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Full Length Research Paper

Study on increasing the solubility and dissolution rate of sulfamethoxazole by cyclodextrins

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The aim of this study was to enhance the solubility and dissolution rate of sulfamethoxazole (SMZ) by preparing the inclusion complexes with β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HPβ-CD) and γ-cyclodextrin (γ-CD). The effect of the type of cyclodextrins used for the preparation of complexes, on the *in vitro* dissolution profiles and solubilities in different mediums (pH 4.5 and 7.0) was also evaluated. The interaction between SMZ and cyclodextrins in solution was studied by the phase-solubility method. Inclusion complexation was confirmed by the results from the studies of infrared spectoroscopy (IR) and differential scanning calorimetry (DSC). The effect of water-soluble polymers, that is, polyethylene glycols 4000, 10000, 20000 and non-ionic surfactants, that is, polysorbate 20, 40 and 60 on the complexation of SMZ with CD_s were also investigated by the same methods. The rates of release of the active material from the complexes were determined from dissolution studies using USP XXII paddle method. As a result of this study, it was found that the solubility of SMZ was significantly enhanced by inclusion of β-CD, especially when the water soluble additives are added (from 0.086 to 0.377 mg/ml with SMZ:β-CD:PEG20000).

Key words: Cyclodextrins, sulfamethoxazole, solubility, dissolution rate.

INTRODUCTION

Sulfonamides are bacteriostatic agents which are systematically used in the treatment of bacterial infections. SMZ, a derivative of sulfonamide, inhibits the synthesis of folic acid, which is an important metabolite of bacteria's DNA synthesis (Martindale, 1993; Raja et al., 2009). SMZ is absorbed from gastro-intestinal area; however, its absorption and bioavailability are limited with its dissolution rate, due to its low solubility like all the other sulfonamide groups. The aim of the study was to prepare the inclusion complexes of SMZ using CD_s to enhance the solubility, dissolution rate and oral bioavailability.

CD_s which are made up of 6D (+) glucopyranose units at minimum, with α 1-4 glycoside bonds and formed as a result of degradation of starch by the help of glucosyltransferase (CGT) enzyme, are cyclic oligosaccharides with hydrophobic cavity and hydrophilic outer surface (1).CD_s can be defined as enzymatically modified starch made of glucopyranose units (Szejtli, 1991a). The commonly available CD_s are α-, β- and γ-

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cyclodextrins, which consist of six, seven and eight glycopyranose units, respectively (Gabriel et al., 2016; Uekama and Otagiri, 1987). The more the glucose units, the bigger the dimensions of CD_s. CD_s are molecules with a polar hydrophilic outside, and an apolar hydrophobic cavity, which provides a guest-host relation to hydrophobic drugs in hydophilic medium which is called "inclusion complex" (Brewster and Loftsson, 2007; Jambhekar and Breen, 2016).

Some CD_s contain 9 and more glucose units, but they are not used since it is difficult to obtain them in pure form and they have low complex forming capacity. Those containing less than 6 units of glucose, on the other hand, cannot be prepared for steric reasons (Bekers et al., 1991; Fromming and Szejtli, 1994).

In these complexes, the drug (guest molecule) is entrapped in the cavity of the cyclodextrin (CD) (host). After the complexation, some physical properties of the guest molecule can be changed. For instance, the strength against oxidation, hydrolysis and photochemical reactions can increase, the evaporation speed of volatile substances can significantly decrease. Also when poor soluble drugs are in complex form, the solubility increases. For this reason, in order to enhance the water solubility of poor soluble substances, complex forming with CD's are widely used (Duchene and Woussidjewe, 1990; Sharma and Baldi, 2016; Sinha et al., 2007; Szejtli, 1988).

The stoichiometries of active material:cyclodextrin complexes and the numerical values of their stability or binding constants are frequently obtained the from the plots of drug solubility against cyclodextrin concentration. This phase-solubility technique was first developed by Higuchi and Connors (1965). Phase-solubility diagrams are categorized into A and B types. A type phase-solubility profiles are obtained when the solubility of the active material increases with an increase in cyclodextrin concentration. The A-type profile is further subdivided into three profiles. The A_L profile indicates that there is a linear increase in solubility as a function of cyclodextrin concentration; the A_P profile indicates an isotherm, wherein the curve deviates from linearity in a positive manner, suggesting that a ligand or a solubilizer is proportionally more effective at higher concentrations. Conversely, the A_N type relation indicates a negative deviation from linearity, which means that the cyclodextrin is proportionally less effective at higher concentration (Jambhekar and Breen, 2016).

In general, the water-soluble CD_s form A-type phase solubility profiles, whereas the less soluble natural CD_s generally form B-type profiles. Type B phase-solubility profiles indicate the formation of a complex with naturally occurring CD_s, in particular β-CD (Brewster and Loftsson, 2007).

It is accepted that water soluble polymers or non-ionic surfactants have significant effects on the formation of the inclusion compounds (Hirlekar et al., 2009; Loftsson et al., 1991, 1994, 1996). The effects of commonly used water soluble polymers, polyethylene glycol 4000 (PEG4000), polyethylene glycol 10000 (PEG10000), polyethylene glycol 20000 (PEG20000), hydroxypropyl methylcellulose (HPMC) and the non-ionic surfactants polysorbate 20 (PS20), polysorbate 40 (PS40), and polysorbate 60 (PS60) on the dissolution rate and solubility of SMZ from the inclusion complexes were also investigated.

In this study, the solid dispersions were prepared by coprecipitation method (Dhanaraju et al., 1998; Elgindy et al., 2011; Özdemir and Erkin, 2012; Polonosamy and Khonam, 2011; Patel and Rajput, 2009). The rates of release of the active material from the complexes were determined from dissolution studies using USP XXII paddle method (USP XXII, 1990).

MATERIALS AND METHODS

Sulfamethoxazole (SMZ) was obtained from Fako Medical (Turkey); in addition, the following were used in the study: β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HPβ-CD), γ-cyclodextrin (γ-CD) (Fluka), polyethylene glycol 4000 (PEG4000), polyethylene glycol 10000 (PEG10000), polyethylene glycol 20000 (PEG20000), hydroxypropyl methylcellulose (HPMC) polysorbate 20 (PS20), polysorbate 40 (PS40) and polysorbate 60 (PS60) (Merck). All other chemicals were of analytical grade.

