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RESEARCH PAPER

In vitro anticancer and hepatoprotective activity studies of *Garcinia xanthochymus*

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ABSTRACT

Natural products are very well known to exhibit anticancer activities. The present study aimed at assessing the potency of various extracts of *Garcinia xanthochymus* against cancer using *in vitro* cell lines. MCF 7, HepG2, HeLa, PC3, A549 and Vero cell lines were employed for the assessment. The ability of extracts to exert toxic insult on cancer cells has been the basis of anticancer activity. GxF was fond to be very potentially toxic (80 mg/ml) to HEP G2 cell lines among all the tested extracts. Also, it was found to be the most toxic compared to GxA and GxF whose average CTC_{50} was found to be 180 and 118 mg/ml, respectively. Among all the tested extracts, GxF was found to be potentially toxic to the MCF 7 cell lines whose CTC_{50} was found to be73 mg/ml. GxA possesses the CTC_{50} of 810 mg/ml. GxR was found to be toxic with average CTC_{50} of303 mg/ml. This was followed by GxF and GxA with average CTC_{50} values of 303 and 456 mg/ml. *In vitro* hepatoprotective activity of the plant extracts was studied by employing primary rat hepatocytes. The drug silymarin was found to exhibit 85.28 per cent protection against paracetamol induced toxicity in primary rat hepatocytes at the tested concentration of 250 mg/ml. It was found that GxF and GxR were found to have comparatively similar protective power like silymarin. These extract exhibited 83.63 per cent and 79.58 per cent protection against paracetamol induced toxicity in primary rat hepatocytes at the concentration of 200 mg/ml, respectively. GxA did not exhibit considerable activity with 55.61 per cent protection.

Key Words : *Garcinia xanthochymus, In vitro*, Anticancer, Hepatoprotective, Cell lines

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Recently there has been a surge in the demand for natural and organic products for use in human diet due to deleterious effects of synthetic food additives and increased understanding of consumer about this problem. Considerably large numbers of studies have evidenced the greater health beneficial effects of various plant species containing phytochemicals that effectively scavenge free radicals (Ambasta, 1986; Baggett *et al.*, 2005 and Baslas and Kumar, 1981). Oxidative stress is a negative effect caused by the free radicals in the organism, and this negative effect can cause various diseases such as arteriosclerosis, diabetes, tumor, heart disease and aging (Bawa and Khanum, 2003; Benneth and Lee, 1989). However, healthy humans can detoxify or eliminate these free radicals by enzymes such as superoxide dismutase, catalase, and peroxidase, and also by food-derived antioxidants (Canter *et al.,* 2005). There is an increasing interest in natural sources of antioxidant molecules for use in the food, because the synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxytoluene (BHT) accumulate in the body and result in

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liver damage and carcinogenesis (Chandalia *et al.,* 2000) *Garcinia xanthochymus*, a perennial medicinal plant native to the south and south-west of Yunnan of China belongs to family Cluceaceae, can grow up to 10-20 m. *Garcinia xanthochymus* trees have dark green leaves and a gummy yellow sap and bear yellow fruits 6-7 cm in diameter with juicy, acidic, yellow pulp containing two seeds. The acidic fruits are used in jams, preserves, and vinegar (Chanmahasathien *et al.,* 2003a) Gamboge is used in watercolors and as a yellow fabric dye (Chanmahasathien *et al.,* 2003b) Gamboge fruits are used in traditional medicine for treating diarrhea and dysentery (Chen *et al.,* 2010). The fruit of *Garcinia xanthochymus* has been used widely as a traditional folk medicine for diarrhea, and dysentery. Previous phytochemical investigations on *Garcinia xanthochymus* resulted in the isolation of benzophenones (Das *et al.,* 2007), flavonoids (Duthie and Brown, 1994), triterpenes (Facciola, 1998) and xanthones (Faisal *et al.,* 1982 and Favero *et al.,* 1993) from the wood, fresh leaves and fruits. Studies (Gustafson *et al.,* 1992 and Gutierrez *et al.,* 2008) evaluated the DPPH radical scavenging activities of compounds from the bark of *Garcinia xanthochymus*, which supports the notion that the plant genus Garcinia is a good source of bioactive compounds. However, the use of the bark has its own limitations. Thus, it is necessary to conduct further bioactive research of other plant parts from *Garcinia xanthochymus*.

RESEARCH METHODOLOGY

Chemicals :

3-(4, 5–dimethyl thiazol–2–yl)–5–diphenyltetrazolium bromide (MTT), Trypan blue, Fetal bovine serum (FBS), Phosphate buffered saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co., St Louis, USA. EDTA, glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl sulfoxide (DMSO) and Propanol from E. Merck Ltd., Mumbai, India.

