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Cytotoxicity of Crude Amino Acids of Four Sudanese Cultivars of *Hibiscussabdariffa* Grown In Sudan

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Abstract

The effect of cytotoxicity of crude amino acids extract on performance of four cultivars of *Hibiscussabdariffa* (Roselle)-(namely: Rahad, Fashir, Kass and Abaid)-were studied by evaluating of anti-tumor activity. Amino acid analyzer, biology test for cytotoxic effect: In vitro measurement of potential cytotoxicity by SBR assay cells, cell-fixation, dying, SRB Assay, by using the human tumor cell lines to identify were used in this study.

It was found there was high toxicity of the crude amino acids extract of all cultivars (Rahad, Fashir, Kass and Abiad) Roselle in different concentration against HEPG2 (Liver carcinoma cell line) also the study showed that Rahad and Abiad cultivars had high toxicicity against the breast cancer cell line. Crude amino acids of Sudanese Roselle cultivars have anticancer activity at low concentration. This plant has gained an interest for study because of the high demand in the world market and is expected to replace chemical products in many industries and medicinal uses

Keywords: *Hibiscussabdariffa*, Roselle, Cytotoxicity, Amino acids, HEPG2 (Liver carcinoma cell line) and breast cancer cell line.

INTRODUTION

Hibiscus species belongs to the general order *Malvales*, family *Malvaceae tribe Hibisceae its* name is *Sabdariffa* and about 17 genera has been identified[1].
Hibiscussabdariffa Roselle is a perennial shrub that grows in tropical and subtropical regions [2, 3,4, 5] . In Sudan where it is considered as an annual shrub it is grown in rain fed areas in the western states in large scale and it is cultivated in small scale in irrigated areas. Almost any part of the plant can be utilized but it is cultivated mainly for its calyces which is valid as the main item of commerce. *Hibiscussabdariffa* a source of medicinal compounds and have components of high nutritive value such as protein, amino acids, carbohydrate, fats, minerals and organic acids [6]. Karkade is widely used as food to make jellies, jam and beverages (Nasir, 2004)[7]. Odigie*et al* (2003) [8], reported that aqueous extract of *Hibiscussabdariffa* attenuates hypertension and reverses cardiac hypertrophy. Hernández et al [9,] reported that *Hibiscus sabadariffa*extract used in the treatment of hypercholesterolemia.

Alcoholic extract of *Hibiscussabdariffa* leaves was studied for its anti-hyperammonemic and antioxidant effect in brain tissues, significantly normalized the levels of ammonia, urea, uric acid, creatinine and non-protein nitrogen in the blood. Also the extract significantly reduced brain levels of lipid peroxidation products such as thiobarbituric acid and reactive substances (TBARS) and hydro peroxide (HP), however, the administration extract significantly increased the levels of antioxidant such as catalase (CAT), superoxide dismutase (SOD) glutathione peroxidase and reduced glutathione (GSH) in brains tissues of hyperammonemic rats (Essa, 2007)[9]. Satue*et al*, (1997)[10], reported that anthocyanin and protocatechic acid (PCA) have antioxidant activity and to offer protection against atherosclerosis and cancer. Wang, *et al*, (1997)[11], reported that compare to common antioxidants such as ascorbate, anthocyanin's were found to be much more potent antioxidants, It is well documented that most medicinal plant are enriched with phenolic compound and bioflavonoids that represent potent antioxidants [12].

MATERIALS AND METHODS

The study was executed at the experimental farm of Medicinal and Aromatic Plants Research Institute at Shambat, Sudan (Latitude 15° 40′ N, Longitude 32 ° 32′ and 360 m above sea level) . The climate is semi-arid with low relative humidity and daily mean air temperature ranging from 25 to 40 °C in summer, and 15 to 21 °C in winter. Four cultivars of *Hibiscussabdariffa* namely Rahad, Fashir, Kass and Abiad were cultivated in the Demonstration Farms of Medicinal And Aromatic Plant Research Institute at Shambat (Sudan). The Plant samples were identified in the department of plant taxonomy in the same institute, collected, dried and kept in carton bags for extraction.

