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Is *G. cambogia* a promising treatment? Effects on cultured nasal epithelial cells

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Abstract. – OBJECTIVE: The purpose of this study is to assess the effects of applying Garcinia cambogia to cultured human nasal epithelial cells.

MATERIALS AND METHODS: A cell culture was set up consisting of human primary nasal epithelial cells harvested during septorhinoplasty from volunteers. The cells came from individuals with no history of rhinosinusitis. One assay for assessing cytotoxicity in cell culture utilises MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). This method allows visualization of fragmented DNA, condensation of nuclei and changes to the external cellular membrane or cytoskeleton. Our study employed this method. Nasal epithelial cells at 37°C were exposed in culture to *G. cambogia* for a period of 24 hours. Afterwards an MTT assay was used in conjunction with confocal microscopy to assess evidence of toxicity. The proliferative capability of the nasal epithelial cells was also evaluated by inducing a scratch injury to cultured cells followed by light microscopic examination.

RESULTS: Testing for cytotoxicity in this manner indicates that *G. cambogia* does not appear harmful to cultured nasal epithelial cells when applied directly. The cells exposed to this plant extract were still fully viable 24 hours afterwards. There was no increase in viability at the level of statistical significance. It was noted, however, that proliferation did increase slightly within the exposure period. The MTT assay and confocal microscopy confirm these findings. Under confocal microscopic examination, a compact morphology with unaltered nuclear and cytoskeletal appearances was observed. Thus, there is no evidence suggesting viability is impaired or that cytotoxicity occurs. Ordinary light microscopic examination showed the area denuded of cells had become re-covered completely within 24 hours in the cultures where *G. cambogia* had been applied. The result suggests that exposure to *G. cambogia* has no significant effect in terms of stimulating or inhibiting cellular proliferation.

CONCLUSIONS: *G. cambogia* may offer clinical benefit as a supplementary topical treatment for inflammation of the nose and sinuses, as seen in chronic and acute rhinosinusitis, or nasal polyps. The plant appears to increase nasal epitheliocytic proliferation slightly, as revealed by the MTT assay. There were no indications of a cytotoxic effect on epithelial cells of the nose.

Key Words:
Garcinia cambogia, Cultured human nasal epithelial cells, Confocal microscopy, Viability, toxicity.

Introduction

The US Federal Food, Drug, and Cosmetic Act (FD&C Act) was amended in 1994 by the Dietary Supplement Health and Education Act (DSHEA). The DSHEA stipulates that a dietary supplement is any vitamin, mineral, herb or other plant or amino acid, taken in order to increase the amount of a specific dietary component[1,2]. Supplements may be prepared as a concentrate, be a metabolite of another substance or form a constituent of another product. They may be an extract or be compounded as a combination of forms[1-3,4"]. Supplements may be sold as tablets, capsules, soft gels, gelatinous capsules, liquids, teas, or powders. The officially approved reference for the documentation of herbal medicinal products in the US is the United States Pharmacopoeia-National Formulary (USP-NF)[1,4]. This document sets out the official public standards for all medicinal products permitted to be manufactured and sold within the USA. It covers both prescription-only and over the counter products[5]. The pharmacopoeia also defines standards relating to ingredients in food and the composition of dietary supplemental products[6].

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Garcinia cambogia, also known as *Garcinia gummi-gutta* is a common ingredient in fish curries. Many people enjoy the powerful tangy taste of the fruit rind. It is used ethnobotanically as an aid to digestion and for alleviating bowel complaints, preventing parasitic infections of the gut and relieving rheumatic complaints. In appearance, the unprocessed plant is a small-sized fruit resembling a miniature pumpkin. It is frequently sold and used as an aid in dieting. Scientific evidence indicates that *G. cambogia* fruit rind extract contains abundant hydroxycitric acid (HCA). This compound exerts an effect on weight loss by altering serotonin levels. This leads to lower intake of calories by increasing satiety. Furthermore, metabolic changes include greater fatty acid oxidation and a reduction in *de novo* lipid synthesis. HCA strongly inhibits the enzyme adenosine triphosphate citrate lyase, preventing citrate's conversion to acetyl-coenzyme A. This conversion is a vital step in lipogenesis, including production of fatty acids, cholesterol and triacylglycerides. Certain other benefits of unrefined extracts of *G. cambogia* have also been demonstrated. It lowers lipid levels in blood, is antidiabetic and reduces inflammation. Furthermore, it decreases malignant potential, is toxic to helminthic parasites, inhibits cholinesterase activity and protects the liver. These effects have been shown both *in vitro* and in animals[7].