Cell lines and culture medium :

MCF-7, HepG2, HeLa, PC3, A549 and Vero cell lines cultured in DMEM supplemented with 10 per cent inactivated Fetal bovine serum (FBS), penicillin (100 IU/ml), streptomycin (100 mg/ml) and amphotericin B (5 mg/ml) in an humidified atmosphere of 5 per cent CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of test solutions :

For cytotoxicity studies, each weighed test extracts were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2 per cent inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT assay :

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used (Francis and Rita, 1986).

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, wash the monolayer once with medium and 100 ml of different test concentrations of extracts were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 3 days in 5 per cent CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 ml of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5 per cent CO_2 atmosphere. The supernatant was removed and 100 ml of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50 per cent (CTC_{50}) values is generated from the dose-response curves for each cell line.

$$\text{\% growth inhibiton} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

Cell lines and culture medium :

HepG2 cell culture was procured from National centre for cell sciences (NCCS), Pune, India. Stock cells of HEPG2 were cultured in DMEM supplemented with 10 per cent inactivated Fetal bovine serum (FBS), Penicillin (100 IU/ml), streptomycin (100 mg/ml) and amphotericin B (5 mg/

ml) in an humidified atmosphere of 5 per cent CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of test solutions :

For hepatoprotective studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2 per cent inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination hepatoprotective activity in HepG2 cell line :

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using DMEM medium containing 10 per cent FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium. 50 ml of DMEM with 2×100mm paracetamol and 2×50 ml of different non-toxic test concentrations of test drugs were added. The plates were then incubated at 37°C for 24 h in 5 per cent CO_2 atmosphere. After 24 h, the cell supernatants were discarded and 50 ml of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5 per cent CO_2 atmosphere. The supernatant was removed and 100 ml of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage cell viability was determined, based on which the percentage protection offered by test and standard drugs was calculated over the paracetamol control.

Isolation of rat hepatocytes :

The primary rat heptocyte culture was prepared as described earlier (Liu *et al.*, 2001). Briefly, abdomen of an adult rat, weighing 200-220 g will be opened with a midline incision. Portal cannula will be placed and the liver will be perfused with HEPES solution fallowed by collagenase type-iv solution. The liver capsule will be disrupted and the resulting cell suspension will be filtered and washed, cell viability will be measured by trypan blue exclusion technique. The cells will be suspended in Ham's F_{12} medium with 20 per cent FBS and incubated at 37^0C for further use. The handling and treatment of animals was as per Control and Supervision of Experiments on animals (CPCSEA), Ministry of environment and forests, Govt. of India rules.

Hepatoprotective study in isolated rat hepatocytes :

Isolated primary rat hepatocytes were suspended in fresh Ham's F_{12} medium containing 20 per cent FBS and incubated at 37^0C for 16h. Medium supernatant was removed carefully and primary hepatocytes were exposed to fresh medium containing toxicant along with / without test product at different doses, along with negative and standard controls. After 24h incubation period, cell supernatant will be aspirated and cell viability was determined by MTT assay.

RESULTS AND REMONSTRATION

The results obtained from the present investigation as well as relevant discussion have been summarised under following heads:

Anticancer activity studies :

In vitro anticancer activity of the extracts was performed by assessing cytotoxicity studies in 6 cell lines among which 5 were cancerous cell lines and one was normal cell line. The cell lines used were MCF-7, HepG2, HeLa, PC3, A549 and Vero cell lines.

GxF was fond to be very potentially toxic (80 mg/ml) to HEP G2 cell lines among all the tested extracts. Also, it was found to be the most toxic compared to GxA and GxF whose average CTC_{50} was found to be 180 and 118 mg/ml, respectively (Table 1 and Fig. 1).

Among all the tested extracts, GxF was found to be potentially toxic to the MCF 7 cell lines whose CTC_{50} was found to be73 mg/ml (Table 2 and Fig. 2).

GxR was found to be toxic with average CTC_{50} of303 mg/ml. This was followed by GxF and GxA with average CTC_{50} values of 303 and 456 mg/ml (Table 3 and Fig. 3).