Apparatus

UV spectrophotometer (Shimadzu UV 1202, Japan), IR spectrophotometer (Jasco FT/IR 420, Japan), differential scanning calorimetry (DSC) (Netzsch Geratebau DSC 204, Germany) and dissolution rate apparatus (Aymes, Turkey) were used.

Phase-solubility studies

Solubility measurements were performed by the method of Higuchi and solutions containing various concentrations of CD_s ranging from 2×10^{-3} to 15×10^{-3} M (the solubility of β-CD is 16.3×10^{-3} M) were shaken with 100 mg of SMZ in sealed flasks in a thermostate water bath at a constant temperature of 37°C (Loftsson and Duchene, 2007). After an equilibrium was attained (approximately after three days), aliquots were with drawn and filtered through 0.45 µm filters. A portion of the filtrate was then diluted with water and analyzed spectrophotometrically. The solubility constant and the ratio of SMZ:CDs in the complexes were calculated from the phase solubility diagram (Connors and Mollica, 1966; Higuchi and Connors, 1965).

The solubilizing effect of water-soluble polymers and non-ionic surfactants was also investigated with phase-solubility studies. For this purpose, an 100 mg active material was added to aqueous solutions containing 0 to 15% of CD_s and 0.50% (w/v) of water soluble polymers (PEG4000, PEG10000, PEG20000 and HPMC) and non-ionic surfactants (PS20, PS40 and PS60). After equilibration, which took at least three days took place at room temperature, the suspensions were filtered through at 0.45 µm membrane fitler and analyzed by UV spectrophotometer. The type of complexation and the apparent stability constants (K_c) of the drug : cyclodextrin complexes were calculated from the slope of the phase solubility diagrams (Loftsson et al., 1991, 1994, 1996).

Solubility studies

The cationic form of SMZ is the dominating form at pH below 1.5, while the nonionized form dominates between pH 2.0 and 6.0 and the anionic form dominates pH above 7.0. Although CD_s are able to solubilize both nonionized and the anionic forms of the drug, they have a small solubilizing effect on the cationic form. After taking these results into consideration, the pH of the mediums to be used for solubility and the dissolution studies were adopted to be either pH 4.5 or 7.0.

Excess amount of active material (more than it can be dissolved) was added to a closed flask with either pH 4.5 or 7.0 and then mixed in a magnetic mixer at 37°C. Thereafter, the liquid phase was filtered through 0.45 µm filters and the amount of active material in the solution was determined. Solubility of the active material was calculated by the point measured during the formation of the equilibrium status.

Preparation of inclusion compounds

Coprecipitation method given below was used to prepare SMZ's inclusion compounds with CD_s.

Coprecipitation method

SMZ was dissolved in acetone and added to the aqueous solution of CD at 1:1 molar ratio. The solvent was allowed to evaporate and then dried under vacuum at 40°C for 24 h and sieved to obtain the optimum particle size (Dhanaraju et al., 1998).

The solid complexes including solubility enhancer such as water-soluble polymers (PEG4000, PEG10000, PEG20000, HPMC) and non-ionic surfactants (PS20, PS40, PS60) were also prepared with the same method.

Characterization of complexes

The infrared spectroscopy (IR) of SMZ, β-CD and the inclusion complexes were measured with KBr discs at 400 to 4000 cm^{-1}. The amount of active material in each sample was kept constant in each measurement. Differential scanning calorimetry (DSC) was performed using a scanning rate of 10°C/min on a Netzsch Geratebau DSC 204. Samples were heated in a sealed aluminum pans from 50 to 300°C.

Dissolution rate studies

Active material release from all the inclusion compounds prepared were performed by using the USP XXII paddle method at 50 rpm in 37°C for three hours (USP XXII, 1990). It was done in 900 ml and at pH 4.5 and 7.0 mediums. Samples taken at certain intervals were measured spectrophotometrically.

RESULTS AND DISCUSSION

Phase solubility diagram

Data obtained from the phase solubility studies were used to calculate the apparent stability constant (Kc) according to the following equation:

$$K_c = S_t - S_o / [S_o (L_t - (S_t + S_o)] \quad (1)$$

S_t = total concentration of dissolved SMZ; S_o = molar solubility of SMZ in the presence of cyclodextrin; L_t = total cyclodextrin concentration.

A B_s type solubility graph was obtained with β-CD. The stoichiometric ratio of the complex determined from the descending part of the diagram was found to be 1:1 (SMZ:β-CD) and the stability constant was found as 122.3 M^{-1} (Figure 1). A type phase-solubility profiles are obtained when the solubility of the active material increases with an increase in cyclodextrin concentration. A_L type solubility graph was obtained by chemically modified HPβ-CD and the stability constant was too low (39 M^{-1}) (Figure 2). No significant increase in solubility of SMZ was observed with γ-CD and it was concluded that the inclusion complex was not formed (Figure 3). However, high stability constants of the complexes indicate high stability, very high values complicate the solubility of the drug. Comparing the stability constants, it was concluded that the β-CD was the best complex producing CD for SMZ. Thus, in all the remaining studies, only β-CD was used as host molecule (Duchene and Woussidjewe, 1990).

Solubility studies

At pH 4.5 (Figures 4 and 5) and pH 7.0 (Figures 6 and 7), the solubility of the active material was enhanced in the presence of water soluble polymers (PEG4000, PEG10000 and PEG20000) and non-ionic surfactants (PS20, PS40 and PS60) (Table 1). No significant increase in the solubility of active material with HPMC was observed. Hence, this result is not given in Table 1. As can be seen in Figure 5, a higher increase in solubility was observed when PS20 was used rather than PS40 and PS60.