Determination of essential amino acids

The plant samples (5 gm of each) were macerated in 50% alcohol until all pigment was extracted and concentrated under reduced pressure at 40 °C. 10 ml NaCl (10%)

was added to the extract, stirred for one hour then 10 ml of trichloroacetic was added and the mixture was filtrated. The precipitate was deposited by centrifugation, washed and dried in desiccators. 20 mg of protein were refluxed with 6N HCl (10 ml) for 20 hours and the acid removed by evaporation under reduced pressure, the residue was dissolved in 10 % isopropanol for amino acids identification using the method of (Baily, 1967) [13], using (Eppendorf-Germany Lc 3000) Amino acid analyzer.

Bioassay of crude amino acids extracts

In the present study, crude amino acid extracts of four cultivars (Rahad , Fashir, Kass and Abiad) Roselle were chosen as representative examples for evaluation of their anti-tumor activity.

Test for cytotoxic effect: In vitro

A set of sterile test tubes were used, while 2.5x105 tumor cell per ml were suspended in phosphate buffer saline, then 1,2.5,5 ,10 µg/ml of the tested compound were added to the suspension. The mixture was kept at 37 º C for 2 hours. Try pan blue dye exclusion test was then carried out to calculate the percentage non- viable cells.

MEASUREMENT OF POTENTIAL CYTOTOXICITY BY SBR ASSAY

Potential cytotoxicity of the compounds was tested:

Cells.

Preliminary experiments were made using the human tumor cell lines to identify the cytotoxicity of selective synthesized chemical compounds.

Stock cultures were grown in T-75 flaks containing 50 ml of PR Mi-1640 medium with glutamine bicarbonate, and 5% fetal calf serum. Medium was changed at 48 hours intervals. Cells were dissociated with 0.25% trypan. Experimental cultures were plated in micro litter plates (Costar, Cambridge, MA), containing 0.2 ml of growth medium per well at densities of 1.000-200,000 cells per well.

Cell-Fixation:-

Cells attached to the plastic substratum were fixed by gently layering 50µl of cold 50% TCA (4^{o}C) on the top of the growth medium in each well to produce a final TCA concentration of 10%. The cultures were incubated at 4^{o}C for one hour and then washed five times with tap water to remove TCA, growth medium and low-molecular weight metabolites, and serum protein. Plates were air dried and then stored until use. Background optical densities were measured in wells incubated with growth medium without cells.

Dying:-

The anionic dye sulforhodamine B (SRB) was purchased from sigma chemical Co. dissolved in 1% acetic acid for cell staining and then extracted from cells with 10 m M [hydroxymethylaminomethane].

SRB Assay:-

TCA-fixed cell were stained for 30 minutes with 0.4% (wt/vol) SRB dissolved in 1% acetic acid. At the end of the staining period, SRB was removed and cultures were quickly rinsed four times with 1% acetic acid to remove unbounded dye. The acetic acid was directly into the culture. This procedure permitted to be performed quickly so that desertion of protein-bound dye did not occur. Residual solution was removed by sharply flicking plates over a sink, which ensured the complete removal of rinsing solution. Because of the strong capillary action in 96-well plates, draining by gravity alone often failed to remove the rinse solution when plates were simply inverted. After being rinsed, the cultures were air dried until no standing moisture was visible. Bound dye was solubilized with 10 mMunbufferedtris base. (pH 10.5) for 5 minutes on gyratory shaker

RESULT AND DISCUSSION

Effect of Cytotoxicity

Figure (1) showed that the cytotoxicity of crude amino acids of Rahad cultivar at concentration 0.54µg against HEPG2 (Liver carcinoma cell line).Figure (2) showed that the cytotoxicity of crude amino acids of Rahad cultivar at concentration 4.3 µg against the breast cancer cell line. Whereas Figure (3) showed that the cytotoxicity of crude amino acids of Abiad cultivar at concentration 1.34 µg and showed cytotoxic effect against HEPG2 (Liver carcinoma cell line) and eventually Figure 4 exhibited the cytotoxicity of crude amino acids of Abiad cultivar at concentration 4.7µg against breast cancer cell line. In Figure 5, crude amino acids of Fashir cultivar at concentration 10µg showed cytotoxic effect against HEPG2 (Liver carcinoma cell line). Whereas Figure (6) crude amino acids of Kass cultivar at concentration 8.66 µg showed cytotoxic effect against HEPG2 (Liver carcinoma cell line). Fig 1, 2, 3, 4, 5, and 6 indicate that the crude amino acids of Sudanese Roselle cultivars have anticancer activity at low concentrations , our results are in agreement with (Tseng et al., 1997) [14] and Farombi, 2003) [15], who reported that crude extracts of the dried flowers of *(Hibiscussabdariffa),* have strong antioxidant potential as they inhibited xanthene's oxidase activity, formation of Moline dialdhyde (MDA), moreover crude extract can inhibit the un scheduled DNA repair synthesis induced by ThioBarhyburic acid (T.BHP) in the rat hepatocyte from (T.BHP) induced cytotoxicity and genotoxicity by mechanism these results were in agreement with ours. The high toxicity of crude amino acid Rahad, Abiad, Fashir and Kass may be also due to the presence of protocatechic acid (PCA) compound in flowers since it was proved to be anticarcinogenic (Tanaka *et al*., 1994)[16]. But the mechanism by which PCA exerts its suppressing effect on chemical carcinogenesis is unknown. These cultivars need further study to explore this mechanism.