Hepatoprotective Activities :

The drug Silymarin was found to exhibit 96% protection aginstParacetamol induced toxicity in Hep G2 cells at the tested concentration of 250 mg/ml. It was found that GxF

Table 1 : Cytotoxicity of *Garcinia xanthochymus* fruit, aerial parts and root extracts against HEP G2 cell line by MTT assay

Sr. No.	Test sample	CTC_{50} (µg/ml) ± S.D.
1.	GxF	80.00±10.00
2.	GxA	180.00±10.00
3.	GxR	118.33±7.64

Values are mean ± standard error of three replicates

Table 2 : Cytotoxicity of *Garcinia xanthochymus* fruit, aerial parts and root extracts against MCF 7 cell line by MTT assay

Sr. No.	Test sample	CTC_{50} (µg/ml) ± S.D.
1.	GxF	73.33±15.28
2.	GxA	810.00±10.00
3.	GxR	>1000

Values are mean ± standard error of three replicates

Table 3 : Cytotoxicity of *Garcinia xanthochymus* fruit, aerial parts and root extracts against HeLa cell line by MTT assay

Sr. No.	Test sample	AVG CTC_{50} (µg/ml) ± S.D.
1.	GxF	326.67±25.17
2.	GxA	456.67±15.28
3.	GxR	303.33±15.28

Values are mean ± standard error of three replicates

Table 4 : Cytotoxicity of *Garcinia xanthochymus* fruit, aerial parts and root extracts against PC 3 cell line by MTT assay

Sr. No.	Test sample	AVG CTC_{50} (µg/ml) ± S.D.
1.	GxF	623.33±5.77
2.	GxA	536.67±5.77
3.	GxR	596.67±5.77

Values are mean ± standard error of three replicates

Table 5 : Cytotoxicity of *Garcinia xanthochymus* fruit, aerial parts and root extracts against A 549 cell line by MTT assay

Sr. No.	Test sample	AVG CTC_{50} (µg/ml) ± S.D.
1.	GxF	170.00±0.25
2.	GxA	500.00±1.00
3.	GxR	610.00±2.5

Values are mean ± standard error of three replicates

Table 6 : Cytotoxicity of *Garcinia xanthochymus* fruit, aerial parts and root extracts against Vero cell line by MTT assay

Sr. No.	Test sample	AVG CTC_{50} (µg/ml) ± S.D.
1.	GxF	93.33±5.77
2.	GxA	813.00±20.00
3.	GxR	341.67±2.89

Values are mean ± standard error of three replicates

Table 7 : Hepatoprotective activities of *Garcinia xanthochymus* fruit, aerial parts and root extracts in HepG2 cell line

Sr. No.	Plant parts	Test concn. in µg/ml	% Protection offered over control
1.	GxF	200	99.5±0.07
2.	GxA	200	58.4±0.09
3.	GxR	200	97.0±0.12
4.	Silymarin	250	96.2±0.02
5.	Cell control	-	100.0
6.	Paracetamol (10mm)	-	0

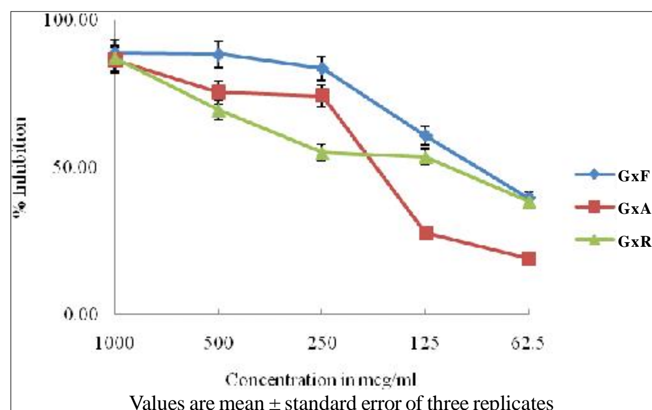
Values are mean ± standard error of three replicates

Table 8 : Hepatoprotective activities of *Garcinia xanthochymus* fruit, aerial parts and root extracts in primary rat hepatocytes

Sr. No.	Test drug	Test concn. in µg/ml	% Protection offered over control
1.	GxF	200	83.63±0.04
2.	GxA	200	55.61±0.05
3.	GxR	200	79.58±0.09
4.	Silymarin	250	85.28±0.03
5.	Cell control	-	100
6.	Paracetamol (10mm)	-	0

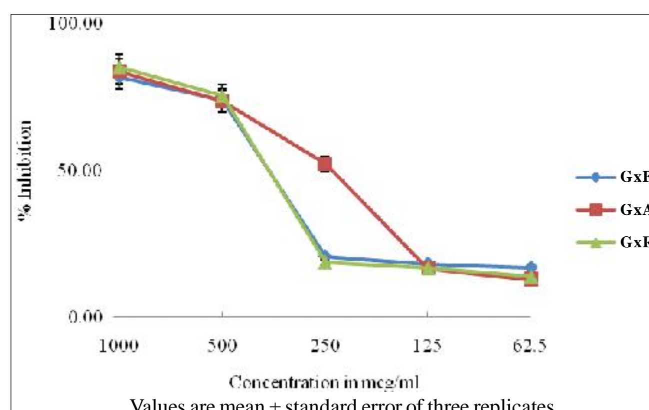
Values are mean ± standard error of three replicates

and GxR were found to have comparatively higher protective power than Silymarin. These extract exhibited 99 and 97% protection against Paracetamol induced toxicity in Hep G2 Cells at the concentration of 200 mg/ml. However, other extracts did not exhibit superior activity at the tested concentration of 200 mg/ml.



