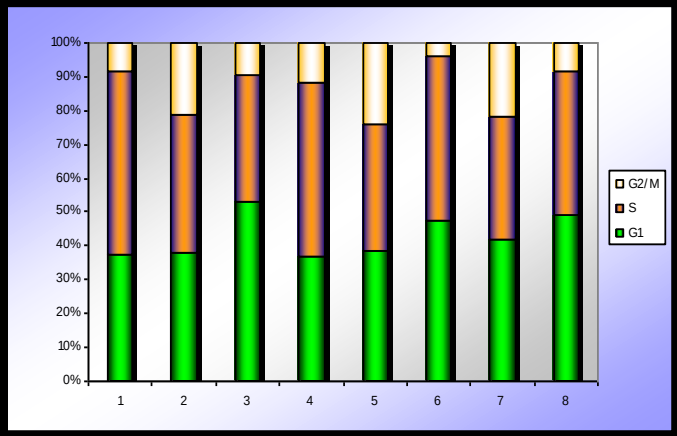
B

24hrs



C

42 hrs



Constructs

1. Rps27a
2. Antisense Rps27a
3. Rps27a + Antisense Rps27a
4. CEP
5. Antisense CEP
6. CEP + Antisense CEP
7. Rps27a + Antisense Rps27a +Antisense CEP
8. pSG5

Effect of Rps27a and CEP on cell cycle.

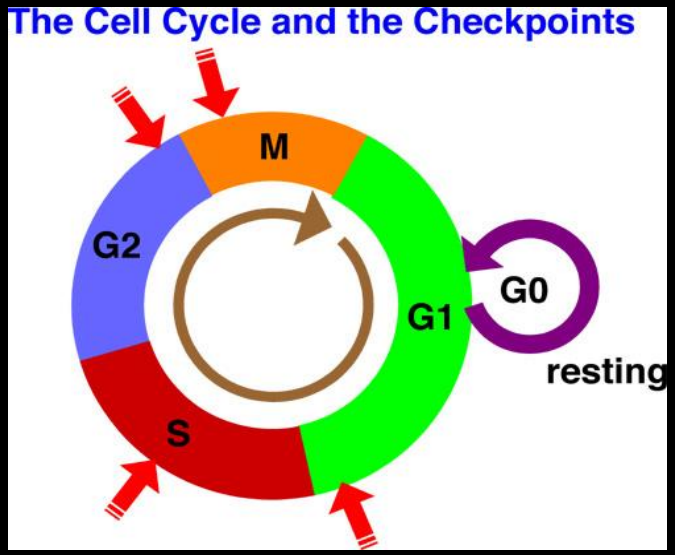
Huh7 cells were transfected with Rps27a, CEP, anti-Rps27a and anti-CEP constructs at 60-70 % confluency and cells were serum syncronized and collected at various time points of 12hrs (A) , 24hrs (B) and 42 hrs (C). The percentages of cells at different phases of the cell cycle were analysed by FACS and values are shown on the left (Y-axis) bar diagram.

Report-Deletion Rps6 (40s RS)
(Rps27a & CEP) ↓

Cell proliferation

G2/M phase arrest

Cyclin B1
(Yu et.al., 2002)



Cyclin E (sherr et.al.,1999)

Rps27a- early growth response gene G1 phase cell division

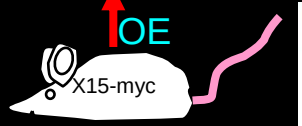
Induction of
Cyclin D-CDK4
Cyclin D-CDK2

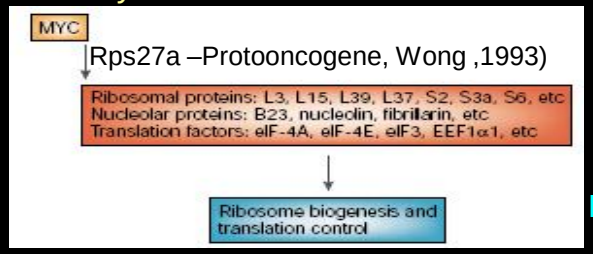
"S"PHASE ↑
DNA replication

(Rps27a & CEP) ↑

OE

OE





DeBenedetti *et al.,* 1990

In yeast, UB13 deletion-Slow growth of phenotype (1.6 hr-6.8hr) (Finley et.al., 1989)
Many small & Large Rib.Sub unit Protein ↑ variety of p.Tumor- Ex RPL36A. OE-Over Expression

Summary

- Rps27a, a ubiquitin precursor protein fused to the 80 amino acid carboxyl extension protein (CEP)

- The flow cytometry analysis reveled that Rps27a overexpressing cells accelerated cells to enter G1 phase to S phase with enhanced cell proliferation

- Immunohistochemical staining of Rps27a expression was moderately reduced from 3 months to 18 months old Transgenic liver tissues.