The DSHEA enshrines the responsibility of the US FDA to establish safety concerns and respond to warnings about supplements deemed to endanger patient safety once they enter the market. Supplements which entered the market before 1994 may still be sold. However, for these products, there is a lack of rigorous scientific data regarding efficacy and safety. Their continued sale reflects an approach based upon trial-and-error. It is not a legal requirement for companies which manufacture or distribute dietary supplements to seek registration as a manufacturer. They also do not need approval before manufacturing or distributing supplements[1,8]. However, they must report any known adverse effects and are obliged to ensure supplements remain unadulterated. There is FDA monitoring of claims about supplements and labelling of particular products. In addition, a system exists for reporting of side effects related to dietary supplements, although participation is voluntary. There have been FDA warnings issued for supplements found to be hepatotoxic[9]. How manufacturers may advertise dietary supplements is under the regulation of the Federal Trade Commission[8].

There is a requirement in the DSHEA that the safety of supplements be proven prior to their being marketed. It is the responsibility of the manufacturer to label the product truthfully and in ways that are not open to misinterpretation. The manufacturing company must be clearly identified, and the ingredients must be listed in their entirety. Claims that cannot be clearly justified must also be avoided. This covers purported benefits on health, nutritional values and claims regarding effects on body structure or function[10].

Claims about structure or function refers to statements that a particular benefit to physiological function or healthy anatomy is linked to use of the supplement. For example, a manufacturer may claim use of a calcium-containing product results in stronger bones. Functional claims may also be made about how a supplement may achieve these benefits, e.g., that high fibre content promotes regular bowel habits. Claims are sometimes made more vaguely, such as stating that a particular nutrient promotes greater well-being. A manufacturer may-claim that a product protects against a disorder caused by a nutrient deficiency, such as vitamin C used to prevent scurvy. In these cases, for US-marketed supplements, the consumer must be informed how common a particular disorder actually is. Claims related to structural or functional benefit must carry the following disclaimer, as mandated by the DSHEA: "This statement has not been evaluated by the FDA. This product is not intended to diagnose, treat, cure, or prevent any disease[6]".

G. cambogia is a tree of considerable economic importance. Its fruit, when smoked and sun-dried, enjoys widespread culinary use, particularly to flavour fish curries. Dried fruit rind prevents bacterial growth and can be used, together with salt, to cure fish. It is used in this way in India and Sri Lanka[11-13]. It may sometimes be used as a replacement for Goa butter made from *Garcinia indica* and is often added to meals to increase their bulk[11,14]. The fruit peel is employed as a herbal medicine to treat joint pains and gastrointestinal disorders. It also works as a purgative, promoting watery discharge from the bowels, is toxic to helminthic parasites and can cause vomiting. It may be used in animal medicine as a mouth rinse in cows[15]. Although the pulp can be eaten, it is very tart-tasting and is not generally consumed uncooked[11,13]. In India, *G. cambogia* is used to prepare a tonic with a high level of vitamin C which is then used to treat a number of cardiac disorders[16].

