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Cytotoxic Effect of Celecoxib and Portulacaoleracea Leaf and Seeds Extract on Rat Hepatocytes

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Abstract: Cancer is an uncontrollable and unchecked division of cells. Cancer can occur in any part of body . Most cancer patients die from cancer that recurs after spreading to a different tissue, rather than from their original tumor All cancers originate from a single cell that starts to behave abnormally, to divide uncontrollably, and, eventually, to invade adjacent tissues. Mechanistic studies indicated that celecoxib exhibits pro-apoptotic effects by inhibiting 3-phosphoinositide-dependent kinase-1 (PDK-1) and the downstream protein kinase B (Akt) signaling pathway in human colon cancer cells . A recent study demonstrated that celecoxib downregulates specificity protein 1 by inhibiting c-Jun N-terminal kinase, thus enhancing the radiation sensitivity and inhibiting the migration and invasion of cancer cells. Purslane (Portulacaoleracea) is a weed naturally found in driveways, lawns, and fields and edible in many regions of Europe, Asia, the Middle East, Africa, and Australia. Previous studies supported that it owns many pharmacological activities, including antidiabetic activity, anti-cancer activity, hepatoprotective activity, anti-microbial effect, and antioxidant activity. Plant materials have been used for the treatment of malignant diseases for centuries and extensive epidemiological surveys have demonstrated that food habits or food components are critical factors in terms of cancer incidence and prevention.Therefore, the present study was conducted to compare and correlate the morphological and cytotoxic effect of celecoxib and portulacaoleracea leaf and seeds extract on rat hepatocytes. Material and methods: water based extraction of portulacaoleracea from both leaves and seeds was performed. Primary cell culture of rat liver tissues were cultured under in 10% supplemented RPMI and incubated at 37 C, 5 % CO2. Cells were exposed to various concentrations of celecoxib and portulacaoleracea leaf and seeds extract. Morphological examination was observed daily. The cells were treated with MTT at concentration for 3hr. DMSO 1% was added to each well after incubation (37 C, 5 % CO2) period and the absorbance was measured at 492 nm using microplate reader. Data was collected and analysed in triplicates and an average was obtained for each reading. The half maximal inhibitory concentration (IC50) was calculated. Results: our results show that strong and apparent morphological changes are seen under on hepatic primary cells when exposed to different concentrations of celecoxib. Furthermore the cytotoxic effect of celecoxib increases with dose response and half maximal inhibitory concentration (IC50) was reached under 59.7 µg/ml of celecoxib. Hepatic Cellular response under exposure to portulacaoleracea leaf and seeds extract was non-significant. Morphological appearance was stable under high conditions. Cytotoxic effect of portulacaoleracea leaf and seeds extract was only seen under higher concentration of seed extract. Half maximal inhibitory concentration (IC50) was not reached under the studied concentrations of portulacaoleracea leaf and seeds extract. Conclusion: We conclude that celecoxib is a potent cytotoxic agent and portulacaoleracea leaf and seeds extract is a weak anticancer agent compared to celecoxib. However a combination of both celecoxib and portulacaoleracea seed extract may both serve as a potent cytotoxic agent.

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INTRODUCTION

Cancer is an uncontrollable and unchecked division of cells. Cancer can occur in any part of body [1]. Most cancer patients die from cancer that recurs after spreading to a different tissue, rather than from their original tumor [2]. All cancers originate from a single cell that starts to behave abnormally, to divide uncontrollably, and, eventually, to invade adjacent tissues. The aberrant behavior of this single cell is due to somatic mutations—changes in the genomic DNA produced by the activity of different mutational processes [3]. Celecoxib oral capsule is a prescription drug that's available as the brand-name drug Celebrex. It's also available in a generic version. Generic drugs usually cost less. In some cases, they may not be available in every strength or form as the brand-name version. Celecoxib only comes as a capsule you take by mouth. Celecoxib, a COX-2 selective inhibitor, has been utilized for over 20 years, particularly as an anti-inflammatory, analgesic and antipyretic medication. However, to date, its antineoplastic properties have not been sufficiently investigated.