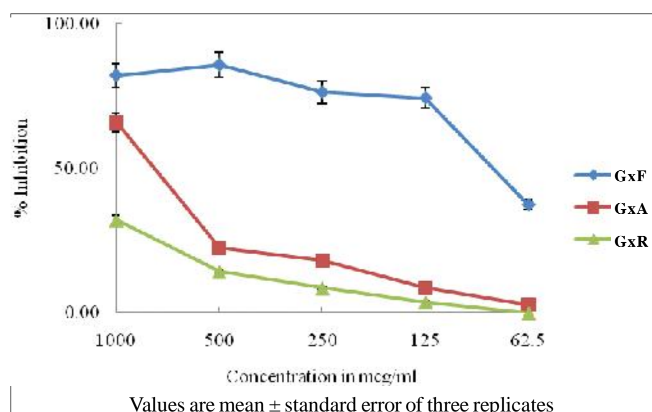
Values are mean ± standard error of three replicates

Fig. 1 : Percent inhibition of HEP G2 cell line by *Gracinia xanthochymus* fruit, aerial parts and root extracts



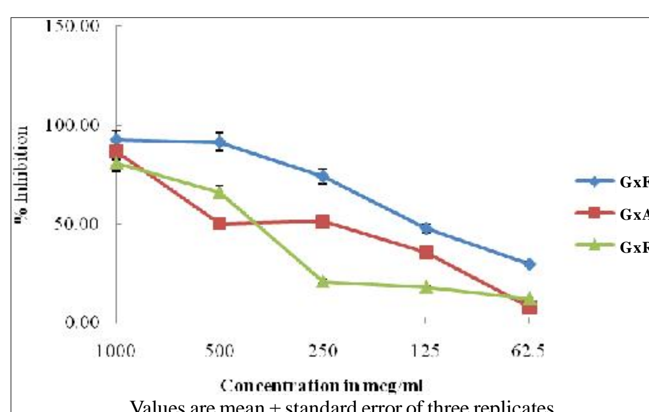
Values are mean ± standard error of three replicates

Fig. 4 : Per cent inhibition of PC 3 cell line by *Gracinia xanthochymus* fruit, aerial parts and root extracts



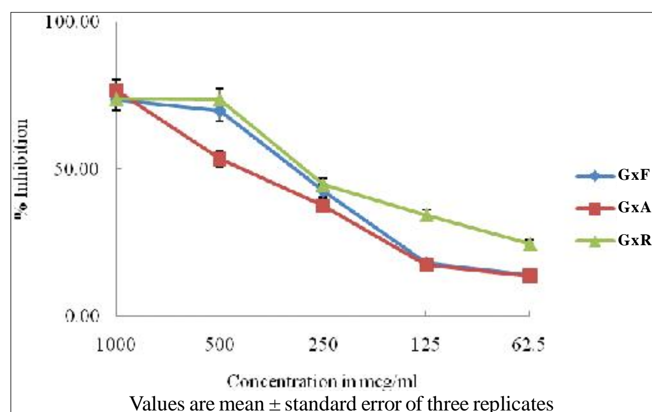
Values are mean ± standard error of three replicates

Fig. 2 : Per cent inhibition of MCF 7 cell line by *Gracinia xanthochymus* fruit, aerial parts and root extracts



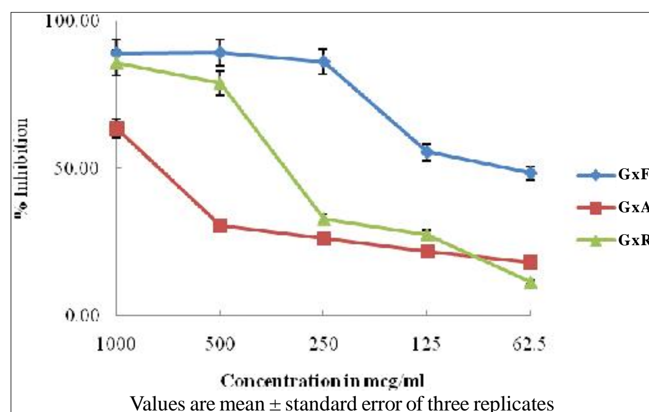
Values are mean ± standard error of three replicates