It has already been shown that an extract of 60% HCA obtained from *G. cambogia* is capable of preventing adipocytes developing and lipid being

stored in the cytoplasm of cells. This was shown in cell cultures using the 3-isobutyl-1-methylxanthine, dexamethasone, and insulin (MDI)-induced mouse embryonic fibroblast-adipose-like cell line (3T3-L1). The extract significantly reduced the levels of peroxisome proliferator-activated receptor (PPAR)-γ2, CCAT/enhancer-binding protein a (CEB-Pa), and adipocyte protein aP2[17]. According to Blunden's research[18] carried out *in vitro*, the plant extract prevents lipids being accumulated in adipocytes but does not affect liposynthesis. A mouse model, where the animals were fed a lipid-enhanced diet to induce obesity, showed that *G. cambogia* extract decreased gain in body mass. It also decreased the deposition of adipose tissue around the viscera, lowered lipid levels both within the liver and in the circulation, and lowered the circulating leptin and insulin concentrations. The same extract was noted to offer benefit in terms of genetic expression in adipocytes induced by a lipid-rich diet. It lowered levels of adipocyte protein 2, sterol regulatory element-binding factor 1c, PPAR-y2 and CEB-Pa[19,20].

The current study aims to assess how an extract of *G. cambogia* affects nasal epithelial cells in cell culture when applied directly to them. This is the first study to investigate the effects of topically applied *cambogia*, including the potential for toxicity.

Materials and Methods

This study was a collaborative effort between the ENT Department of EskişehirOsmangazi University and the Department of Biology in the Science Faculty of Eskişehir Technical University. The primary nasal epithelial cells used were sourced from healthy tissue excised during the course of rhinoplasty. Written consent for the tissue to be used in this way was provided by the patients concerned. Once harvested, the mucosae were kept in a cyto-preservative medium for transportation to the Cell Culture Laboratory at the Eskişehir Technical University.

Methodology

Primary cell culture

Nasal epithelial tissues were surplus tissues obtained routinely at operation in five patients undergoing septoplasty. They were transferred into cell culture. None of these patients exhibited rhinosinusitis. To obtain individual nasal epithelial cells, the tissues were dissected into small fragments in sterile Petri dishes, then transferred into a medium containing freshly constituted DMEM-F12. This medium contained 1% penicillin-streptomycin and 10% bovine foetal serum. The cells were maintained at 37ºC in high humidity with 5% carbon dioxide. Any excess fragments were washed off on Day 7. The cells that remained adherent to the plates were washed in phosphate-buffered saline (PBS) then treated with trypsin prior to being passaged onto T25 cell culture plates. The experiment proper was deemed feasible when 85% of the cells on the plate formed a confluent sheet[21].

MTT Cytotoxicity Assay Following Application of G. Cambogia

After being treated with trypsin, primary epithelial cells were transferred to a plate containing 96 individual wells. The number of epithelial cells within each well was around 5,000. The test reagents were employed at different concentrations, the most diluted being at a concentration of 0.15 mg/ml, whilst the highest concentration was 5 mg/ml.These plates remained at a temperature of 37°C in a humid atmosphere with 5% carbon dioxide.

The *G. cambogia* extract (Natural Factors, Coquitlam, Canada) was prepared for topical application by being first mixed with dimethylsulfoxide, giving a concentration of 5 mg/ml. Varying concentrations of the cambogia-DMSO mixture were applied to the cells in each well, the range being from 0.15 to 5 mg/ml. The mixture then remained for 24 hours. After this incubation was complete, 20 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in phosphate-buffered saline (PBS, Invitrogen) was placed in each well. The MTT concentration was 5 mg/ml. Without altering the culture conditions in any other way, the plates then remained under incubation for 4 more hours, after which the cell culture medium was replaced by 200 µL DMSO. The evaluation used an ELISA plate reader, the wavelength of which was set to 570 nm. The absorbance recorded allowed calculation of the percentage viable tissue remaining and the value of IC_{50}, i.e., the half maximal inhibitory concentration[21].

Confocal Microscopic Examination

Nasal epithelial cells were cover-slipped and placed in a 6-welled plate. Each of the wells held around 30,000 epithelial cells. The IC_{50} dose for an extract of *cambogia* had previously been ascertained. A volume of *G. cambogia* extract delivering this concentration was applied to the epithelial cells for a 24-hour period. The controls consisted

of epithelial cells not exposed to the extract. After the incubation period, the medium was removed and the epithelial cells were washed with PBS. The cells were then fixated for quarter of an hour in 2% glutaraldehyde at ambient temperature. The fixated cells were washed once more with PBS and staining was then performed. The staining agents were Alexa Fluor-488 phalloidin and acridine orange. A Leica SP5-II microscope was employed for confocal microscopic examination. In particular, fragmented DNA, condensed nuclei or changes in the cellular outer membrane or cytoskeletal framework were considered indicators of cellular toxicity[21].