Mechanistic studies indicated that celecoxib exhibits proapoptotic effects by inhibiting 3-phosphoinositide-dependent kinase-1 (PDK-1) and the downstream protein kinase B (Akt) signaling pathway in human colon cancer cells. A recent study demonstrated that celecoxib downregulates specificity protein 1 by inhibiting c-Jun N-terminal kinase, thus enhancing the radiation sensitivity and inhibiting the migration and invasion of cancer cells [4].

Purslane (Portulacaoleracea) is a weed naturally found in driveways, lawns, and fields and edible in many regions of Europe, Asia, the Middle East, Africa, and Australia [5]. This plant is used diffusely as a potherb, being added in soups or salads; and has also been used in traditional medicine in many countries. Purslane possesses a number of nutritional benefits due to the presence of its constituents: omega-3 fatty acids, flavonoids, terpenoids, alkaloids, sterols, vitamins, proteins and minerals. Previous studies supported that it owns many pharmacological activities, including antidiabetic activity, anti-cancer activity, hepatoprotective activity, anti-microbial effect, and antioxidant activity. Therefore, the World Health Organization (WHO) listed this plant as one of the most used medicinal plants and named it as "Global Panacea" [6].

Plant materials have been used for the treatment of malignant diseases for centuries and extensive epidemiological surveys have demonstrated that food habits or food components are especially critical factors in terms of cancer incidence and prevention [7].

MATERIALS AND METHODS

Preparation of celecoxib

Celecoxib 200 mg was purchased from (Pfizer, USA). Stock concentration of (1000 µg/ ml) was made from 0.01g of celecoxib and dissolved in 0.1ml of

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DMSO and 9.9 ml of distilled water. The mixture was allowed to dissolve before filtering through a 0.22 um syringe Millipore [8] Various concentrations (2.5, 5.0, 10.0, 20.0, 40.0, 80.0 µg/ ml) were made from stock solution[9].

Preparation of portulacaoleracea seeds and leaves extract

Portulacaoleracea extract was extracted using water based method. Stock concentration (1000 µg/ ml) was made from 0.01g of portulacaoleracea and dissolved in 0.1ml of DMSO and 9.9 ml of distilled water. The mixture was allowed to dissolve before filtering through a 0.22 um syringe Millipore. Various concentrations (31.1, 62.5, 10.0, 125.0, 250.0, 500.0 µg/ ml) [10] were made from stock solution.

Primary Cell culture of rat hepatocytes

Primary Rat liver culture was achieved using fresh rat liver tissues. Rat liver Tissues were dissected and incubated in trypsin (0.25 %) for 20 min at 37 °.C Tissues were shaken vigorously and mechanically pippetted.Cell were inhibited by adding (RPMI 1640 and 10 % fetal bovine serum FBS and Penicillin / streptomycin)was prepared and incubated at 37°C, The dissociated tissue was layered over supplemented media. Cells were plated in non-TC treated 24 plates Cells and cultured for 7 days, fresh media was replaced daily [11-13].

Cell viability determination using MTT assay :

Cells were seeded at 1× 105 cells/mL in 96 well micro titer plates in RPMI medium. The cells were incubated overnight for attachment. Different concentrations of celecoxib and portulacaoleracea seeds and leaves extract were dissolved in media and were added in triplicate and incubated for 48 hrs. Thereafter, the cells were treated with MTT at concentration 2µg/ml for 3hr. After the incubation time, all the contents of well aspirated. DMSO 1% was added to each well after incubation (37 C, 5 % CO2) period and the absorbance was measured at 492 nm using microplate reader [14-17]. Data was collected and analysed for each well. The half maximal inhibitory concentration (IC50) was calculated.

RESULTS

Primary cultured hepatic rat cells were cultured from freshly isolated liver tissues and were allowed to grow to reach a confluence 80% confluence level and then further subcultured and exposed to various concentrations of celecoxib and extract from both leaves and seeds.