Overall, The sensitivity of over expressed Rps27a in HCC might be a general biomarker for tumor proliferation.

Evaluation of the antitumorogenic/anti-angiogenic activities of the floral isolates of *B. monosperma*

Butea monosperma (Flame of the Forest)

Family	:	Fabaceae
Orgin	:	India
Type/Uses	:	flowering tree
Size	:	50feet
Growth Rate	:	Slow growing at first
Lighter Requirments	:	full sun
Water Requirments	:	average, drier in the winter
Min.Temp.	:	mid 30° s
Flower	:	late winter, spring

Test Material	A003	F008	F009	Adriamycin	Mitomycin	Tamoxifen	5-Flurouracil
Concentration used:	100 mg/L	100 mg/L	100 mg/L	$1x10^{-5}$ M	$1x10^{-5}$ M	$1x10^{-5}$ M	$2x10^{-5}$ M
Cell lines							
Breast – MCF-7	6	-	-	72	-	-	-
Breast – T47D	4	22	0	34	-	-	-
Breast – ZR75-1	0	-	-	46	-	-	-
Cervix – SiHa	0	9	0	-	-	-	19
CNS - SK N MC	23	57	2	-	-	27	-
CNS - SK N SH	43	-	-	82	-	-	-
CNS - SNB78	2	-	-	20	-	-	-
Colon - Colo205	87	19	0	-	-	-	40
Liver - Hep2	51	58	35	-	88	74	-
Lung - A549	19	27	11	58	-	17	-
Lung - NCI H23	0	-	-	-	59	-	-
Oral - KB	16	-	-	-	-	-	9
Ovary – NIH OVCAR3	0	-	-	-	31	-	-
Ovary – OVCAR5	5	37	6	-	-	-	-
Prostate – DU145	0	-	-	-	69	-	-

Percent growth inhibition of human cancer cell lines with the aqueous extract (A003) of flowers of *Butea monosperma* and its fractions (F008 and F009) [Mathan *et.al.*,] [Communicated]

Hepatoprotective Herbal Extracts

- Tredational Herbal Medicine & CAM becoming popular among cancer patients (Molassiotis *et al*., 2005; Yates *et al*, 2005)

- Commonly used herbal preparations for treating Liver Diseases-Silymarin (Mayer *et al*, 2005), TJ-9 (Oak et al., 1995), Liv-52 (Huseini *et al*, 2005), Phyllanthus (Liu *et al*., 2001), Glycyrrhizin (Kumada *et al*, 2002), curcumin (Aggarwal et al., 2003; Campbell et al., 2005), Calotropis *procera* (Choedon & Mathan, 2006).

- RRL (Regional Research Laboratory, CSIR), Jammu

- **The traditional Indian medical system of Ayurveda, the flowers of *B. monosperma* have been used in the treatment of hepatic disorders and viral hepatitis (Schuppan *et al*., 1999; Dhiman *et al*., 2005)**

- **Chemopreventive potential of methonolic extract of *B.monosperma* flower on chemically induced oxidative damage in rats were reported (Sehrawat *et al.*, 2006)**

- **The mechanism of its action has not been elucidated.**

Flowchart of *Butea monosperma* floweral extracts and its fractions.

Butea monosperma

↓

Flowers (Dried)

