KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)
(Established Under Section 3 of UGC Act,1956)
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In vivo Toxicity Report

Study I: *In vivo* acute oral toxicity study (OECD 423)
Animals: Wistar albino rats (both male and female)
Dose: Single dose
Sample: HERBOLIV+
Observation: Mortality/ Behaviour changes
Procedure:

RESULT:

No behavior change or mortality observed during the study. The test sample is safe at 100% concentration.

Study II: *In vivo* sub-acute oral toxicity (OECD 407 – Repeated dose 28-day oral toxicity study)

Animals: Wistar albino rats (both male and female)

Dose: Repeated dose for 28 days

Observation: Biochemical parameters, Haematological parameters and histopathological changes.

Procedure: Animals were treated with HERBOLIV+ (100%, 75% and 50% dilutions with water) for 28 days. Serum was collected after 28 days treatment for biochemical and haematological study. Animals were euthanized and major organs were collected for histopathological examinations.

Grouping		**Number of Wistar rats**
Group I	Control	6
Group II	HERBOLIV+ (100%)	6
Group III	HERBOLIV+ (75%)	6
Group IV	HERBOLIV+ (50%)	6
	TOTAL	**24**

Results:

i) No biochemical changes observed (Annexure I)

ii) No haematological changes observed (Annexure II)

iii) No histopathological abnormalities observed (Annexure III)

All experiments on animals were conducted in accordance with the CPCSEA guidelines and after getting approval from IAEC (approval number: **KAHE/IAEC/2019/15-06/007**.

21/06/19

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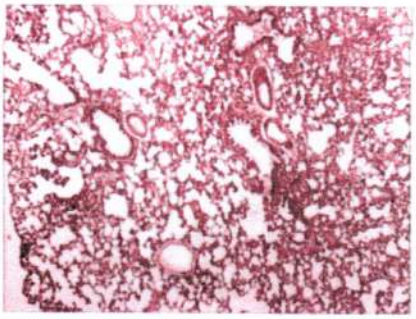
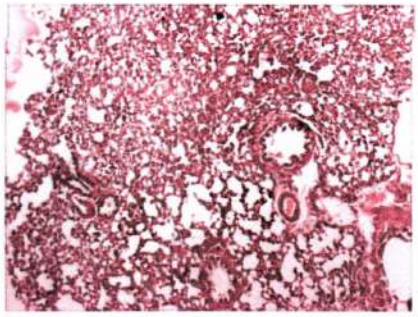
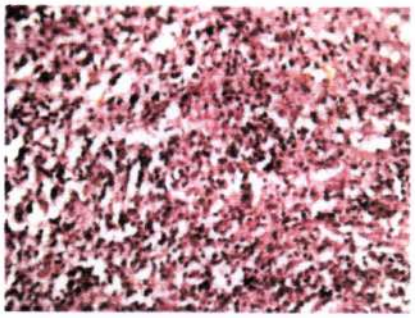
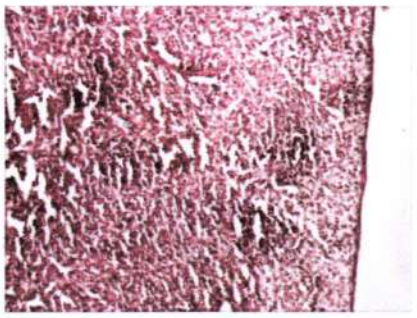
Lung





Spleen





Interpretation:

Heart	Group I (Control) Heart shows normal cardiac muscular bundles. Normal architecture. Group II (100% HERBOLIV+): Heart shows normal cardiac muscular bundles. Normal architecture.
Kidney	Group I (Control): Shows thin glomeruli with normal vascularity. Many collecting tubules look normal. Group II (100% HERBOLIV+): Shows glomeruli which are highly hypercellular, the tubules and inter-tissues looks normal.
Liver	Group I (Control): Normal architecture, showing central vein, the hepatocytes are arranged in regular manner. The portal triad looks normal. Group II (100% HERBOLIV+): Normal architecture, showing central vein and hepatocytes are arranged in regular manner.
Lung	Group I (Control): Lung with normal architecture. Group II (100% HERBOLIV+): Normal architecture with few of the acinus dilated.
Spleen	Group I (Control): Normal architecture, mild congestion. Group II (100% HERBOLIV+): Shows normal architecture with mild congestion.

The histopathological slides show that the treatment sample (HERBOLIV+) is safe up to 100% concentration.

11/06/19

Authorized signatory

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Annexure III

Micrographs of different tissues of control and treated animals: Histopathology Study

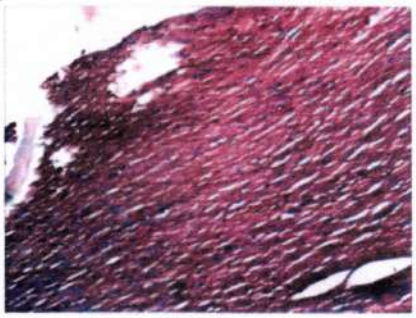
Sample: HERBOLIV+

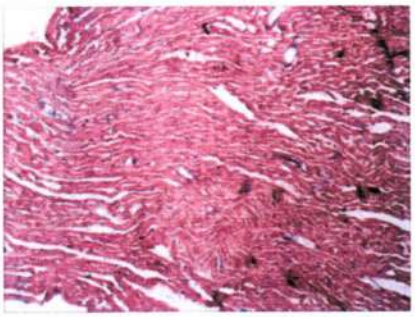
Study: Sub-acute oral toxicity study (according to OECD 407)