Wound Healing Assay

Plates containing 6 wells were seeded with nasal epithelial cells, each well containing 30,000 cells. The incubation lasted 24 hours. A single layer of cells forming a confluent layer was noted. This layer was then disturbed by scratching with the sterile tip of a pipette, of the size used to transfer between 20 and 200 microlitres of fluid. The scratched cultures were washed with PBS. Control cells had 3 ml of fresh medium applied, whilst the test cells had 3 ml of medium plus cambogia extract. All these wells were visualised by light microscopy to record the initial appearances. The light microscope was then used at 8-hour intervals to record progression of healing. Non-scratched epithelial cells formed a further control group[22].

Statistical Analysis

A statistical analysis was undertaken. One-way analysis of variance (ANOVA) was employed for multiple comparisons. All calculations were performed in the GraphPad prism 6.0 for Windows software, manufactured by GraphPad Software Inc. (La Jolla, CA, USA).

Results

MTT Assay Results

No evidence of toxicity arising from topical application of *G. cambogia* was seen. The primary nasal epithelial cells remained fully viable after 24 hours' incubation. Although there was no increase in cellular viability at the level of statistical significance, there was a slight increase in the rate of cellular proliferation within the incubation period ($p>0.05$) (Figure 1).

Results of Confocal Microscopic Examination

The results obtained using confocal microscopy are in agreement with those seen in the MTT assay. The epithelial cells which were not exposed to the extract (i.e., control cultures) exhibited a compact morphological appearance. The overall shape of the cell was fusiform, and the nucleus possessed a clear boundary (see Figure 2A). The epithelial cells that were incubated with the *G. cambogia* extract were also seen to possess a compact morphology. They exhibited no indications of damage to the nuclear envelope or the cytoskeletal elements. Thus, it appears the extract is not cytotoxic and does not impair cellular viability. These findings accord well with the results of the MTT assay (Figure 2B).

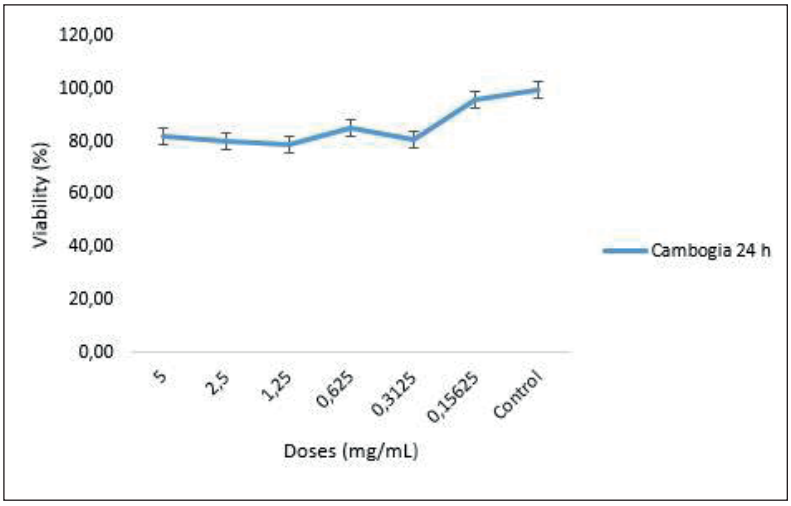

Figure 1. Curve of viability of primary nasal cells exposed to different concentrations of G. cambogia for 24 hours.

