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In vitro antioxidant and cytochrome p450 inhibitory activity of dark chocolate mediated zinc oxide nanoparticles

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ABSTRACT

Dark chocolate provides enormous health benefits owing to its enriched antioxidants, such as polyphenols and flavonoids, which can encounter free radicals in the body. These antioxidants can lower blood cholesterol, lowering your risk of heart attacks, cancer, high blood pressure, and stroke. This study was aimed to investigate both the free radical scavenging property and inhibitory property of dark chocolate mediated zinc oxide nanoparticles on activity of cytochrome P450 (CYP) isoform. The DC-ZnO was evaluated against free radicals formation using DPPH and ABTS free radical assays. Besides, the ZnO nanoparticles were also evaluated for CYP34A inhibitory activity. From the results it was demonstrated that there was a dose dependent increase in the inhibition of DPPH and ABTS free radicals and CYP3A4 inhibitory activity by the dark chocolate formulation mediated zinc oxide nanoparticles. The maximum inhibitory activity was observed at maximum concentration of 160µL. It may be thus concluded that the synthesized dark chocolate mediated zinc oxide nanoparticles possess potent antioxidant activity with significant inhibitory effect on Cytochrome P3A4 enzyme.

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INTRODUCTION

Chocolate is a confection made up of cocoa mass, cocoa butter, and sugar. Dark chocolate, milk chocolate, and white chocolate are the three varieties of chocolate. With a cacao concentration of more than 60%, dark chocolate is one of the" chocolate couverture's products. Dark chocolate provides health benefits because of its antioxidant enrichment, such as polyphenols and flavonoids that have been reported to inhibit free radical formation in the body. These antioxidants have shown to possess potential health benefits against various diseases and disorders including diabetes, cardiovascular disorders, cancer, neurodegenerative diseases etc., (1). Chocolate is processed from its main source cocoa beans that have high polyphenols content in it. However, during the processing steps the polyphenols are damaged, oxidized, and their bioavailability is decreased. Therefore in order to avoid the progressive decrease in polyphenol content and to improve the bioavailability, nanotechnology may be applied (2). The notion of preserving the protection of hydrophobic and hydrophilic substances allows for the use of materials with nanoscale levels ranging from 1 to 100 nanometers, improving their properties, stability, and availability, particularly in the delivery system (3).

KEYWORDS:
Dark chocolate, Zinc oxide, DPPH, ABTS, CYP3A4, inhibitions

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Zinc oxide (ZnO) Nps have recently attracted a lot of interest because of their potential health benefits. ZnO Nps possesses strong pharmacological effects, including anti-cancer, anti-microbial, and antioxidant activity, according to published studies (4). Our team has extensive knowledge and experience in this research area that has been translated into high quality publications (5-14). Further, owing to its nutritional and reported potentials, dark chocolate was selected to synthesize ZnO. Further the present study investigated the antioxidant property of the dark chocolate synthesized ZnO nanoparticle and also evaluated the enzyme inhibitory activity of synthesized ZnO nanoparticles on cytochrome P450 isoform.

MATERIALS AND METHODS

Antioxidant Assay

Free radical scavenging activity of dark chocolate mediated ZnO nanoparticles was determined by DPPH and ABTS radical scavenging assays described earlier (15,16).

DPPH Free radical scavenging assay

In DPPH radical scavenging assay, 10µL different concentrations of the synthesized nanoparticles (5, 10, 20, 40, 80 & 160µL) was added to 190µL of DPPH (150µM prepared in ethanol). The reaction mixture was shaken thoroughly and incubated in dark for 30min at 37°C. After incubation, the absorbance was measured at 517 nm using Biotek synergy H4 hybrid microplate reader, USA. The reaction mixture without the nanoparticle was used as control and ascorbic acid was used as standard. The % inhibition of DPPH free radical formation was calculated as follows: [(Control - Test)/Control]*100

ABTS radical scavenging assay

The ABTS (2,2'-azino-di [3-ethylbenzthiazoline sulphonate]) assay was performed as follows: 10µL different concentrations of the synthesized nanoparticles (5, 10, 20, 40, 80 & 160µL) was added to 10µL of metmyoglobin and 150µL of 2mM ABTS. The reaction was initiated by adding 40µL of H202 (441µM). The reaction mixture without the test drug was kept as control and ascorbic acid was used as standard. The absorbance was read at 690 nm using Biotek synergy H4 hybrid microplate reader, USA. The % inhibition of ABTS radical formation was calculated as follows: [(Control - Test)/Control]*100

CYP34A inhibitory activity

Briefly, various concentrations of the synthesized nanoparticle, potassium phosphate buffer, CYP450 reagent and substrate 7-Benzyloxy-4-trifluoromethylcoumarin (BFC) were added to a 96-well plate. The mixtures were pre - incubated for 20 min at room temperature. The reaction was started by a mixture of reconstituted substrate and NADP+ and incubated at room temperature for 30-60 min. The reaction was stopped by the Tris-HCl buffer, pH 10.5. The fluorescent intensities of the products were measured by Biotek synergy H4 hybrid microplate reader using an excitation and emission wavelength of 405 nm and 460 nm, respectively.