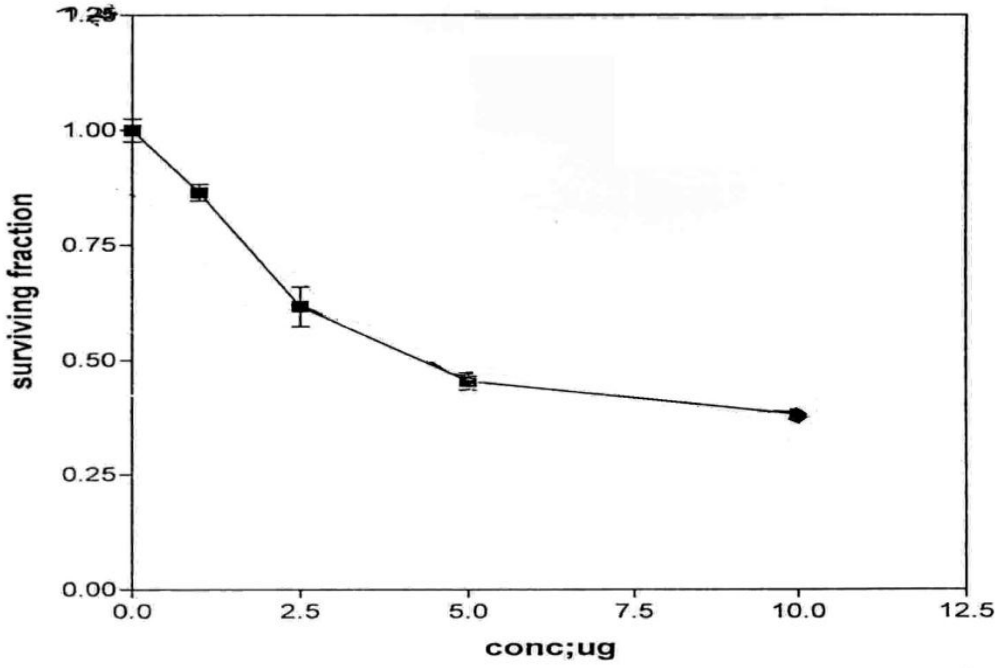

Figure 1: Cytotoxic activity of *Hibiscussabdariffa* (Rahad cultivar) on Liver carcinoma cell line (HEPG2)