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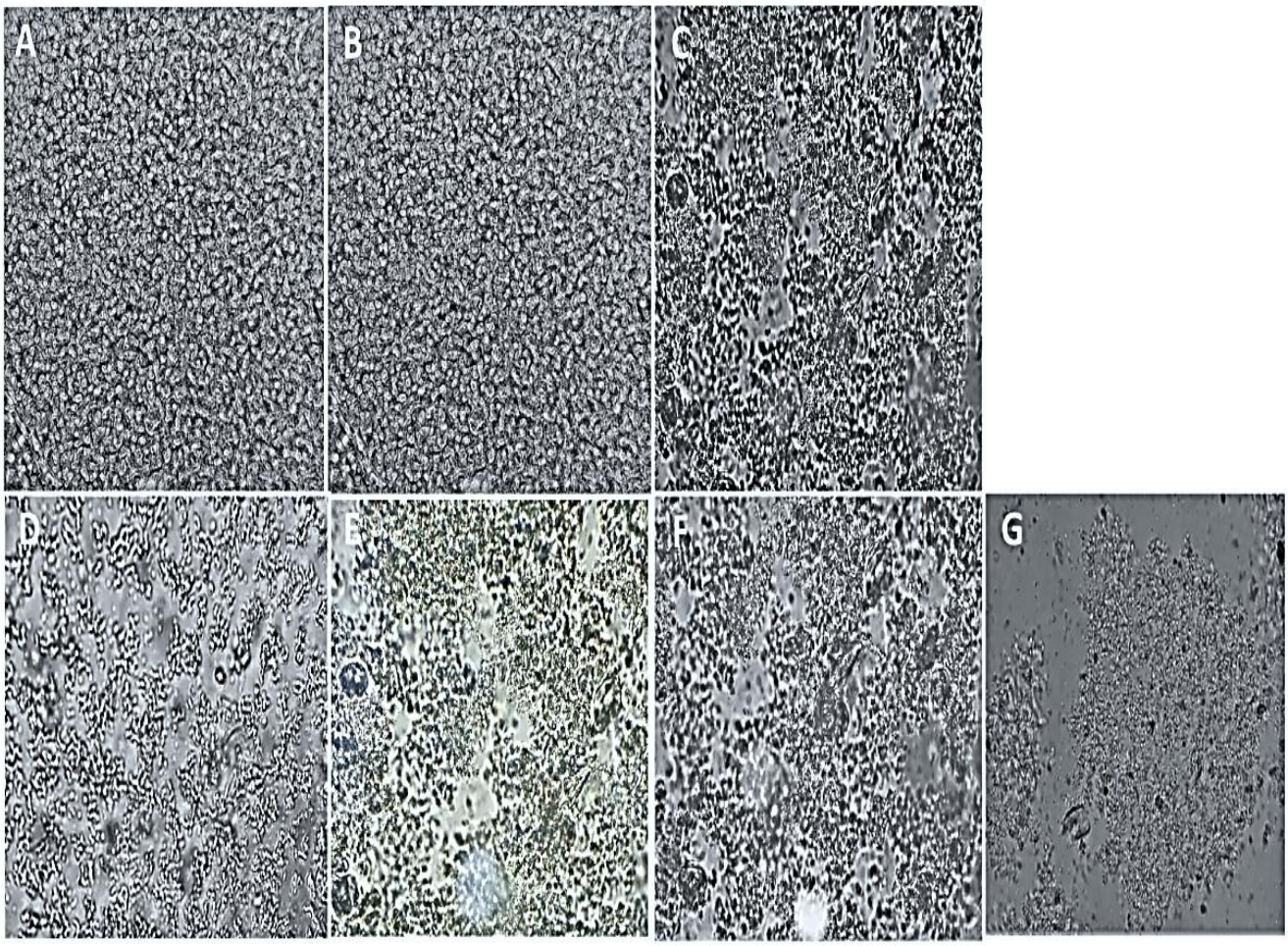

Figure 1: A series of photographic images (400X) of cultured rat liver cells exposed to different concentration of celecoxib. Image A are hepatic cells under control conditions with epithelial appearance. Images (B-G) show cells under increasing concentration of celecoxib (2.5-80 µg/ml). Noticeable cellular changes are seen including apoptotic changes induced by celecoxib as well as cellular morphological changes including pigmentation and vaculation.