RESULTS

DPPH and ABTS radical scavenging assay

The absorption maxima of dark chocolate mediated zinc oxide nanoparticles was found at 365 nm which confirmed the formation of ZnO nanoparticles (Figure 1). By using the DPPH method, the antioxidant activity of zinc oxide nanoparticles mediated by dark chocolate was examined. As shown in Figure 2, the dark chocolate mediated zinc oxide nanoparticles showed significant and dose dependent inhibition on free radical formation. At 5µL concentration the zinc oxide nanoparticles showed free radical inhibition of 5% and a maximum of 79 % inhibition was obtained at 160µL concentration (Figure 2). Similarly in the ABTS assay, the free radical scavenging activity of ZnO nanoparticles was found to be significantly high at its maximum concentration volume of 160µL concentration. A dose - dependent inhibition was found ranging from 10% to 80% from 5µL - 160µL concentration (Figure 3).

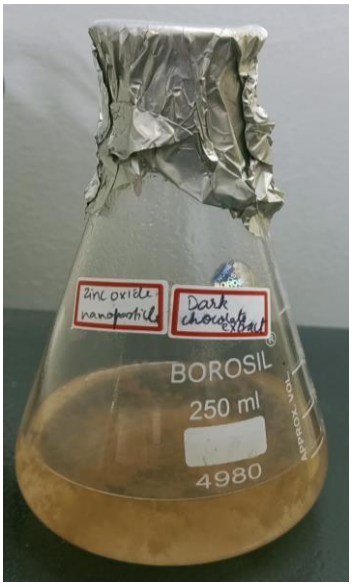

Fig.1 showing the synthesized dark chocolate mediated zinc oxide nanoparticles

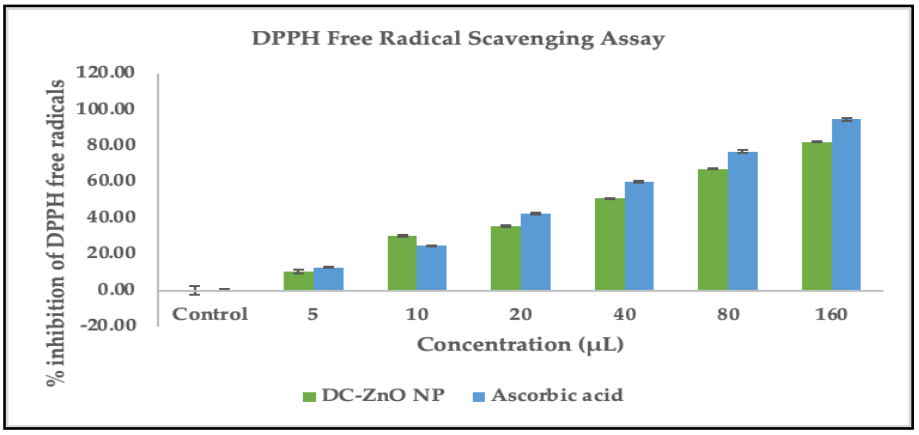

Fig.2: Effect of dark chocolate mediated zinc oxide nanoparticles on DPPH free radical formation

Data expressed as Mean±SEM (n=3)

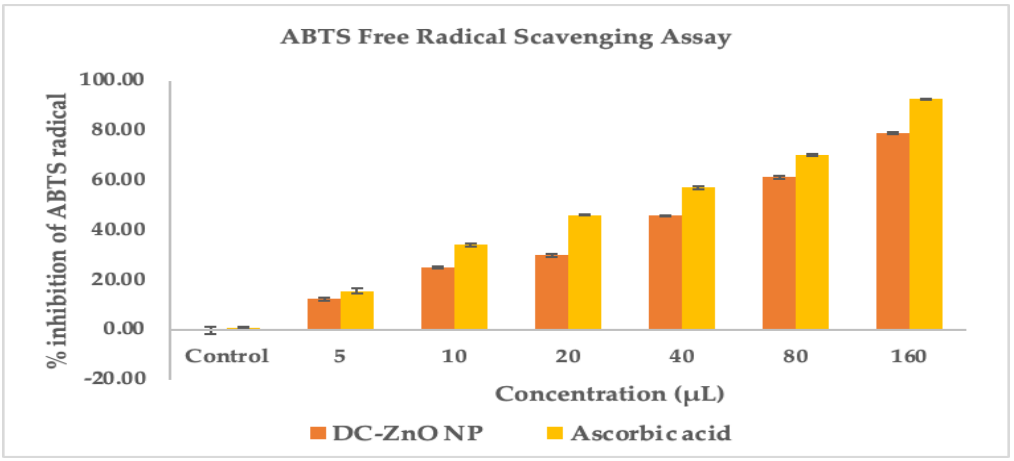

Fig.3: Effect of dark chocolate mediated zinc oxide nanoparticles on ABTS radical formation
Data expressed as Mean±SEM (n=3)

Cytochrome (CYP3A4) inhibitory activity

The results of the in vitro cytochrome inhibitory activity of DC-ZnO nanoparticles showed that the nanoparticle on increasing concentration significantly inhibited the CYP34A enzyme activity (Figure 4).