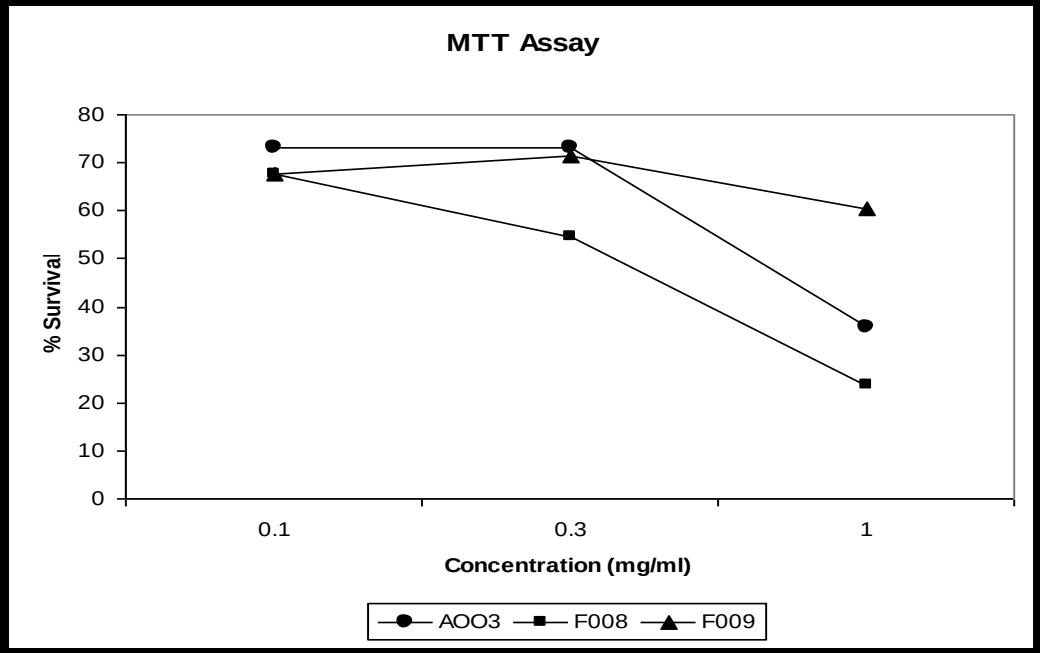
↓ **Extraction**

A003 Aqueous (Active *in vivo*)

↓ **Fractination**

F008 n-Butanol (Active *in vitro*)

F009 Aqueous Active *in vitro* & *in vivo*)



Cytotoxic effect of *Butea monosperma* extract on cancer cell lines. **Huh-7 cells were incubated with different concentrations of *Butea monosperma* and analyzed for cell viability at 24h or 48h (*n*=6), mean±SE. a.*P*<0.001; b. *P*<0.01**

- **IC_{50} in Huh7 cells – F009- less toxic (65% cell survival at 1g/L)**
  _ A003 & F008 (0.5 – 1g/L) & Doxorubicin (0.1g/L)

X 15-myc Transgenic mouse model

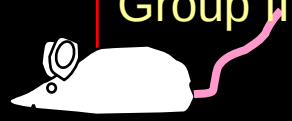
(4 weeks old matched age group, n=6)

Group I

12 weeks

Receives 9 ip doses of A003, F008 & F009 in saline on biweekly (100 mg/kg)

Group II



20 weeks

Serum VEGF
Tumor weight
Histopathology
Immunohistochemistry for Rps27a
Morphometry

Anti- tumorigenic effect of *B. monosperma* in transgenic mice

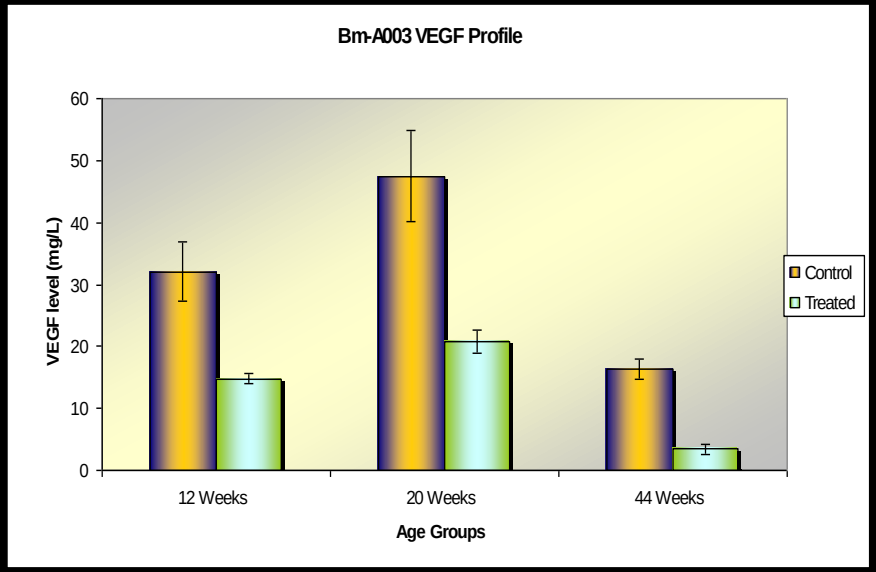
A.

	Period (wks)	Group: Treated(n=6)	Group: Control (n=6)
A003	12	14.8±0.8[b]	32.1±4.8
	20	20.8±1.9[a]	47.5±7.3
	44	3.45±0.8[a]	16.3±1.6
F009	12	13.5±0.6[NS]	14.4±0.5
	20	16.2±0.6	17.1±0.8[NS]
Non transgenic control		7.00 ± 0.55	

Values are shown as mean± SE. [a] p<0.001; [b] p<0.01; [NS] p = 0.15

Tg-2 to 4 fold↑

B.