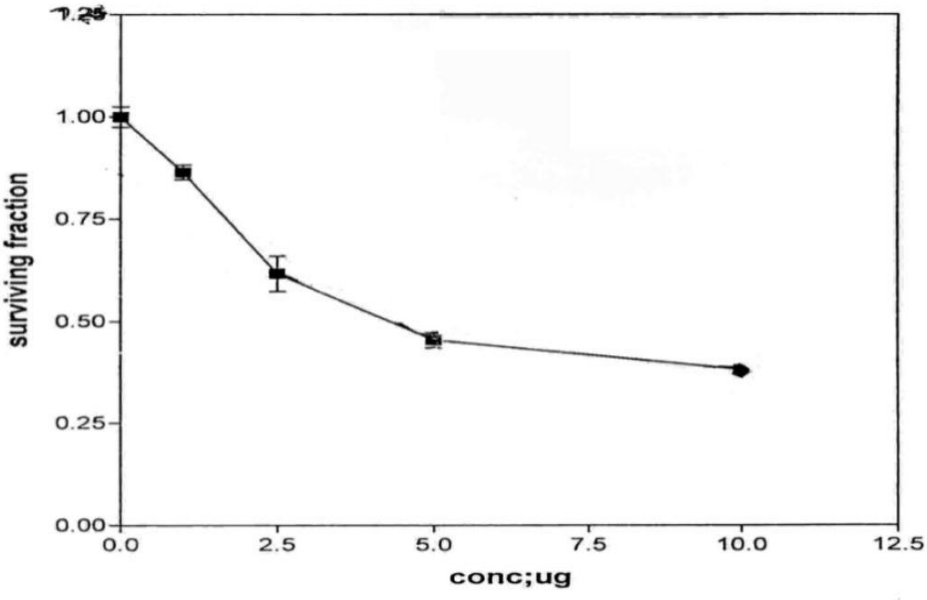

Figure 2: Cytotoxic activity of *Hibiscussabdariffa* (Rahad cultivar) on Breast carcinoma cell line (MCF7**)**

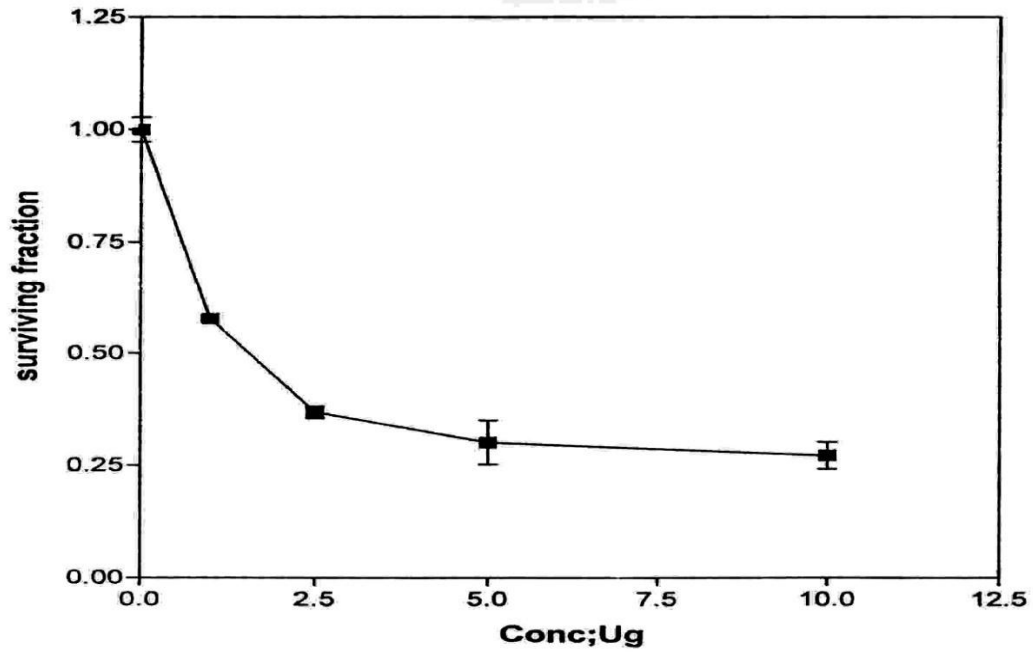

Figure 3: Cytotoxic activity of *Hibiscussabdariffa* (Abiad cultivar) on Liver carcinoma cell line (HEPG2)