Group I: Control

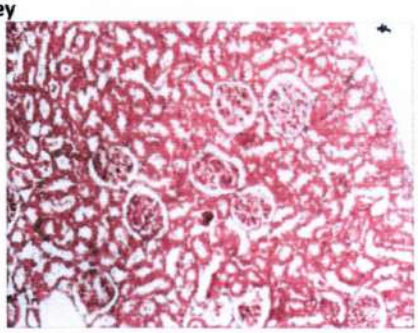
Group II: Treatment with HERBOLIV+ (100%)

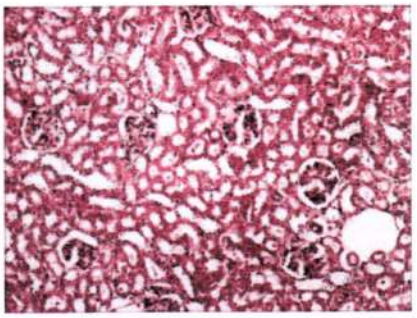
Heart



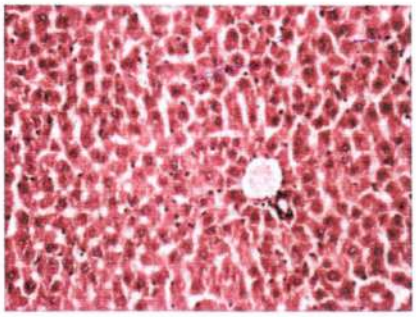


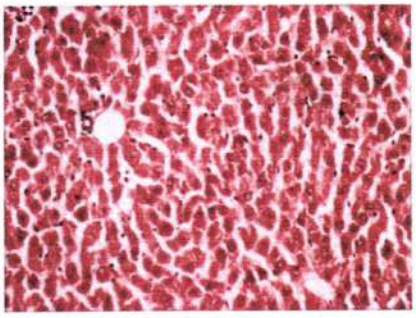
Kidney





Liver





Annexure I

Biochemical parameters in serum

Parameters	Groups			
	Group 1 (Control)	Group 2 (100%)	Group 3 (75%)	Group 4 (50%)
Glucose (mg/dL)	95.42 ± 5.27	89.1 ± 5.87	93.5 ± 4.49	88.77 ± 5.67
Cholesterol (mg/dL)	77.07 ± 7.65	72.98 ± 6.64	75.94 ± 10.48	74.14 ± 8.3
Triglycerides (mg/dL)	129.01 ± 14.42	120.44 ± 24.72	110.42 ± 18.2	133.18 ± 22
Uric acid (mg/dL)	1.26 ± 0.11	1.38 ± 0.14	1.53 ± 0.25	1.34 ± 0.2
Creatinine (mg/dL)	0.31 ± 0.07	0.27 ± 0.06	0.29 ± 0.03	0.3 ± 0.07
Bilirubin (mg/dL)	0.15 ± 0.01	0.16 ± 0.02	0.14 ± 0.01	0.13 ± 0.02

No significant difference observed between the control and treated groups.

21/06/19

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Annexure II

Haematological parameters

Parameters	Groups			
	Group 1 (Control)	Group 2 (100%)	Group 3 (75%)	Group 4 (50%)
RBC ($x10^6$ cells/mm^3)	6.79 ± 0.27	6.72 ± 0.17	7.57 ± 0.38	7.04 ± 0.11
WBC ($x10^3$ cells/mm^3	3.4 ± 0.34	3.11 ± 0.2	4.06 ± 0.52	3.46 ± 0.25
Haemoglobin (g/dl)	13.35 ± 0.2	14.13 ± 0.25	13.45 ± 0.24	13.61 ± 0.49
Hematocrit (%)	41.18 ± 1.57	40.51 ± 1.37	41.16 ± 1.39	39.85 ± 2.25
MCV (μm^3/red cell)	57.76 ± 2.41	58.54 ± 3.31	57.86 ± 2.36	57.98 ± 2.41
MCH (pg/red cell)	19.16 ± 0.54	21.17 ± 0.69	18.97 ± 0.55	21.01 ± 0.33
MCHC (g/dl RBC)	32.72 ± 1.44	35.02 ± 1.59	32.71 ± 1.47	35.86 ± 1.62
Platelets ($x10^3$ cells/mm^3	652 ± 39.59	783.3 ± 35.39	787.7 ± 31.56	697.3 ± 35.3

No significant difference observed in the haematological parameters between control and treated groups.

21/06/19

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Phytochemical analysis report

Qualitative estimation of Biochemical constituents

Extracts	AL	SA	TP	FL	ST	CG	OF	TN	AP	CHO
Ethanol	++	+	+	++	++	++	+	+	+	+

AL – Alkaloids
CH – Carbohydrates
ST – Steroids
CG – Cardioglycosides
FL – Flavonoids
SA – Saponins
TP – Tannin & Phenolic compounds
OF – Oils & Fats
AP – Amino acids & Proteins
TN – Terpenoids

'+' Present '_' Absent

The result shows the presence of all compounds such as alkaloids, flavonoids, steroids and cardioglycosides.

Quantitative estimation of Biochemical constituents

Parameters	Amount present
Total flavonoid (mg/g of Quercetin equivalent)	98.5±14.57
Total phenol (mg/g of Gallic acid equivalent)	115.8±18.28
Total tannin (mg/g of Tannic acid equivalent)	88.1±12.28

2/10/19
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