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Anti-Proliferative Activity of Rutin in Cancer

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***Abstract*: Rutin, a powerful antioxidant, is said to have anti-cancer properties. Our study aims to prepare rutin-loaded chitosan nanoparticles (RCNPs) by an ion gelation method. These particles are characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction spectroscopy (XRD), scanning electron microscopy (SEM). The FTIR spectrum of RCNP also showed the existence of various chitosan effective groups and peaks corresponding to rutin effective groups, indicating the successful encapsulation of rutin into chitosan ultrafine particles. By applying the Debye-Scherrer equation to his XRD data, his RCNP size was found to be 33.12 nm. SEM images revealed the surface porosity and rough analysis of RCNPs. The electromagnetic ability was found to be +0.68 mV, indicating the stability of the ultrafine particles. The entrapment efficiency (EE%) was found to be 68.7%, the loading efficiency (LE%) was 49.09%, and the recovery of RCNP was 41.08%. Based on the above test values, RCNP was found to be useful in various cancer cell lines, namely HeLa, MDAMB231, Panc-1, PC, was further tested in the MTT assay against exposed to antiproliferative activity, SK0V3. These results showed that rutin and RCNP had anti-proliferative effects only on the cell lines of SK0V3, and the effect of RCNP was greater than that of pure rutin. Based on this, we can hypothesize that rutin-loaded chitosan nanoparticles (RCNP's) have a promising, prominent role in revolutionizing the treatment of cancer diseases.**

***Keywords*: Rutin, Nanoparticles/Ultrafine particles, Loaded Chitosan, Anti-cancer activity, Ovarian cancer, Pancreatic cancer.**

1. Introduction

Cancer is the second leading cause of death and includes 277 types of cancer [15]. Cancer cells are characterized by abnormal growth and uncontrolled division [8]. The main treatment options are chemotherapy, surgery, radiotherapy, and hormone therapy [44]. Alopecia, anaemia, fatigue, nausea are the main side effects. Moreover, the curative application of the same; alopecia, anaemia, fatigue, nausea is declining due to their low efficacy, high cost and high toxicity [41]. Scientists around the world rely on therapies based on plants. Considering the importance of all plant components, phytochemical in particular play an important role in the treatment of chronic diseases. The phytochemical 7-methylgallate plays a role in the treatment of neurodegenerative diseases [33]. Other flavonoids, such as rutin, vinca alkaloids, and etoposide, are used to treat cancer because plant-derived compounds are more tolerant and non-toxic to normal human cells [26]. Most flavonoids are polar compounds and are naturally water soluble, which limits their absorption. There are two important factors that reduce bioavailability. One of them being molecular size, that hinders passive absorption, and the other factor is low lipid solubility, that limits the entry through phospholipid layer. The challenge, therefore, is to increase the bioavailability of these phytochemicals to improve their curative efficacy [6], [23], [24], [40]. Rutin, also known as quercetin-3-rutinoside [27], is commonly found in tea, passionflower, apples and buckwheat [14]. It has many pharmacological activities, namely cardioprotective [4], neuroprotective [32], anticonvulsant [29], antidepressant [25], analgesic [36], antinociceptive [39] and anticancer [2]. reported. against HL-60 human leukaemia cells [24], LAN-5 human neuroblastoma cells [11], colorectal cells [5], OVCA 433 ovarian cancer cells [38], pancreatic cancer cells [28] It has been reported to have anticancer effects.

Considering the various pharmacokinetics of rutin and its anticancer cell action profile, a great deal of studies have been conducted to increase its clinical application to enhance its bioavailability [20]. Rutin-loaded nanophytosomes were designed to have better antioxidant capacity and better bioavailability [16]. Targeted delivery to the brain to treat cerebral ischemia has been reported with rutin-loaded chitosan nanoparticles [1], [6]. Nano formulations of chitosan play an important role in enhancing the bioavailability of many herbal medicines. It is a polymer derived from the deacetylation [35] of chitin, a fibrous material found in the exoskeleton of the cell walls of crustaceans, fish scales and fungi [45]. Most of the biomedical applications of this polymer are based on its nontoxic, biodegradable, mucoadhesive, and biocompatible applications [42]. In addition, it has the ability to adhere to mucosal layers and transiently open epithelial tight junctions [34]. Based on the above discussion, the present study was designed to improve therapeutic efficacy by encapsulating rutin in chitosan to improve its bioavailability.

2. Anti-Proliferative Activity of RCNPs by MTT Assay

The IC50 values for rutin and RCNP against the SK0V3 cell line were 62.28±19.42 and 86.45±19.46, respectively. Thus, for the first time, the efficacy of rutin and RCNP against ovarian cancer cell lines has been reported. However, in our study, both Rutin and He-RCNP failed to show activity against HeLa, MDA-MB-231, Panc-1, and PC3. A graphical summary describing the antiproliferative activity of rutin and RCNPs is presented.