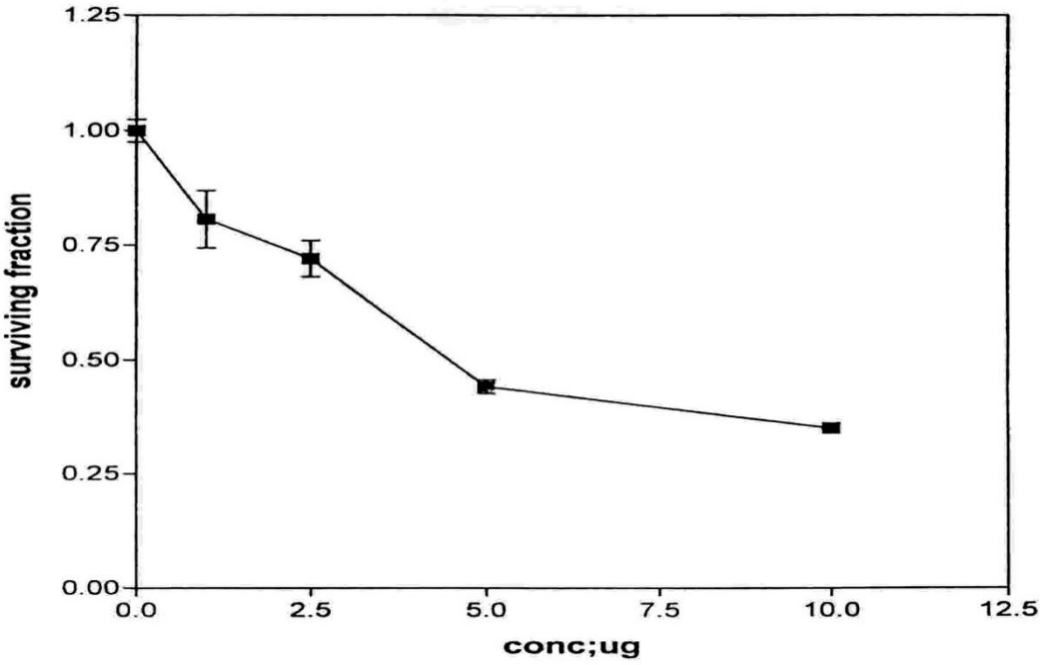

Figure 4: Cytotoxic activity of *Hibiscussabdariffa* (Abiad cultivar) on Breast carcinoma cell line (MCF7)

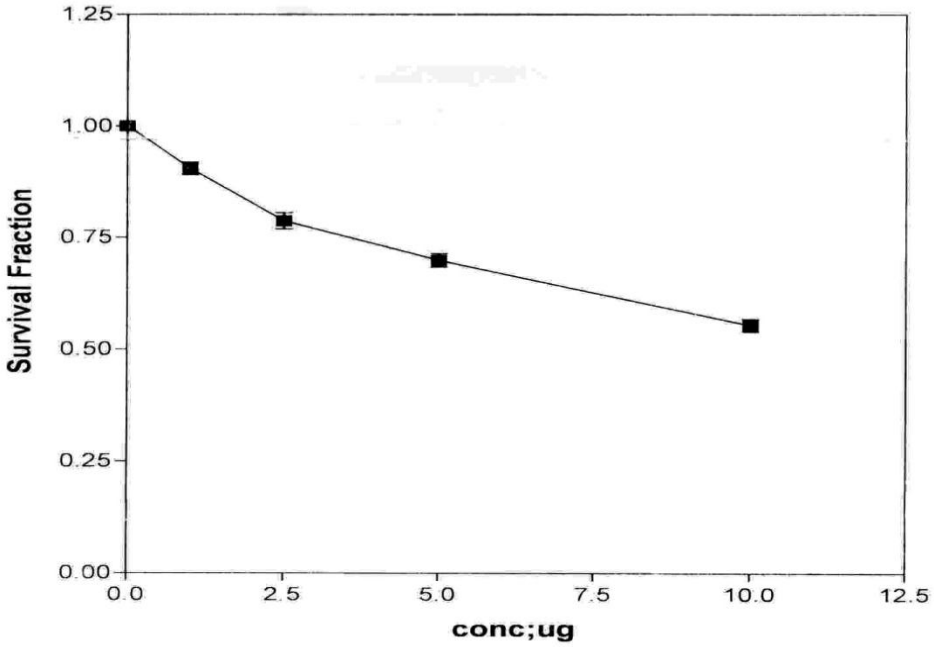

Figure 5: Cytotoxic activity of *Hibiscussabdariffa* (Fashir cultivar) on Liver carcinoma cell line (HEPG2)