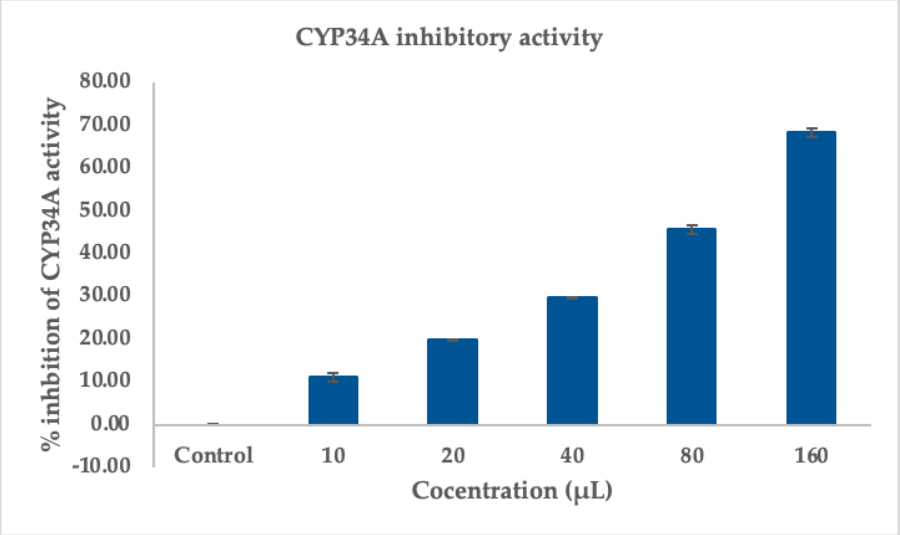

Fig.4: Inhibitory potential of DC-ZnO NP on CYP3A4 activity
Data expressed as Mean±SEM (n=3)

DISCUSSION

Worldwide, a lot of people use items made from botanical medicine. By 2050, the anticipated global market for medicinal plants is expected to exceed $5 trillion, demonstrating the growing acceptance of botanical medicine (17). Although plant based herbal drugs are easily available and possess potent health benefits, these drugs may not be necessarily safe. Concomitant use of herbal drugs and allopathic medicine may result in unwanted herb-drug interactions and is of major clinical concern (18). In vitro CYP assays offer an accurate, and relatively inexpensive, first stage assessment tool for gauging the potential for herb-drug interactions. They are useful for initial risk assessment of medicinal plants capable of causing adverse drug reactions (ADRs) when taken concomitantly with pharmaceutical drugs metabolized by the same enzyme (19). This may help clinicians and patients to avoid concomitant use of herbs and drugs that may lead to potential or actual herb-drug interactions. CYPs 1A2, 2D6, and 3A4 are three of the key enzymes involved in drug metabolism. The present study results showed that the green synthesized dark chocolate mediated zinc oxide nanoparticles significantly inhibited the enzyme activity of CYP34A. The significant CYP34A inhibitory activity of many of the herbal plants have shown to be attributed to their secondary metabolites, particularly the polyphenols (20). In accordance with the CYP34A inhibitory activity observed in the present study may also be attributed to the flavonoids present in dark chocolate.

Further, the ability of polyphenols to chelate metals and their capacity to reduce or inhibit certain enzymes can both be used to determine their bioactivity (21). Furthermore, multiple techniques must be utilized to test a chemical's antioxidant activity because the antioxidant qualities rely on the kind of solvent used in the extraction and the complexity of the compound (15). In accordance with this in the present study we have used both ABTS and DPPH free radical scavenging assay to substantiate the antioxidant capacity of the DC-ZnO nanoparticles. Antioxidant-active polyphenolic substances typically have many functions and operate via most of these mechanisms. Hence it may be suggested that the polyphenol and the flavonoid content present in the dark chocolate may be the determinants for its antioxidant activity as well as the CYP inhibitory activity.

CONCLUSION

The synthesized dark chocolate mediated zinc oxide nanoparticles showed potent antioxidant activity with significant inhibitory effect on Cytochrome P34A enzyme.The findings of this study imply that zinc oxide nanoparticles mediated by dark chocolate may potentially be utilized for further clinical and biomedical applications.

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Conflict of interest-NA

Informed Consent- None declared

Authorship contribution

P compiled the manuscript RVG conducted the study PRP designed the study

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