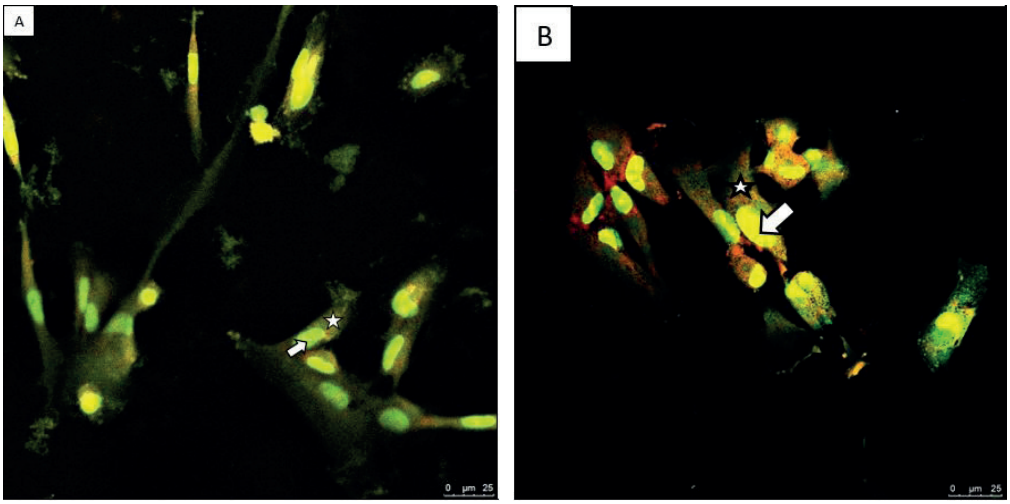

Figure 2. Confocal microscopy images of primary cultured nasal cells. **A**, Control group and test cells exposed to *G. cambogia* (**B**). Arrows indicate nucleus, whereas asterisks indicate the cytoskeleton.

Wound Healing Assay Results

The width of the induced scratch injury was noted prior to and following exposure for 24 hours to the *G. cambogia* extract. The area denuded of cells by the pipette tip had been partially re-covered by epithelial cells at the end of the 24 hours incubation period. The initial and final appearances are shown in Figures 3A and 3B, and 4A and 4B, respectively. The appearances at the end of the 24 hours in which the epithelial cells were in contact with the *G. cambogia* extract suggest that this extract neither impairs nor increases cellular proliferation rates (Figure 4B).

Discussion

The plant previously and still frequently referred to in the scientific literature as *Garcinia cambogia* (Gaertn.) Desr. (Clusiaceae), is now more properly termed *Garcinia gummi-gutta* (L.) Roxb. It may also be called the Malabar tamarind. This species is indigenous to Southeast Asia. The rind of the fruit is often utilised in preserving food, adding flavour or increasing the volume of food[23]. It has been used medicinally to remedy constipation, haemorrhoids, joint pains, swelling and irregularity of the menstrual cycle. It is also

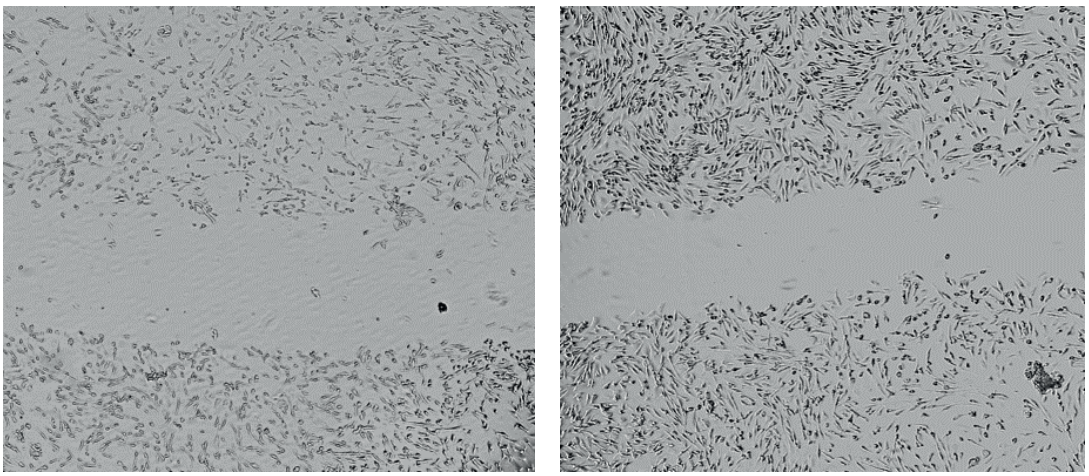

Figure 3. Wound healing images of primary nasal cells at the initial stage (0^{th} hour). **A**, Untreated (Control) cells and cells exposed to *G. cambogia* (**B**).