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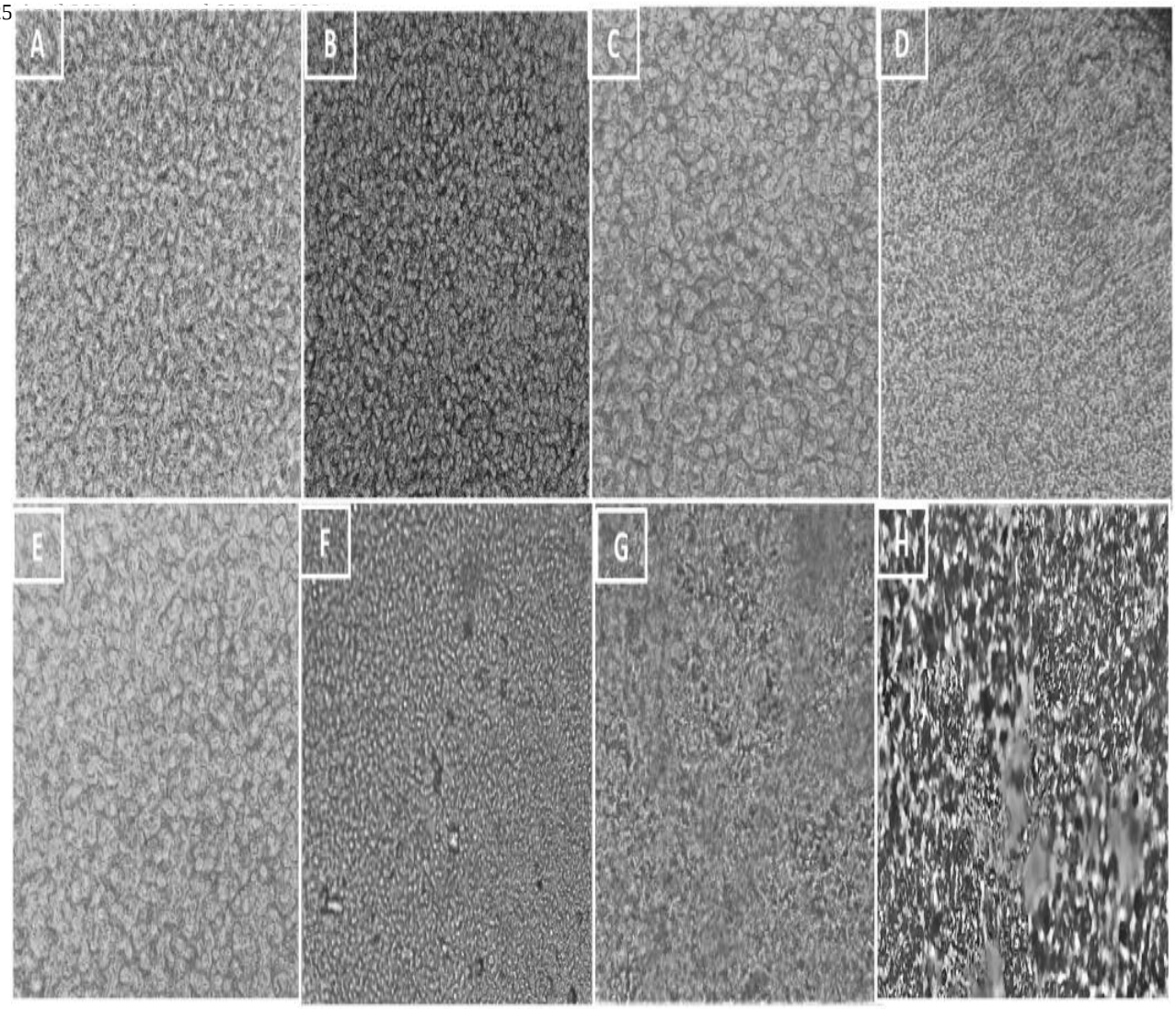