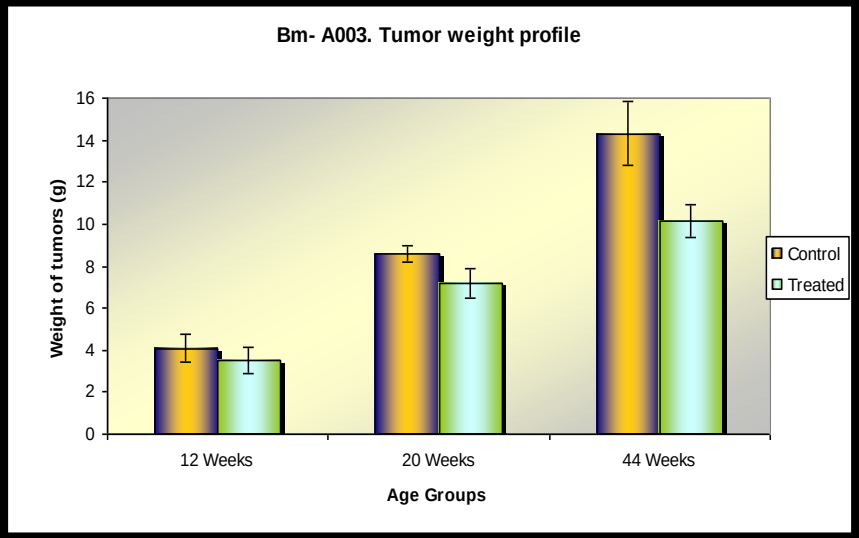
A. Serum VEGF levels (mg/L) in the X-15 *myc* mice after treatment with the aqueous extract (A003) or aqueous fraction (F009) of *Butea monosperma (BM).* B. BM-A003 treated, 20 and 44 wks old, Transgenic animals are showing the significant reduction (p<0.001) in their VEGF level.

A.

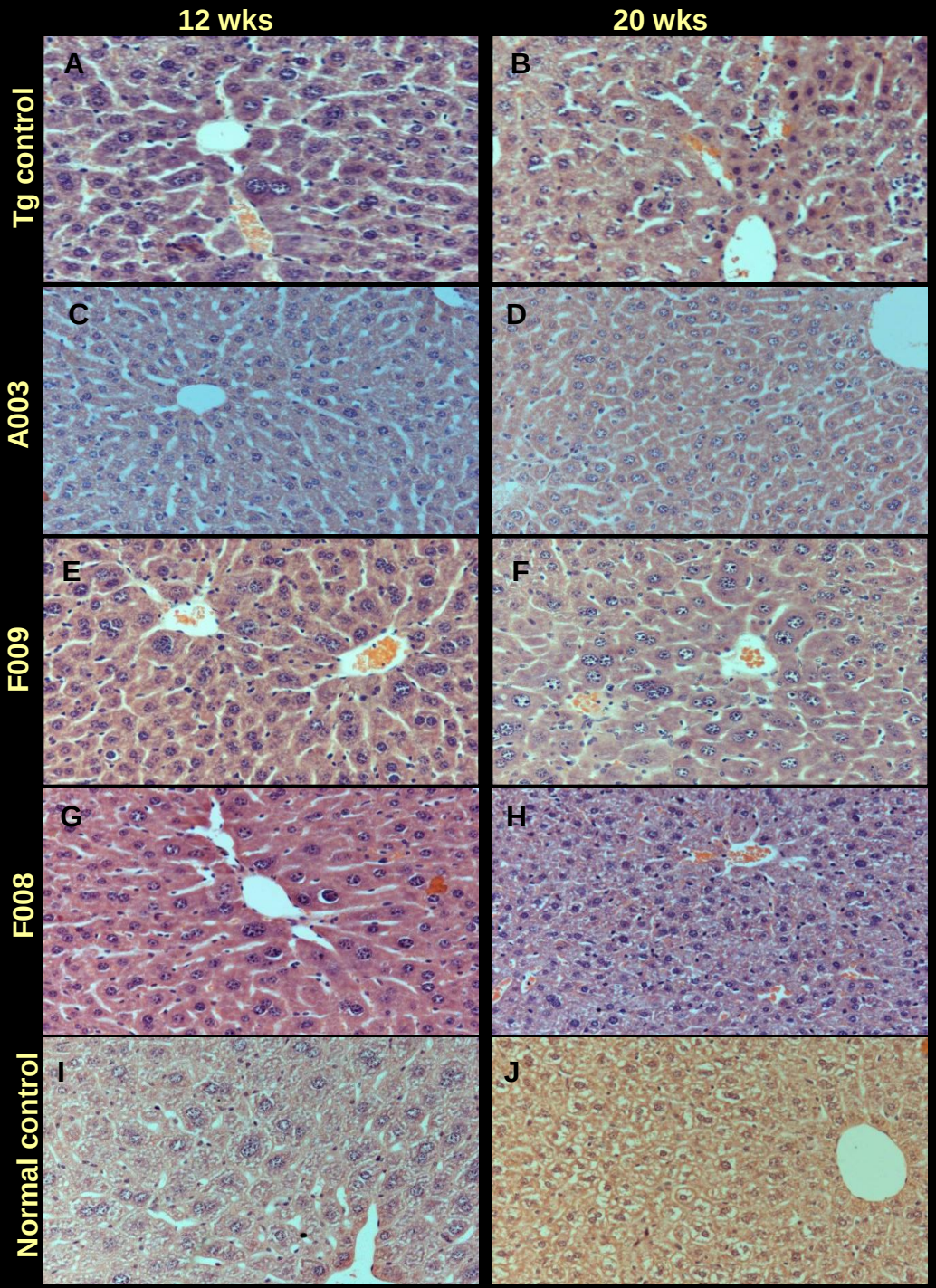
	Period (wks)	Group: Treated(n=6)	Group: Control (n=6)
A003	12	3.50±0.63	4.08±0.66
	20	7.17±0.72[b]	8.57±0.39
	44	10.15±0.81[a]	14.32±1.53