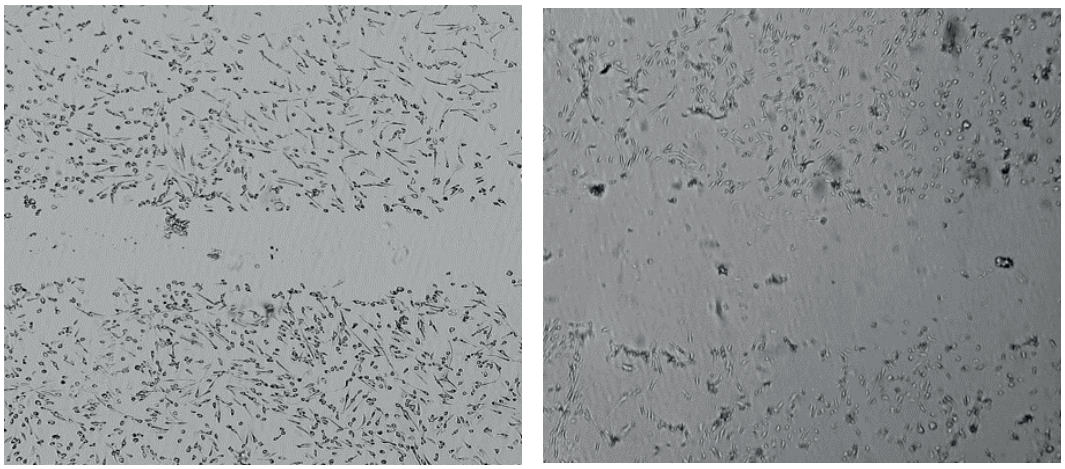

Figure 4. Wound healing images of primary nasal cells at the end of incubation period, i.e., at 24th hour. **A**, Untreated (Control) cells and cells exposed to *G. cambogia* (**B**).

employed in treating parasitic intestinal infections across Asia[24]. Phytochemical analysis has identified the following as compounds of interest: organic acids[25], benzoyl benzene[26] and 9-oxoxanthene[27] and related molecules. These compounds appear to possess bioactivity with potential benefit in obesity[19,20], hyperlipidaemia[28] and malignancy[29], as well as other conditions.

Dietary supplements manufactured from *G. cambogia* are widely sold. They are promoted as enhancing weight loss. However, with increasing use, there have been reports in the USA of acute hepatitis and even acute liver failure, for which hepatic transplantation was required[30-32]. The compound responsible for hepatic toxicity has been speculated to be HCA and this is now amongst the compounds blacklisted by the US FDA on account of potential liver toxicity. The same agency has also advised against use of certain preparations where *G. cambogia* is a constituent, since these products were contaminated with sibutramine[33]. Sibutramine is itself hepatotoxic and has been denied marketing authorisation due to toxic effects on the cardiovascular system[34].

G. cambogia-containing dietary supplements usually consist of between 20 and 60% HCA. They also frequently contain constituents derived from sources other than just *G. cambogia*[35]. The varied nature of *G. cambogia* extracts needs to be borne in mind when making judgements about quality, safety, and efficacy. There are numerous reports claiming that many such extracts contain less HCA than the normally expected concentration[36].