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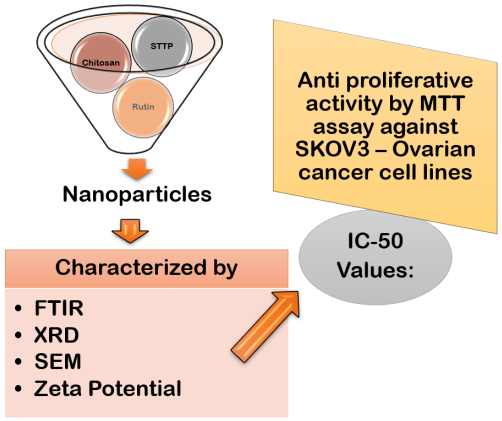

Fig. 1. Summary of graphs illustrating the antiproliferative activity of rutin and RCNP

Table 1
Antiproliferative activity of Pure rutin and RCNPs

Compounds	HeLa	MDAM B231	Panc-1	PC3	SKOV3
Pure Rutin (50µM)	NA	NA	NA	NA	62.28±19.42
RCNP (50µM)	NA	NA	NA	NA	86.45±19.46

Antiproliferative activity of pure rutin and RCNP by MTT assay. All values are expressed as Mean ± SEM, NA=Not Active.

3. Discussion and Conclusion

RCNP was prepared by adding rutin to the chitosan solution. STPP is then added dropwise to the above solution. After stirring overnight, the above solution is centrifuged at 8000 rpm, then the supernatant is removed to separate the nanoparticles, washed with ethanol and saved for further studies. EE% can also be increased by optimizing the amount of chitosan, drug, and optimizing temperature, rotation speed, and pH. LE% is the actual amount of drug loaded into RCNP. We found the RCNP yield to be 31.08%. The results obtained by us were consistent. Briefly, Patil and Joban Putra achieved a yield of 36.72% and 32.24% EE% in the production of rutin-loaded chitosan nanoparticles. Recoveries were constant, but, EE% was much better in our study. Additionally, optimizing the chitosan to STTP ratio can increase both efficiency and yield.

In the FTIR, 1525.30cm^{-1} peak of the chitosan ultrafine particles indicates the interaction between STTP and chitosan, and 1529 cm^{-1} indicates the -NH bending mode of the primary amine. For the RCN, 3241 cm^{-1} and 1650.50 cm^{-1} indicate -OH and C=O stretches, respectively. 1564 cm-1, 1203 cm^{-1} and 1060 cm-1 indicate the C=C, P=O and C-O-C vibrations of rutin, respectively. This confirms the loading of rutin in chitosan ultrafine particles. 1496 cm indicates the -NH bending mode of the chitosan primary amine [30], the OH stretching vibration is due to a broad peak at 3397 cm, and the interaction between STTP and chitosan is due to the rutin charge A confirmation peak of is indicated by the rutin C-O-C vibration peaking at 1064 cm [7]. The diffractogram showed many XRD peaks below 300 indicating the semi-crystalline nature of chitosan. RCNP sizes were found to range from 21.51 nm to 41.37 nm using the Debye-Scherrer equation, with an average size of 32.15 nm. The results were consistent with those of other groups.[6]

Based on Scanning Electron Microscope studies, a porous structure of RCNPs has been delineated. This promotes drug inflammation and rapid release. The surface morphology presented in the study is consistent [10]. The low crosslink density of ultrafine particles is attributed to their porous, rough, loose and open surface morphology [9]. In contrast, zeta potential measurements manifested the decreased stability of rutin-loaded chitosan ultrafine particles. [U2] This is due to a number of factors, including the type and amount of biopolymer, the ionic strength, and the pH of the solution. Values close to zero may be due to negatively charged rutin groups [7]. RCNPs are active against SKOV3 cells. Rutin is a citrus flavonoid found in many plants. Long-term high intake of flavonoids reduces the incidence of ovarian cancer [13]. Results showed that RCNP was more potent than rutin. This increased activity of the rutin-loaded chitosan ultrafine particles is due to the targeted delivery of the ultrafine particles. Moreover, pure rutin and RCNP are inactive against other cell lines, namely HeLa, MDAMB231, Panc-1 and PC3.

A recent study demonstrated a simple method to incorporate the insoluble plant component rutin into chitosan ultrafine particles. The synthesized RCNPs are stable and water soluble. Moreover, successful encapsulation of rutin in ultrafine particles enhances the bioavailability of rutin, thereby enhancing its therapeutic efficacy. The results obtained more clearly confirm the efficacy of the rutin-loaded chitosan ultrafine particles. This could be ascribed to anti-proliferative effects, intracellular agglomeration, and increased cellular assimilation. Thus, chitosan-loaded rutin ultrafine particles could be a powerful tool for rutin encapsulation and remittance and an efficient therapeutic option for cancer by enhancing rutin's stability and water solubility. Further studies should be conducted to investigate its anti-cancer effects when used at high concentrations. Evaluation of RCNP compared to other cell lines such as; epithelial; human breast cancer (MDAMB231), pancreatic carcinoma (Panc-1), cervical carcinoma (HeLa), and adenocarcinoma (PC3). RCNP in vivo work against ovarian cancer is also guided by the results of this study. Rutin's anti-proliferative effects appear promising for cancer research, but its long-term effects however have to be investigated.

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