Values are shown as mean± SE. [a] p<0.001; [b] p<0.01

B.

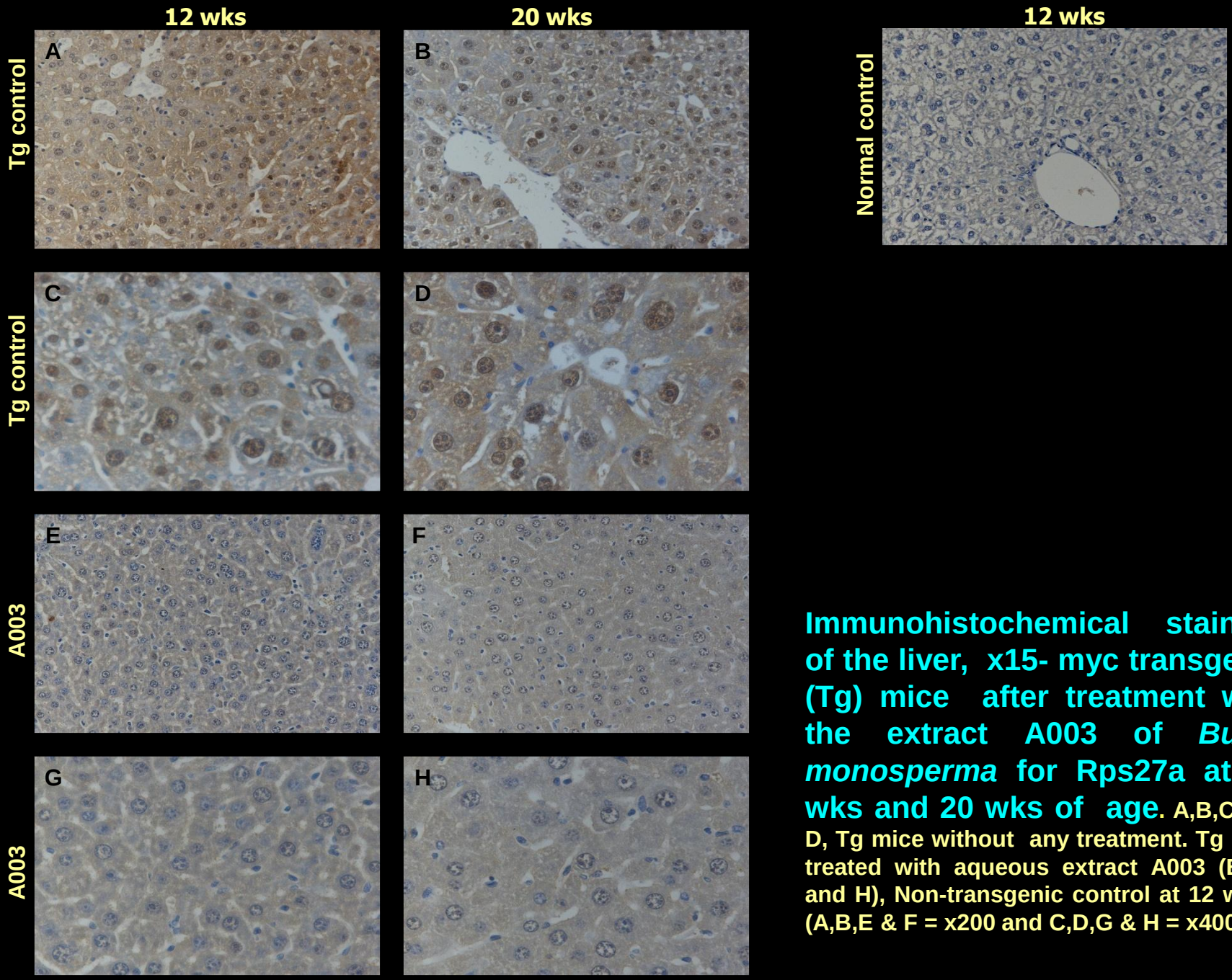


A. Liver tumor weight (g) of X-15 *myc* mice after treatment with the aqueous extract (A003) of *Butea monosperma (BM).* B. BM-A003 treated, 44 wks old, Transgenic animals are showing the significant reduction (p<0.001) in their tumor weight.



Histological analysis of the liver of X15-*myc* transgenic (Tg) mice at 12 wks and 20 wks of age after treatment with the extract and fractions of *Butea monosperma*.

(A&B), Tg mice without any treatment. Tg mice treated with aqueous extract A003 **(C&D),** aqueous fraction F009 **(E&F)** and butanolic fraction F008 **(G&H); (I&J),** Non-transgenic control at 12 wks and 20 wks. **(All x200)**



Immunohistochemical staining of the liver, x15- myc transgenic (Tg) mice after treatment with the extract A003 of *Butea monosperma* for Rps27a at 12 wks and 20 wks of age. A,B,C and D, Tg mice without any treatment. Tg mice treated with aqueous extract A003 (E,F,G and H), Non-transgenic control at 12 wk (I) (A,B,E & F = x200 and C,D,G & H = x400)

12- wks

Parameters	Treatment Groups				
	Normal Control	Tg Control	A003	F008	F009
Area	26.9±11.8[a]	45.54±22.0	22.7±10.6[a]	42.46±26.4[c]	42.36±23.6[c]
Diameter(mean)	5.53±1.20[a]	7.26±1.81	5.00±1.30[a]	6.84±2.10[a]	6.90±1.93[a]
Perimeter	18.05±3.72[a]	24.18±6.89	16.40±4.10[a]	22.26±6.75[a]	22.50±6.50[a]
Size (length)	6.20±1.45[a]	8.60±2.45	5.60±1.40[a]	7.60±2.34[a]	7.65±2.20[a]
Size (width)	5.24±1.00[a]	6.50±1.64	4.80±1.25[a]	6.53±1.98	6.50±1.85