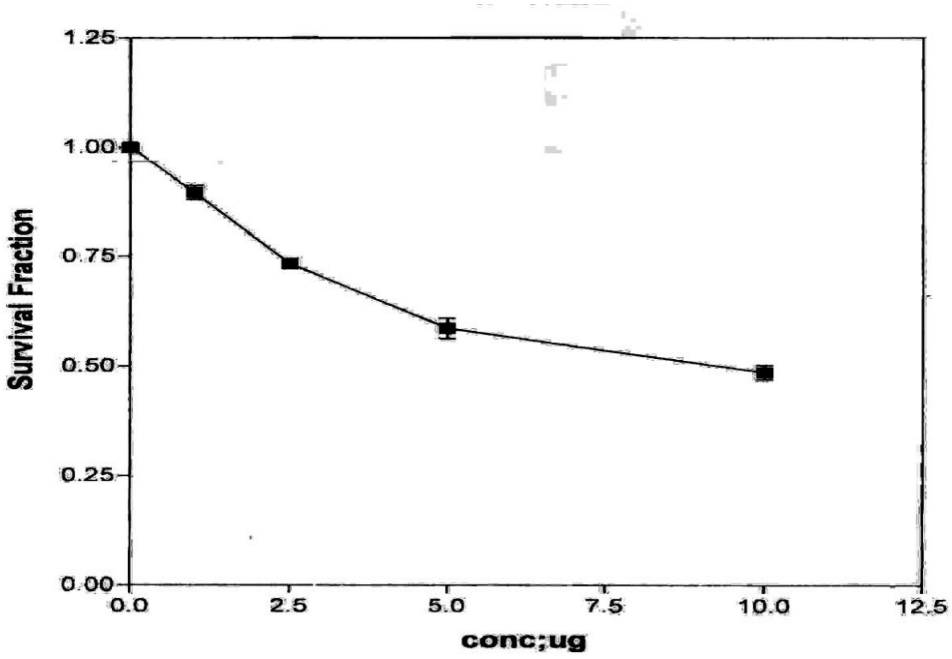

Figure 6: Cytotoxic activity of *Hibiscussabdariffa* (Kass cultivar) on Liver carcinoma cell line (HEPG2)

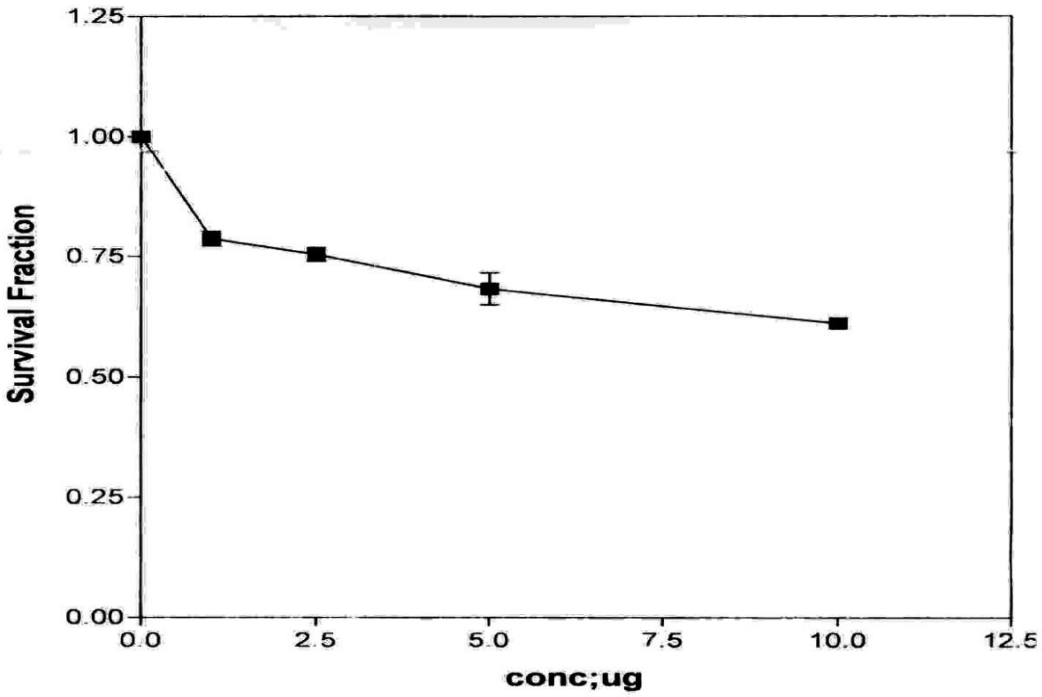

Figure 7: Cytotoxic activity of *Hibiscussabdariffa* (Fashir cultivar) after storage six months on Liver carcinoma cell line (HEPG2)

CONCLUSION

Based on the results of this study it can be concluded that cytotoxicity of crude amino acids of all cultivars (Rahad, Fashir, Kass and Abiad) of *Hibiscussabdariffa* L. had highly toxic effect against HEPG2 Liver carcinoma cell line and the breast cancer cell line**.**

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