However, it is HCA which is the key constituent of *cambogia* extracts in terms of promoting weight loss[37,38]. HCA is an α-, β-dihydroxy tricarboxylic acid[37]. Between 10 and 30% of the fruit consists of HCA, which occurs as an unconjugated acid, as hydroxycitrate or in lactone form[39]. HCA is marketed in the form of calcium, magnesium or potassium hydroxycitrate[40]. HCA is also synthesised by a number of different types of bacteria. It may be extracted in future from a bacterial source[41]. HCA may be formed in the laboratory by using citric acid as feedstock. To achieve this conversion, a water molecule is removed from citric acid, resulting in aconitic acid, which is then oxidised to HCA[42]. The mechanism by which HCA contributes to weight loss is through inhibition of a key enzyme in aliphatic acid lipogenesis, ATP citrate lyase. This enzyme catalyses the breaking down of citrate into oxaloacetate and acetyl-CoA. The latter is a building block for manufacturing aliphatic acids[43].

It has been demonstrated by Bilal et al[44] that, although rats fed with a specially designed diet resulted in atherosclerosis. On the contrary, feeding the animals *G. Cambogia* 4.5% w/w reduced the development of obesity and atherosclerosis[44]. This effect potentially occurred *via* an increase in circulating non-esterified fatty acid levels, reflecting raised lipolysis. The same researchers also discovered later[28] that providing *G. cambogia* as 4.5% of the total dietary mass for a period of 30 days to rats resulted in raised circulating apolipoprotein A1 and the overall cholesterol level. Apolipoprotein A1 is protective against atherogenesis. The ratio of apoliprotein A to B was unaffected. When a similar *G. cambogia*-containing diet was fed to rats, metabolism of lipids and proteins by the heart was reduced[45].

One study[46] looked at the effects of *G. cambogia* fruit rind extract on inflammatory bowel disease. Rats had colitis artificially induced using TNBS (2,4,6-trinitrobenzene sulfonic acid). A preparation administered at a dose of 500 or 1,000 mg/kg, and containing 51.2% of the negative isomer of HCA resulted in improvements visible to the naked eye, as well as lower levels of expression of MPO, COX-2 and iNOS. There were also lower levels of PGE2 and IL-1β in the animals' colon. There was no evidence of toxicity[46]. The authors concluded that *G. cambogia* may offer benefit in treating inflammatory disorders of the intestines secondary to imbalance of the immune system within the mucosa[7].

The objective of the current study was to ascertain whether cytotoxicity occurs when *G. cambogia* extract is applied to nasal epithelial cells in cell culture. Our findings suggest that *G. cambogia* does not cause cytotoxicity in primary epithelial cells. Incubation with the fruit extract did not diminish the viability of cells after 24 hours. Although viability was not increased at the level of statistical significance, there was a mild increase in proliferation within the incubation period. This increase was evident on both MTT assay and confocal microscopy. The confocal microscopic appearances of the control epithelial cells, namely compact morphology, clear nuclear outline and intact cytoskeletal framework were the same as those seen in the epithelial cells exposed to *cambogia*. This finding implies that cytotoxic effects do not occur.

The findings from the scratch assay depend upon light microscopy-guided interpretation of the extent to which the epithelial cells re-close the denuded area within the 24-hour incubation exposure. The findings here were that the denuded area was re-covered to the same extent by control and exposed cell cultures. Thus, the extract neither increases nor decreases cellular proliferation.

There are a number of phytochemical compounds found in *G. cambogia* with the potential to suppress inflammation. Garcinol (5 μM) can prevent NF-κB and/or JAK/STAT-1 from being activated. This effect was shown in RAW264.7 macrophages in mice which had been activated by exposure to bacterial lipopolysaccharides[47]. Garcinol also suppresses the synthesis of iNOS and COX-2 in histiocytes rendered active by lipopolysaccharide exposure, as well as suppressing the production of reactive oxygen species within the cell. When 1μ M garcinol 40-50% was administered to the cell lines HT-29 and HCT-116, which are derived from human adenocarcinomas, and IEC-6, derived from immortalised normal rat gut cells, these cell lines no longer synthesised the products of arachidonic acid metabolism. It is hypothesised that these metabolic alterations occur due to an inhibitory effect on the addition of phosphoryl groups to cPLA2 (hence lower levels of arachidonic acid metabolites). Furthermore, STAT-1 does not become active, which then reduces synthesis of inducible nitric oxide synthase[48].