Feret (mean)	5.80±1.18[a]	7.62±2.00	5.22±1.30[a]	7.09±2.13[a]	7.14±2.00[a]
Density(mean)	124.7±10.8[a]	119.43±11	132.22±9.8[a]	108.7±12.3[a]	126.43±15[a]
Density (std.dev)	13.83±2.63[a]	16.03±4.43	17.00±4.20	18.40±6.62[a]	20.45±7.16[a]
Heterogeneity	0.064±0.05[a]	0.109±0.1	0.149±0.10[a]	0.154±0.12[a]	0.209±0.14[a]

Values are shown as mean± SE (n=500).**'a' = p<0.001; 'b' = p<0.01; 'c' = p<0.05.**

Mean values and standard deviation for the parameters of area, diameter (mean), perimeter, size (length & width), feret (mean) Density (mean), density (std.dev) and heterogeneity (in millimeter) of the liver cell nuclei of 12 wks old, X15- myc transgenic mice treated with *Butea monosperma* (A003) and its fractions (F008 and FOO9) with their relevant Tg control.

20- wks

Parameters	Treatment Groups				
	Normal Control	Tg- Control	A003	F008	F009
Area	26.85±11.8[a]	47.96±24.6	23.95±15.0[a]	26.85±14.5[a]	41.64±24.6[a]
Diameter(mean)	5.526±1.20[a]	7.370±1.90	5.071±1.60[a]	5.439±1.52[a]	6.760±2.10[a]
Perimeter	18.04±3.72[a]	24.20±6.24	16.56±5.17[a]	17.84±4.94[a]	22.14±6.91[a]
Size (length)	6.194±1.45[a]	8.433±2.32	5.669±1.75[a]	6.230±1.75[a]	7.567±2.40[a]
Size (width)	5.237±1.00[a]	6.879±1.70	4.844±1.60[a]	5.088±1.46[a]	6.395±2.00[a]
Feret (mean)	5.763±1.18[a]	7.684±1.95	5.286±1.64[a]	5.691±1.56[a]	7.039±2.15[a]
Density(mean)	124.7±10.8[a]	113.52±13	122.4±10.2[a]	105.6±17.2[a]	121.7±11.1[a]
Density (std.dev)	13.83±2.63	14.25±4.35	17.86±5.03[a]	21.83±8.54[a]	16.44±4.26[a]
Heterogeneity	0.064±0.05	0.073±0.07	0.160±0.12[a]	0.198±0.16[a]	0.126±0.10[a]