The solubility of SMZ was significantly increased with the addition of β-CD from 0.086 to 0.175 mg/ml at pH 4.5 and 0.188 mg/ml at pH 7.0, respectively. When PEG4000, PEG10000 and PEG20000 were used with SMZ:β-CD, the solubility increase were greater than the SMZ:β-CD alone (Figures 4 and 6). When the effects of polymers and surfactants on the solubility of active material were examined, it was found that the solubility of SMZ was increased by 4.4 times with the formulation including SMZ:β-CD:PEG20000 (from 0.086 to 0.377 mg/ml at pH 4.5 and to 0.406 mg/ml at pH 7.0) as compared to pure SMZ. The higher solubility (0.377 mg/ml at pH 4.5 and 0.406 mg/ml at pH 7.0) were obtained with PEG20000 than the other PEG_s because of higher hydrophilic nature. The solubility values of SMZ from SMZ:β-CD complexes including PEG10000 or PEG4000 were 0.287 mg/ml at pH 4.5 and 0.193 mg/ml at pH 7.0 for PEG10000 and 0.241 mg/ml at pH 4.5 and 0.100 mg/ml at pH 7.0 for PEG4000, respectively. Furthermore besides the solubility increase in both pH 7.0 and 4.5, there was found, a slight difference between

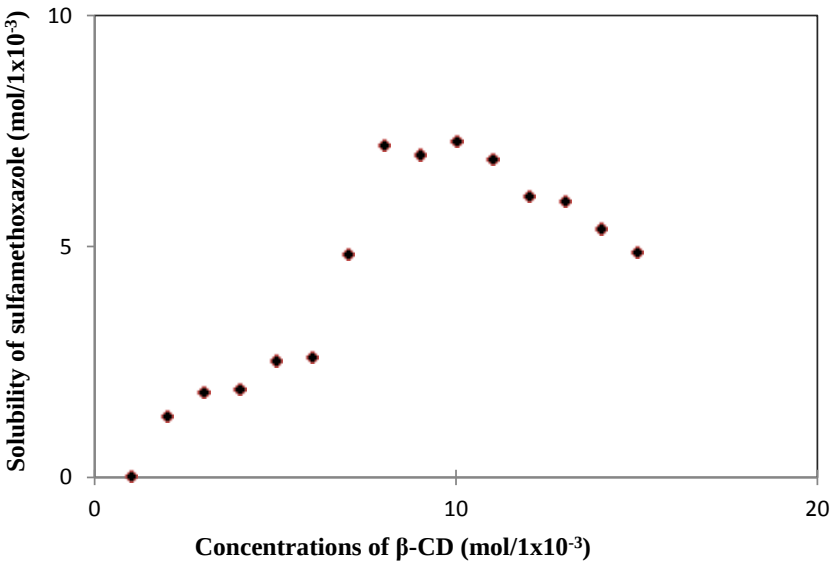

Figure 1. Phase-solubility diagram of SMZ:β-CD system in distilled water.

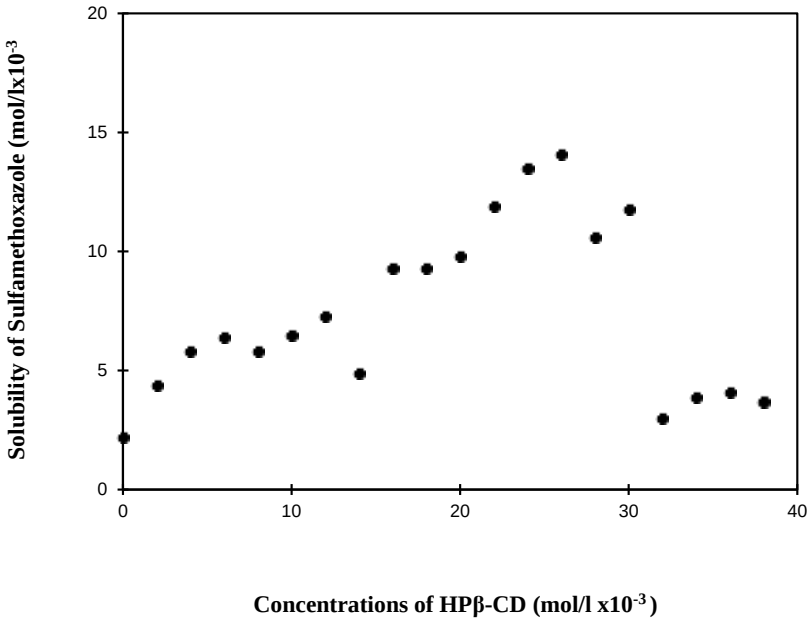

Figure 2. Phase-solubility diagram of SMZ:HPβ-CD system in distilled water.

two mediums and the higher solubility observed in the pH 7.0 medium was attributed to the presence of SMZ in anionic form. The results are agreement with the results published by other authors (Jambhekar and Breen, 2016).

The characterization of inclusion compound formation

Infrared spectrophotometers (IR) and differential scanning calorimetry (DSC) were used to prove the formation of inclusion complexes between the active material and β-CD. The characteristic bonds of SMZ at 1599 ($-SO_2NH$) and 1306 ($-SO_2$) which were observed by IR, seemed to disappear in the inclusion compounds or become modified significantly (Figure 8).

The observed decreases in the intensities of the characteristic bonds of SMZ may be due to its restriction within the β-CD cavity.

Figure 9 shows the thermogram of SMZ, β-CD and the

Table 1. The solubility values of sulfamethoxazole from the inclusion complexes at 37°C.

Formulations	Solubility (mg/ml)	
	pH 4.5	pH 7.0
Sulfamethoxazole	0.086	0.124
Sulfamethoxazole:β-CD (1:1)	0.175	0.188
Sulfamethoxazole:β-CD:PEG20000 (1:1)	0.377	0.406
Sulfamethoxazole:β-CD:PS20 (1:1)	0.349	0.386