Figure 2: A series of photographic images (400X) of cultured rat liver cells exposed to different concentration of portulacaoleracea root and leaves extract. Image A and E are hepatic cells under control conditions showing normal epithelial appearance. Images (B-D) shows hepatic cells under increasing concentration of portulacaoleracea leaves extract (31.1, 10, 500 μg/ml). No apparent cellular changes are seen under increased exposure to portulacaoleracea leaves extract. Images (F-H) shows heaptic cells under increasing concentration of portulacaoleracea seeds extract (31.1, 10, 500 μg/ml). Slight noticelable cellular changes are seen when cells are exposed to 10 and 500 μg/ml of portulacaoleracea

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Cytotoxicity of primary cultured hepatic rat cells under different concentration of celecoxib

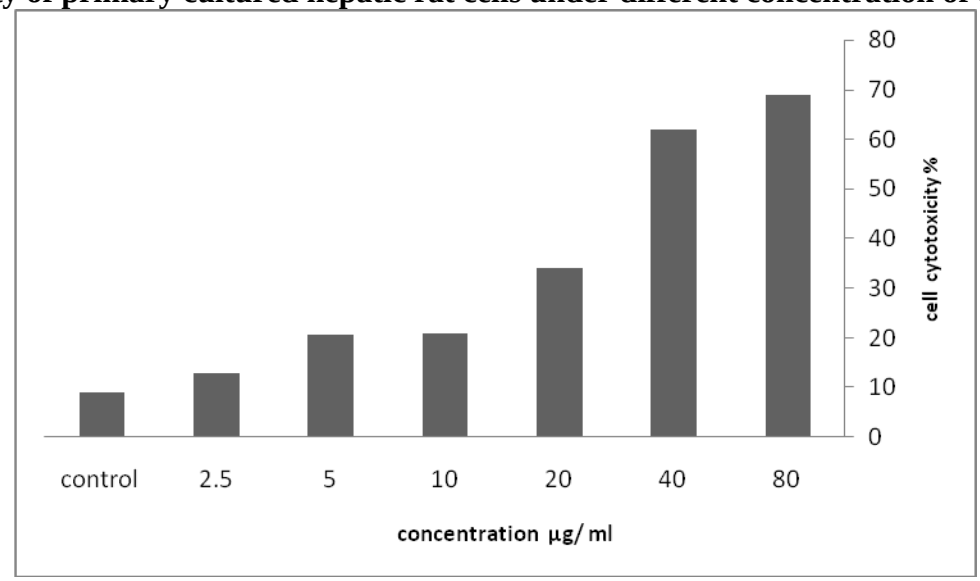

Figure 3: Cell cytotoxicity shows an increase in cellular death among rat hepatic cells when exposed to increasing concentration of celecoxib. Cell cytotoxicity shows a potent effect of celecoxib on hepatic cells. Instant cell death was noticed at lower concentration compared to control conditions. The half maximal inhibitory concentration (IC50) of celecoxib is 59.7 µg/ml. Cellular death rate continues to increase to reach 68 % after exposure to a concentration of 80µg/ml.

Cytotoxicity of primary cultured hepatic rat cells under different concentration *portulacaoleracea* leaf and seeds extract