Fig. 5 : Percent inhibition of A 549 cell line by *Gracinia xanthochymus* fruit, aerial parts and root extracts



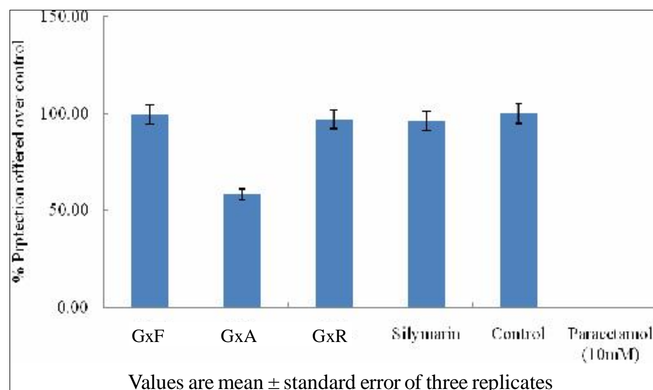
Values are mean ± standard error of three replicates

Fig. 3 : Percent inhibition of HeLa cell line by *Gracinia xanthochymus* fruit, aerial parts and root extracts



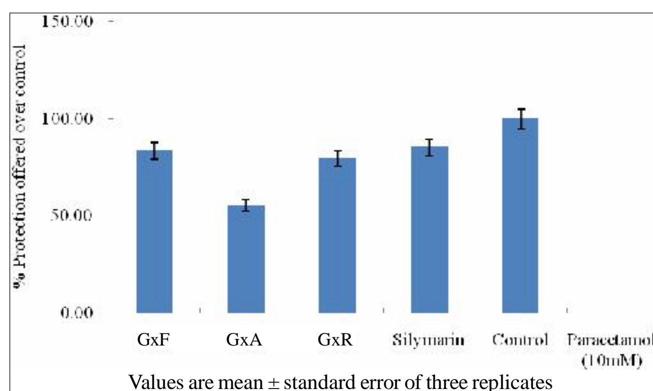
Values are mean ± standard error of three replicates

Fig. 6 : Per cent inhibition of Vero cell line by *Gracinia xanthochymus* fruit, aerial parts and root extracts



Values are mean ± standard error of three replicates

Fig. 7 : Percentage protection offered by *Garcinia xanthochymus* Fruit, Aerial parts and Roots (200 µg/ml) and standard drug Silymarin (250 µg/ml) in HepG2 cell line



Values are mean ± standard error of three replicates

Fig. 8 : Percentage protection offered by *Garcinia xanthochymus* fruit, aerial part and root extracts (200 µg/ml) and standard drug Silymarin (250 µg/ml) in primary hepatocytes

The drug Silymarin was found to exhibit 85.28 per cent protection against paracetamol induced toxicity in Primary rat hepatocytes at the tested concentration of 250 mg/ml. It was found that GxF and GxR were found to have comparatively similar protective power like Silymarin. These extract exhibited 83.63 per cent and 79.58 per cent protection against paracetamol induced toxicity in Primary rat hepatocytes at the concentration of 200 mg/ml, respectively. GxA did not exhibit the much activity with 55.61 per cent protection (Table 8 and Fig. 7).

Conclusion :

GxF was fond to be very potentially toxic (80 mg/ml) to HEP G2 cell lines among all the tested extracts. Also, it was found to be the most toxic compared to GxA and GxF whose average CTC_{50} was found to be 180 and 118 mg/ml, respectively. Among all the tested extracts, GxF was found to be potentially toxic to the MCF 7 cell lines whose CTC_{50} was found to be 73 mg/ml. GxA possesses the CTC_{50} of 810 µg/ml. GxR was found to be toxic with average CTC_{50} of303 µg/ml. This was followed by GxF and GXA with average CTC_{50} values of 303 and 456 mg/ml. *In vitro* hepatoprotective activity of the plant extracts was studied by employing primary rat hepatocytes. It was found that GxF and GXR were found to have comparatively similar protective power like Silymarin. These extracts exhibited 83.63 per cent and 79.58 per cent protection against paracetamol induced toxicity in primary rat hepatocytes at the concentration of 200 mg/ml, respectively. GxA did not exhibit considerable activity with 55.61 per cent protection. Therefore, the results conclusively infer that the GxF may be considered for further studies.

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