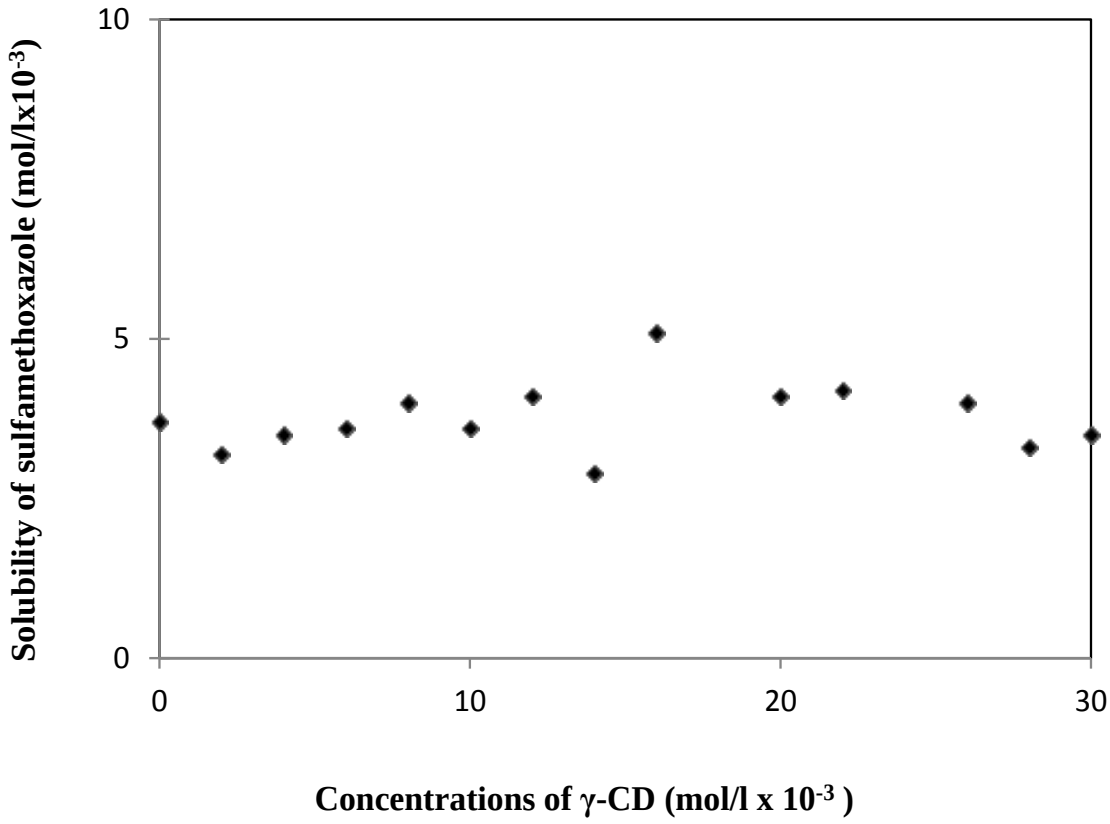

Figure 3. Phase-solubility diagram of SMZ:γ-CD system in distilled water.

inclusion complexes obtained from DSC measurements. The β-CD displayed no peaks in the temperature range between 50 and 300°C, while the SMZ exhibited its characteristic endothermic peak associated with the melting point of the drug around 171°C. Although, inclusion complexes showed broad peaks, the peak of the SMZ disappeared especially in the presence of PEG20000 and PS20 without distinct phase transition around this temperature.

The SMZ:β-CD coprepicipitated complex showed a less intense peak. The absence of the peak in DSC curves may be considered as a strong indication of the inclusion formation of the drug into the β-CD cavity.

Studies on dissolution rate

The studies conducted at pH 4.5 conditions, in which active material exists in non-ionic form, demonstrated an increase in dissolution rates of complexes as compared to pure active drug rates. On the other hand, adding water soluble polymers (PEG4000, PEG10000 and PEG20000) to the formulation by 0.50% (w/v) increased the drug release as compared to the pure drug. 4.7% of pure SMZ were dissolved within 30 min, whereas the measured values for the formulations including water soluble polymers coded as β-CD:SMZ, β-CD:SMZ:PEG4000, β-CD:SMZ:PEG10000 and β-

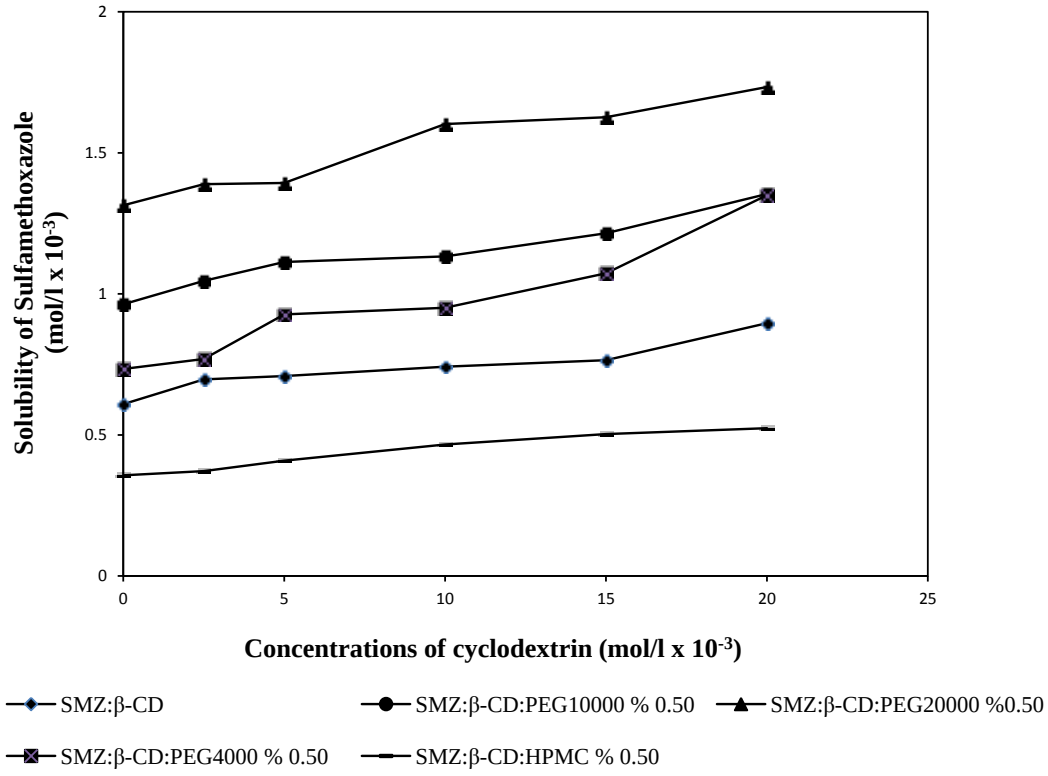

Figure 4. Effect of water soluble polymers (PEG4000, PEG10000, PEG20000 and HPMC) (0.5% w/v), on the solubility of SMZ at pH 4.5.