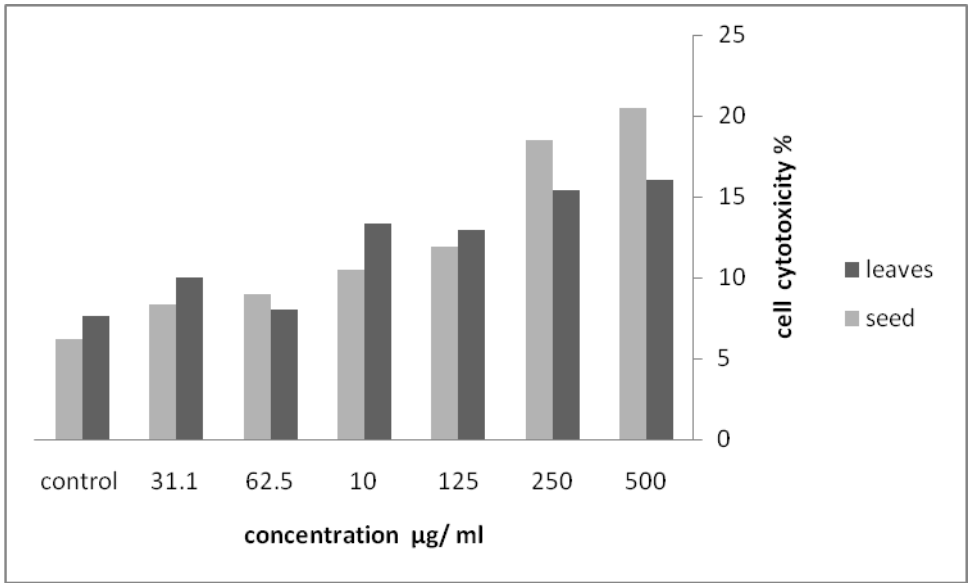

Figure 4: cell cytotoxicity shows an increase in cellular death among rat hepatic cells when exposed to increasing concentration of *portulacaoleracea* from both leaves and seeds. Cell cytotoxicity shows a mild effect of *portulacaoleracea* extract. Slight increase in cell cytotoxicity level is noticed however The half maximal inhibitory concentration (IC50) is not reached under the studied concentrations of *portulacaoleracea* for both leafs and seeds extract.

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DISCUSSION

The result of the study shows that the high concentration of celecoxib causes apoptosis and death in primary cultured rat hepatocytes . The results are attributed to drug action mechanisms which includeeffect of celecoxib was mainly caused by inducing apoptosis rather than disturbing cell proliferation.Celecoxib-resistant cell lines with COX-2 overexpressionexhibit a reduced level of Bax, a pro-apoptosis protein, and increased levels of anti-apoptosis proteins such as Bcl-2 or Bcl-xL. COX-2 knockdown by its specific siRNA significantly decreases clonogenicity and levels of Bcl-xL and Bcl-2 in these cells. In MDA-MB-231and MCF-7 cell lines,[33] celecoxib induces apoptosis by decreasing phosphorylation of Akt, then increasing the expression of Bax and activation of caspase 3 and caspase 7. A study by singh and colleagues [18] showed that celecoxib induces apoptosis of the BC cell line, MDA-MB-231, by inhibiting the NF-κB pathway. Another mechanism of celecoxib-induced apoptosis in most cellular systems involves p53-independent mitochondrial apoptosis pathway, which is COX-2 independent and could not be inhibited by the overexpression of Bcl-2 [18].

The cytotoxic potential of aqueous extracts of *Portulacaoleracea*leaves and seeds shows that cell cytotoxicity shows an increase in cellular death among rat hepatic cells when exposed to increasing concentration of *portulacaoleracea* from both leaves and seeds (fig 2and 4). Cell cytotoxicity shows a mild effect of *portulacaoleracea* extract. Slight increase in cell cytotoxicity level is noticed however the seed extract was more toxic than the leave and *Portulacaoleracea* extract is composed of a wide range of constituents, of which flavonoids, alkaloids, terpenes, phenolic acids, and coumarins are preeminent.Other notable constituents are omega-3-fatty acids, polysaccharides,vitamins, and amino acids.And thecomponent were more concentrated in the seed than in the leaves [19].

CONCLUSION

This study concludes that strong and apparent morphological changes are seen under on hepatic primary cells when exposed to different concentrations of celecoxib. Furthermore the cytotoxic effect of celecoxib increases with dose response and half maximal inhibitory concentration (IC50) was reached under 59.7 μg/ml of celecoxib. Hepatic Cellular response under exposure to portulacaoleracea leaf and seeds extract was non-significant. Morphological appearance was stable under high conditions. Cytotoxic effect of portulacaoleracea leaf and seeds extract was only seen under higher concentration of seed extract. Half maximal inhibitory concentration (IC50) was not reached under the studied concentrations of portulacaoleracea leaf and seeds extract. Conclusion: We conclude that celecoxib is a potent cytotoxic agent and portulacaoleracea leaf and seeds extract is a weak anticancer agent compared to celecoxib. However a combination of both celecoxib and portulacaoleracea seed extract may both serve as a potent cytotoxic agent.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or

financial relationships that could be construed as a potential conflict of interest.

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