It has been previously shown that an extract of *G. cambogia* fruit rind is an effective antioxidant *in vitro*. This has been demonstrated using several different assays, i.e., DPPH, hydroxyl radical scavenging, total peroxyl radical trapping and lipid peroxidation[49]. For these assays the IC_{50} for the fruit extract were 36, 50, 44 and 62 μg/ml, respectively. For comparison, the following values of IC_{50} are available: for ascorbate in the DPPH and lipid peroxidation assays, 10 and 24 μg/ml, respectively; for quercetin in the hydroxyl radical assay, 36 μg/ml; and for TROLOX in the peroxyl radical assay, 18 μg/ml. These authors[49] hypothesised that the antioxidant activity arose due to the presence of phenol moieties. A later study by Shivakumar et al[50] demonstrated antioxidant abilities *in vitro* for a *G. cambogia* fruit extract prepared using a hydroalcoholic or ethanolic extraction method[50]. The researchers employed the DPPH, hydroxyl radical and ferric thiocyanate assays. There was 79% inhibition of DPPH using the hydroalcoholic extract, or 87% with the ethanolic extract. In both cases the concentration was 300 μg/ml. In comparison, 94% inhibition was noted with ascorbic acid, used at the same concentration.

G. cambogia extracts prepared using ethyl ethanoate, ethanol or a hydroalcoholic extraction method have been demonstrated to possess antibacterial action. They are capable of inhibiting the growth of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*. One study found that these extracts produced a zone of inhibition on culture media ranging from 15 to 34 mm across[50]. The extract of highest potency was prepared using ethyl ethanoate, with the ethanol only or hydroalcoholic preparations somewhat less effective in bacterial inhibition. However, an extract prepared using hexane did not have any antibacterial activity, regardless of the bacterium involved. Furthermore, the aqueous and ethanolic extracts of fruit rind also possessed an inhibito-

ry action versus HIV-1 protease and HIV-1 integrase, which are enzymes of major importance in the pathogenesis of HIV. With an aqueous extract, the IC_{50} for HIV-1 protease was 67 μg/ml and 70 μg/ml for the viral integrase. An extract prepared using ethanol had lower potency, the IC_{50} value being 100 μg/ml for either enzyme[51].

So far there have been no other reports of studies investigating how a *G. cambogia* extract can be utilised in the nasal interior and how toxic or otherwise *G. cambogia* is to nasal epithelial cells. However, this plant is of significant medical interest to rhinologists, as it possesses antioxidant[49,50], antimicrobial[50,51] and anti-inflammatory actions[47,48]. Thus, it may be suitable for use in acute or chronic inflammatory conditions affecting the nose and sinuses. This study provides the useful information that local toxicity to *G. cambogia* does not appear to occur within the nasal epithelium.

Conclusions

It is proposed that *G. cambogia* may offer clinical benefit as a supplementary topical treatment for inflammation of the nose and sinuses, as occurs in both chronic and acute rhinosinusitis, as well as with nasal polyps. This is because the plant appears to slightly increase nasal epitheliocytic proliferation, as revealed by the MTT assay, whilst exhibiting no indications of a cytotoxic effect on epithelial cells of the nose.

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Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Approval
This is a cell-culture study. Ethics committee approval was not needed.

Informed Consent
Human primary nasal epithelium was obtained from healthy tissue removed routinely as part of surgery (septo-rhinoplasty) from individuals who gave written consent for their tissue to be used in scientific research.

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Authors' Contributions
Muhammet Dilber: Planning, designing, literature survey, interpretation of the results, active intellectual support, submission.
Nuray Bayar Muluk: Planning, designing, literature survey, interpretation of the results, active intellectual support, writing.
Canan Vejselova Sezer: Planning, designing, data collection, literature survey, interpretation of the results, active intellectual support.
Hatice Mehtap Kutlu: Planning, designing, data collection, performing the study, literature survey, interpretation of the results, active intellectual support, writing.
Cemal Cingi: Planning, designing, literature survey, performing the study, interpretation of the results, active intellectual support, English editing.

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