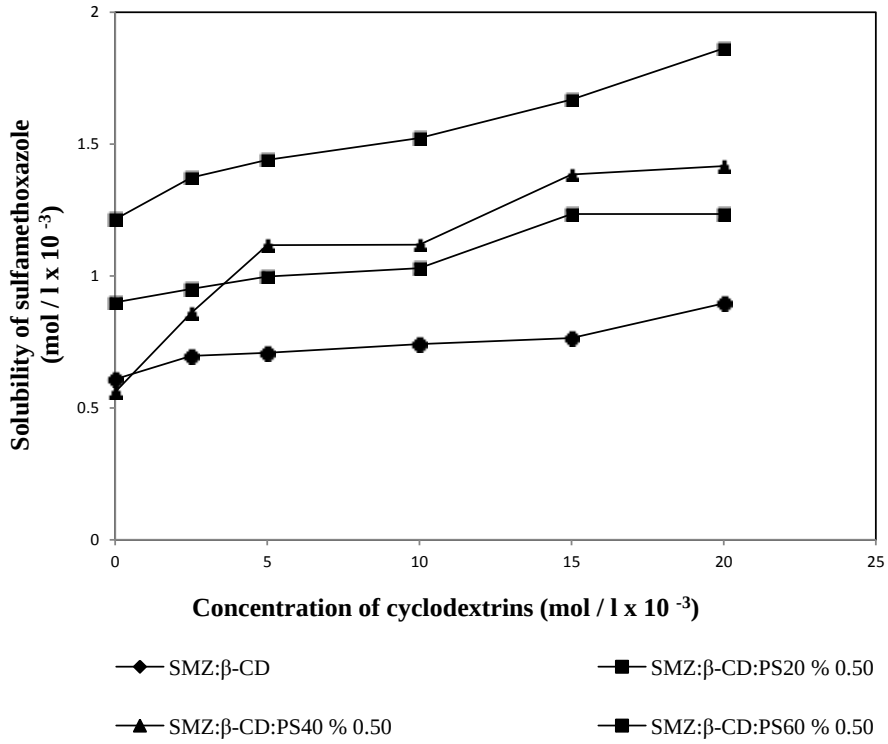

Figure 5. Effect of non-ionic surfactants (PS20, PS40 and PS60) (0.5% w/v), on the solubility of SMZ at pH 4.5.

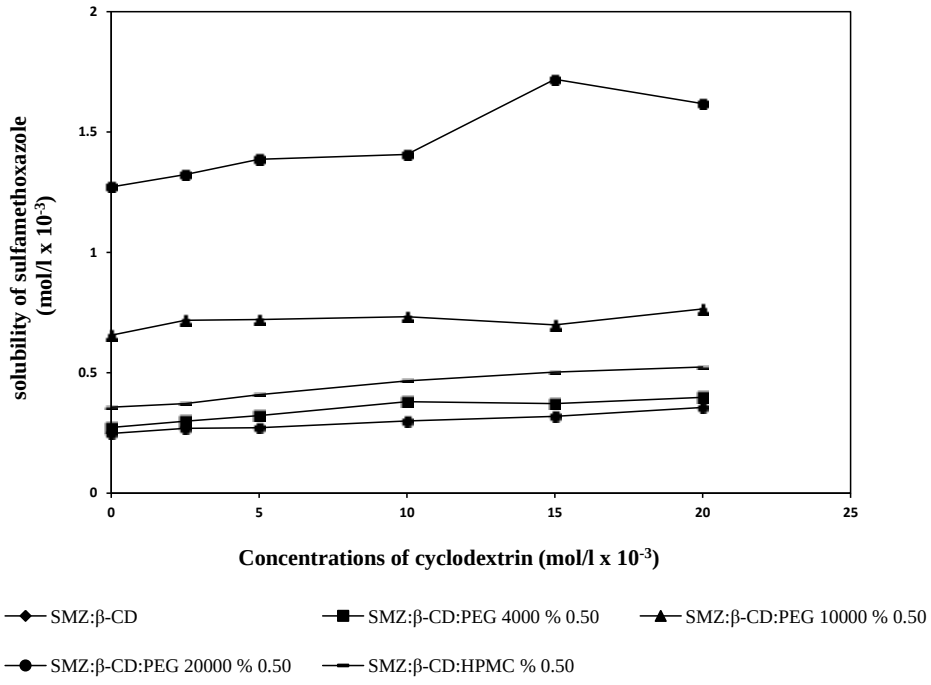

Figure 6. Effect of water soluble polymers (PEG4000, PEG10000, PEG20000 and HPMC) (0.5 % w/v), on the solubility of SMZ at pH 7.0.

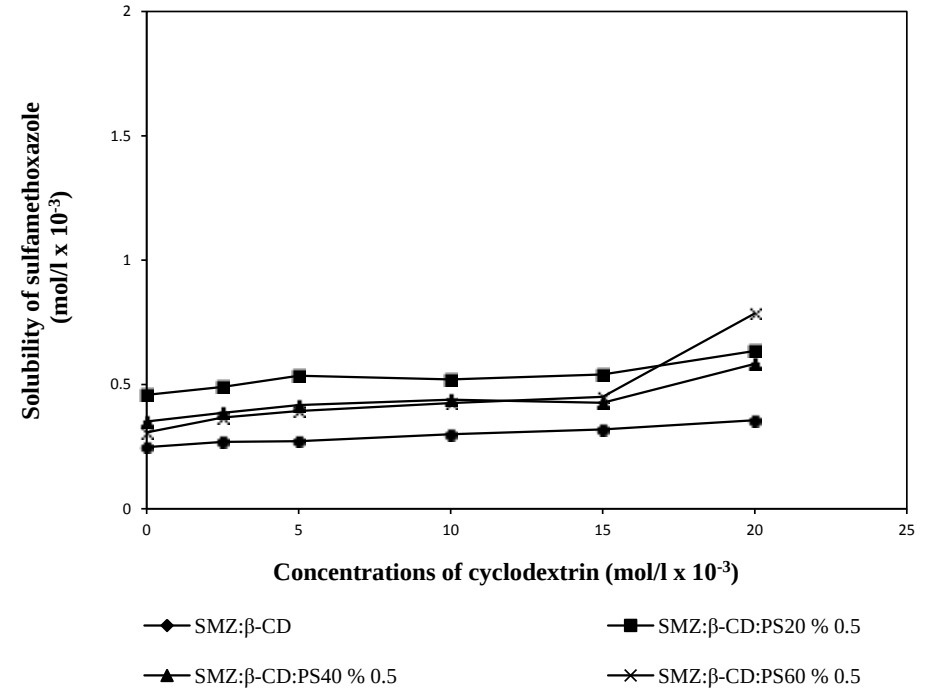

Figure 7. Effect of non-ionic surfactants (PS20, PS40 and PS60) (0.5% w/v) on the solubility of SMZ at pH 7.0.