Values are shown as mean± SE (n=500).**'a' = p<0.001; 'b' = p<0.01; 'c' = p<0.05.**

Mean values and standard deviation for the parameters of area, diameter (mean), perimeter, size (length & width), feret (mean) Density (mean), density (std.dev) and heterogeneity (in millimeter) of the liver cell nuclei of 20wks old, X15- myc transgenic mice treated with *Butea monosperma* (A003) and its fractions (F008 and FOO9) with their relevant Tg control.

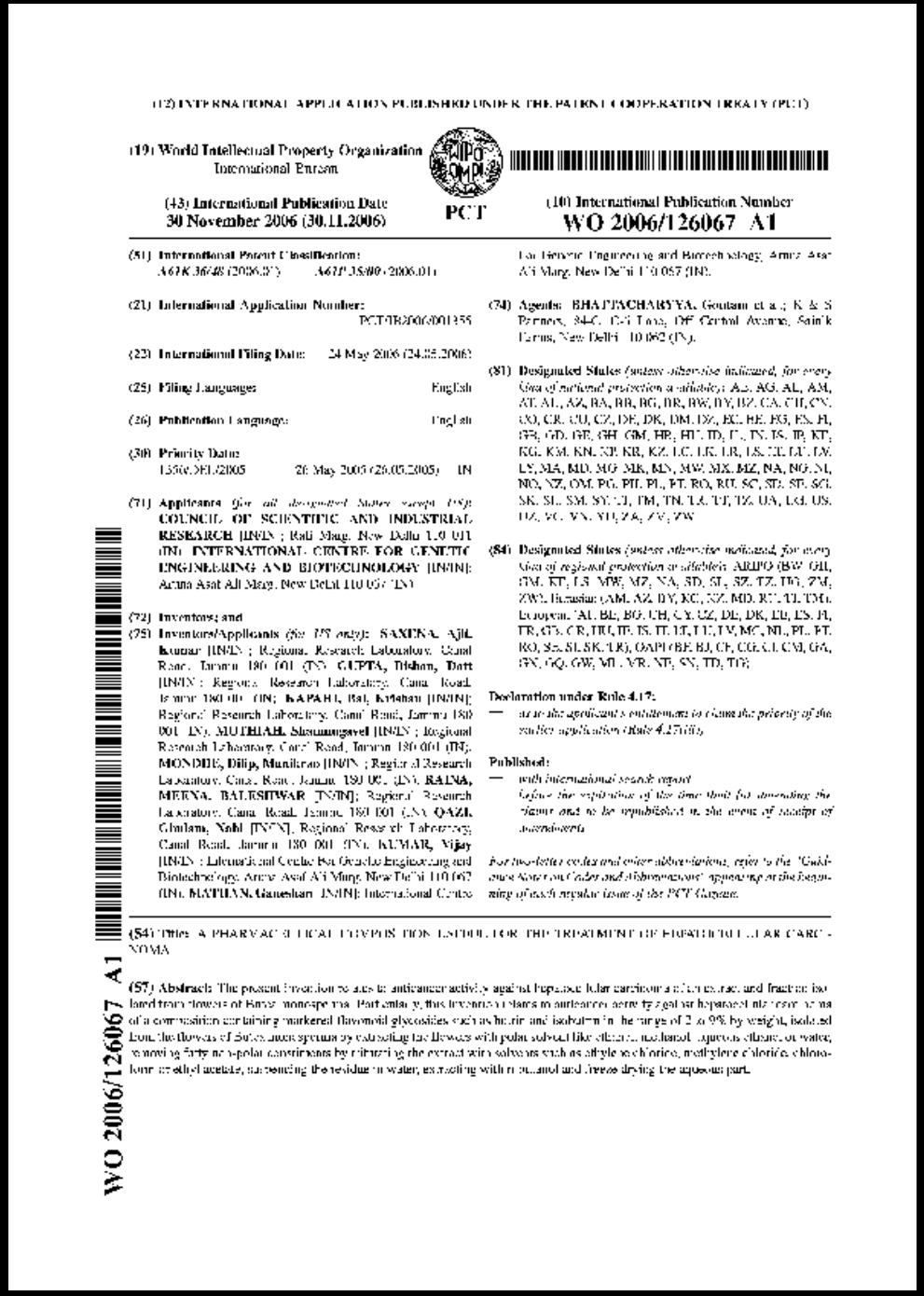
SUMMARY

- *in vitro* cytotoxicity and *in vivo* transgenic animal studies suggested that aqueous extract (A003) and fractions (F008 and F009) of *Butea monosperma* flowers is not only hepatoprotective but also carries anti-proliferative, anti-tumorogenic and anti-angiogenic properties.