CD:SMZ:PEG20000 were 38.0, 22.5, 38.0 and 41.7%, respectively (Figure 10). It was found that, as the PEG molecule weight increases, so does the active material release. The effect of non-ionic surfactants (PS20, PS40

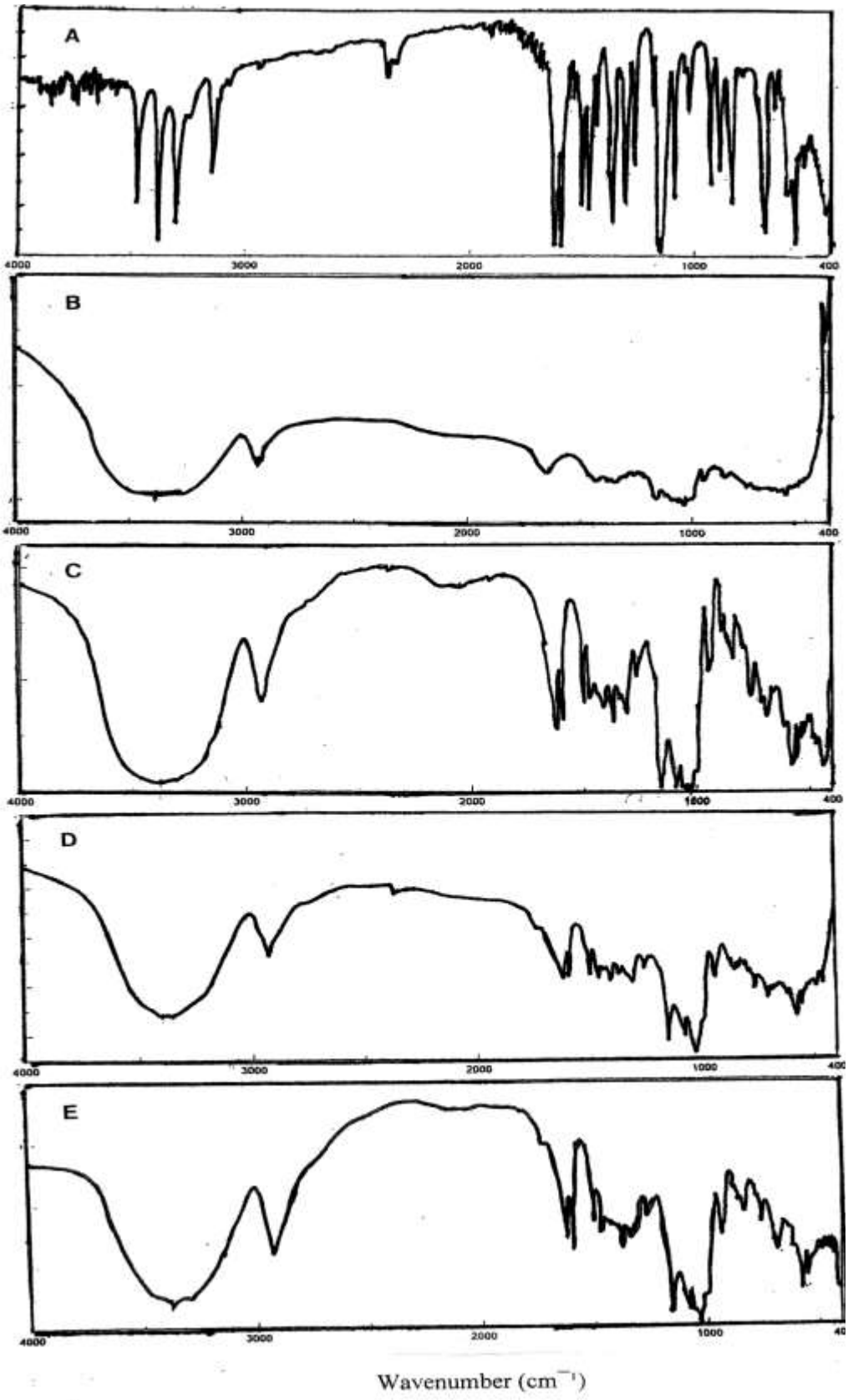

Figure 8. The IR spectra of, A) Sulfamethoxazole, B) β-Cyclodextrin, C) SMZ:β-CD (1:1) coprecipitated inclusion complex, D) SMZ:β-CD:PEG20000 (1:1) coprecipitated inclusion complex, E) SMZ:β-CD:PS20 (1:1) coprecipitated inclusion complex.

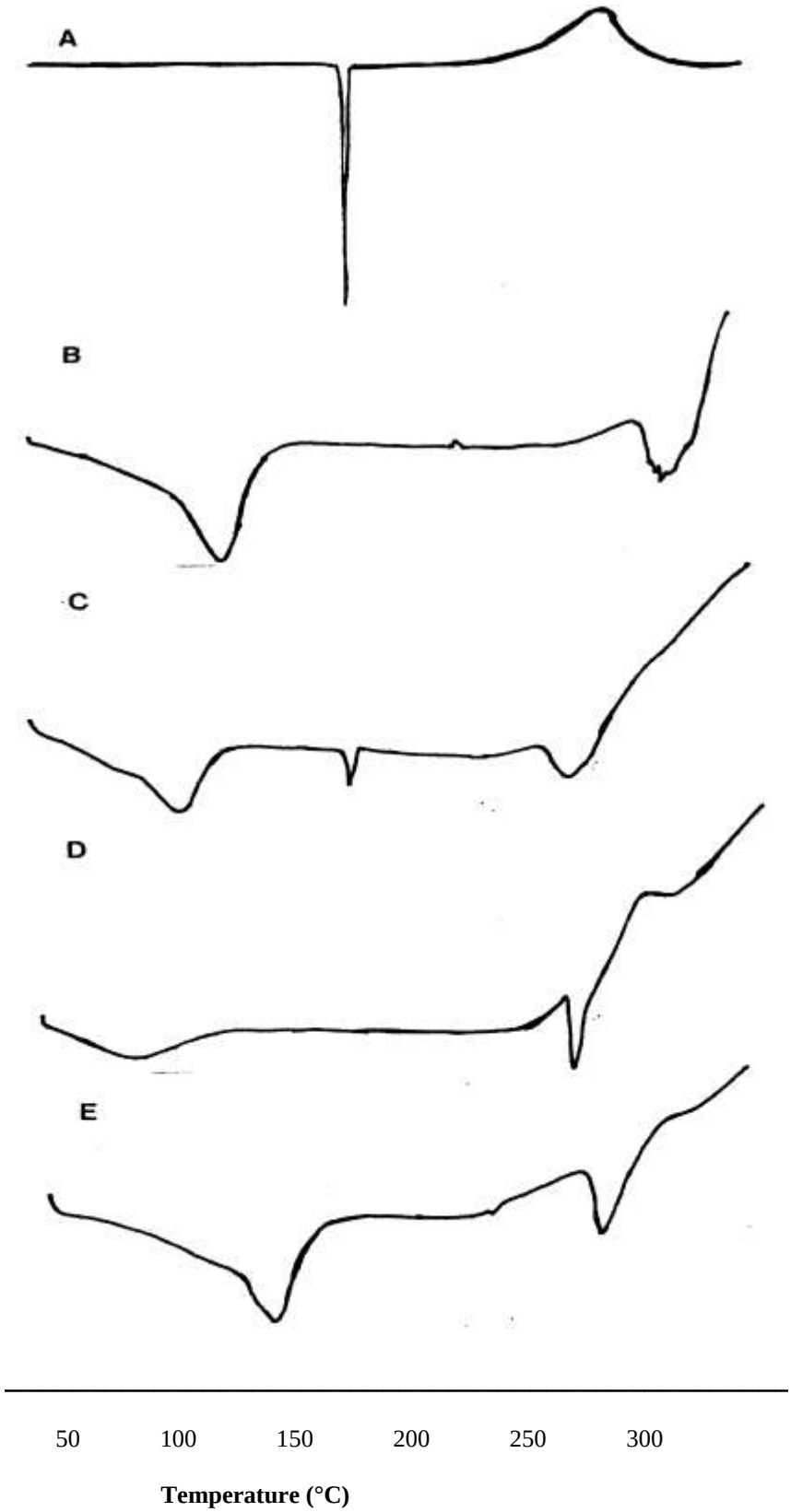

Figure 9. The DSC thermograms of, A) Sulfamethoxazole, B) β-Cyclodextrin, C) SMZ:β-CD (1:1) coprecipitated inclusion complex, D) SMZ:β-CD:PEG20000 (1:1) coprecipitated inclusion complex, E) SMZ:β-CD:PS20 (1:1) coprecipitated inclusion complex.

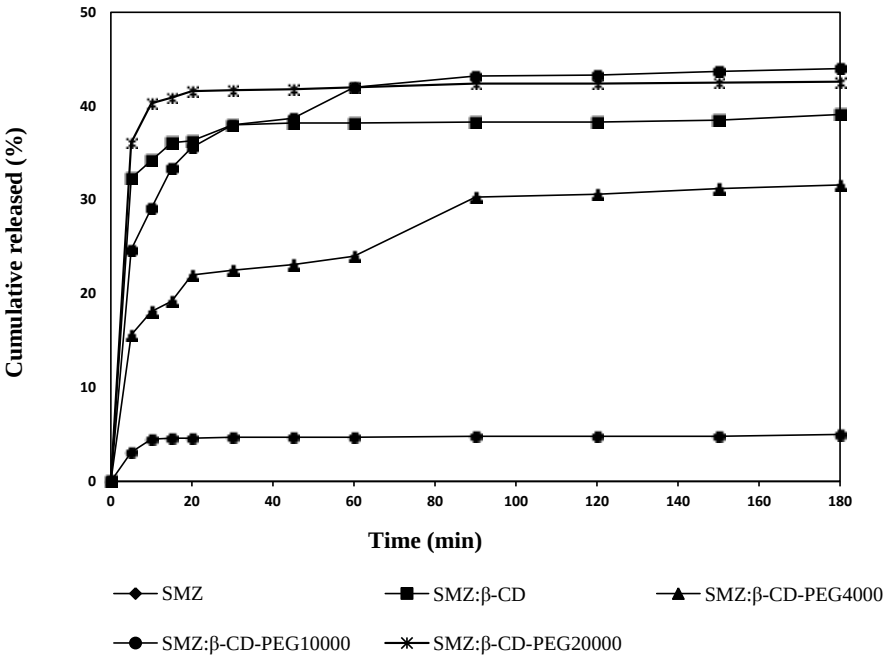

Figure 10. The dissolution profiles of SMZ and its β-CD:PEG complexes in pH 4.5.

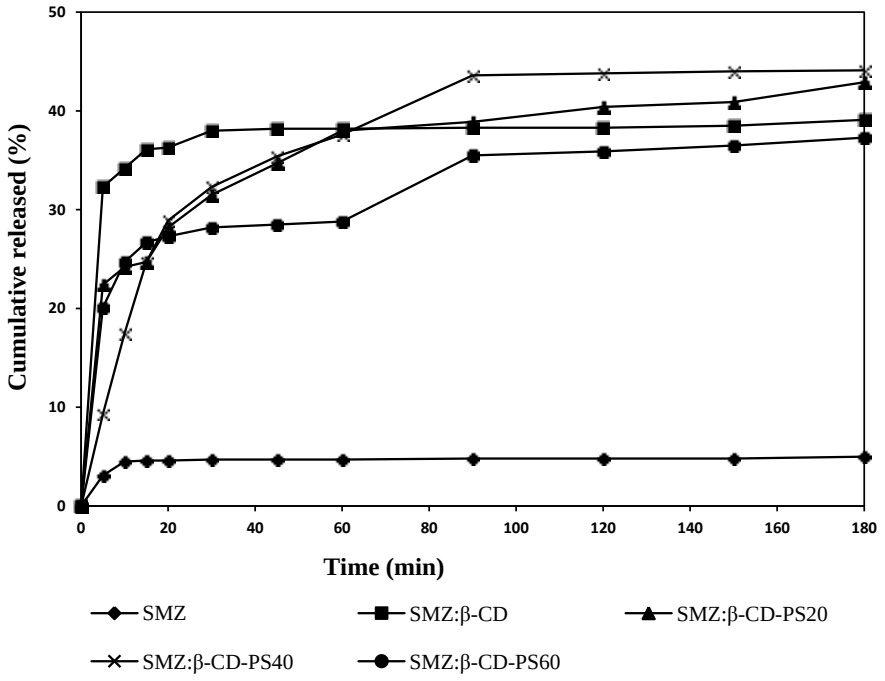

Figure 11. The dissolution profiles of SMZ and its β-CD:PS complexes in pH 4.5.

and PS60) with the concentration of 0.5% (w/v) were also examined with dissolution tests. The active material released at the end of the three hours were 39.1, 42.9, 44.1%, and 37.3% from β-CD: SMZ, β-CD:SMZ:PS20, β-CD:SMZ:PS40, and β-CD:SMZ:PS60 respectively. To conclude, the presence of these substances was observed to have a somehow significant effect on the active material release (Figure 11).