- The chemopreventive action of fractions, F008 and F009 was less prominent as compared to the aqueous extract A003 suggesting either loss or inactivation of some of the key constituents in these fractions.

- Immunohistochemical analysis of Rps27a in the transgenic animals treated with aqueous extract A003 revealed a marked reversal of pathological manifestation including no staining for Rps27a in the liver.

Overall, the aqueous extract of *Butea monosperma* flower has the potential for developing new cancer therapeutics



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 November 2006 (30.11.2006)

PCT

(10) International Publication Number
WO 2006/126067 A1

(51) International Patent Classification:
A61K 36/48 (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number:
PCT/IB2006/001355

(22) International Filing Date: 24 May 2006 (24.05.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
1350/DEL/2005 26 May 2005 (26.05.2005) IN

(71) Applicants *(for all designated States except US)*: COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH [IN/IN]; Rafi Marg, New Delhi 1,0 001 (IN). INTERNATIONAL CENTRE FOR GENETIC ENGINEERING AND BIOTECHNOLOGY [IN/IN]; Aruna Asaf Ali Marg, New Delhi 110 067 (IN).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: SAXENA, Ajit Kumar [IN/IN]; Regional Research Laboratory, Canal Road, Jammu 180 001 (IN). GUPTA, Tilhan, Datt [IN/IN]; Regional Research Laboratory, Canal Road, Jammu 180 001 (IN). KAPAHI, Bal, Krishan [IN/IN]; Regional Research Laboratory, Canal Road, Jammu 180 001 (IN). MUTHIAH, Shanmugavel [IN/IN]; Regional Research Laboratory, Canal Road, Jammu 180 001 (IN). MONDHE, Dilip, Manikrao [IN/IN]; Regional Research Laboratory, Canal Road, Jammu 180 001 (IN). RAINA, MEENA, BALESHWAR [IN/IN]; Regional Research Laboratory, Canal Road, Jammu 180 001 (IN). QAZI, Ghulam, Nabi [IN/IN]; Regional Research Laboratory, Canal Road, Jammu 180 001 (IN). KUMAR, Vijay [IN/IN]; International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi 110 067 (IN). MATHAN, Ganeshan [IN/IN]; International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi 110 067 (IN).

(74) Agents: BHATTACHARYYA, Goutam et al.; K & S Partners, 84-C, Okhla Indl. Estate, Off Central Avenue, Sainik Farms, New Delhi 110 062 (IN).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:
— *with international search report*
before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: A PHARMACEUTICAL FORMULATION USEFUL FOR THE TREATMENT OF HUMAN SOLAR CARCINOMA

(57) Abstract: The present invention relates to anticancer activity against human solar carcinoma cells (in vitro) and fraction isolated from flowers of *Butea monosperma*. Particularly, this invention relates to anticancer activity against human solar carcinoma cells of a composition containing marketed flavonoid glycosides, such as butrin and isobutrin in the range of 2 to 9% by weight, isolated from the flowers of *Butea monosperma* by extracting the flowers with polar solvent like ethanol, methanol, aqueous ethanol or water, removing fatty non-polar constituents by extracting the extract with solvents such as ethyl acetate, methylene chloride, chloroform or ethyl acetate, suspending the residue in water, extracting with n-butanol and freeze drying the aqueous part.

WO 2006/126067 A1

PATENTS

1. Saxena, A. K., Gupta, B. D., Kapahi, B. K., Shanmugavel, M., Mondhe, D. M., Qazi, G. N., **Mathan, G.,** and Kumar, V. Anticancer activity of an extract from *Butea monosperma* and the fraction thereof. Patent application #20060280817; Class: 424757000 (USPTO), 2006.

ACKNOWLEDGEMENT

SCHOOL OF LIFE SCIENCES,
BHARATHIDASAN UNIVERSITY

ICGEB, NEW DELHI

CSIR, NEW DELHI

PROF. CHELLAM BALASUNDARAM
Prof. VIJAY KUMAR Ph.D, FNAS


THANK YOU