A significant increase in drug release was observed in the complexes at pH 7.0 as compared to the pure drug alone. At the end of 30 min, the SMZ release rates were 9.9, 25.5 and 17.1% from pure drug alone, β-CD:SMZ

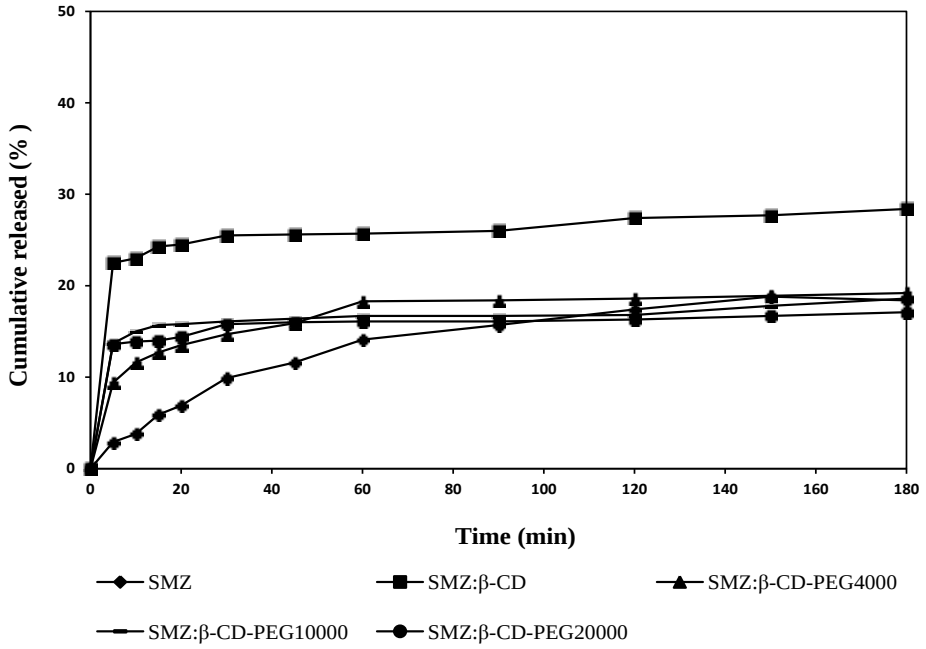

Figure 12. The dissolution profiles of SMZ and its β-CD:PEG complexes in pH 7.0.

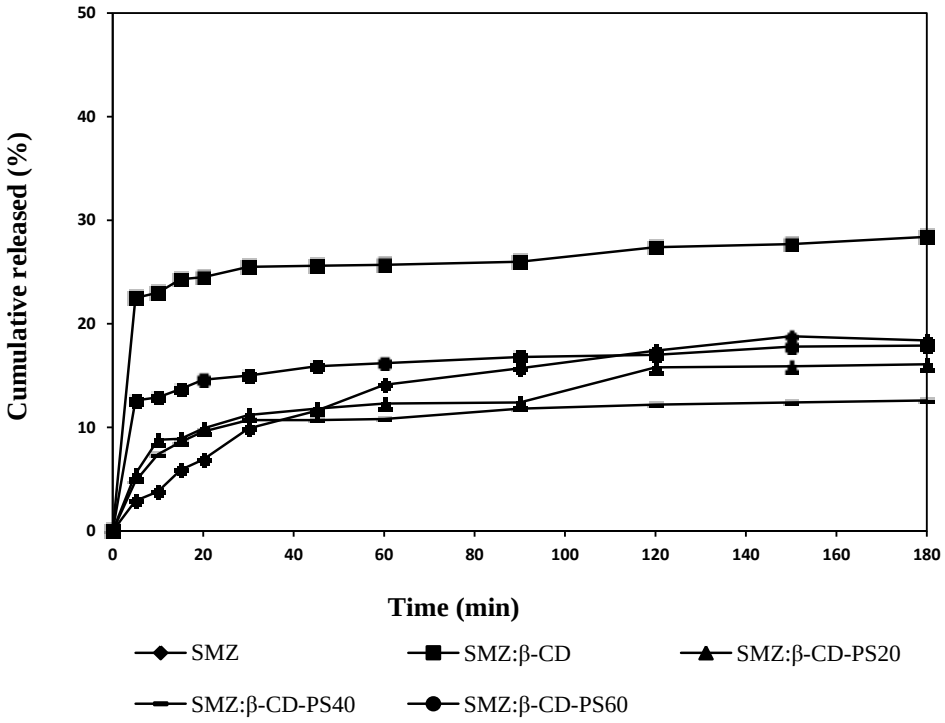

Figure 13. The dissolution profiles of SMZ and its β-CD:PS complexes in pH 7.0.

and β-CD:SMZ:PEG20000, respectively. The release of active material from the formulations including water soluble polymers (PEG4000, PEG10000 and PEG20000) and nonionic surfactants (PS20, PS40 and PS60) were lower than 20% within 3 h. These results point out to the fact that additives had no further effect on the active material release rate at pH 7.0, where the drug substance is in anionic form, as compared to the β-CD:SMZ complex (Figures 12 and 13). The *in vivo* studies also showed that β- CD SMZ:PEG20000: coded formulation,

which achieved the highest increase in the active material solubility and dissolution rate at pH 4.5 when compared with the pure SMZ, β-CD:SMZ coded complexes, increased the bioavailability among healthy volunteer participants (Özdemir and Erkin, 2012).

Conclusion

As a result of this study, it may be concluded that the solubility and the dissolution rate of SMZ was significantly enhanced by the complex formation with β-CD. The water soluble polymers and non-ionic surface active substances added to the solid dispersions increased the solubility and dissolution rate of active material-CD complexes at either pH 4.5 or 7.0.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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Full Length Research Paper

Extrapyramidal and purgative effects of fluphenazine in turkeys

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Fluphenazine is a typical antipsychotic medicine with extrapyramidal effects. It was tested for purgative and neurological effects in turkeys. The results showed that the drug induced purgation at a dose range of 5 to 200 mg/kg body weight causing highest frequency of fecal droppings (7) at 10 mg/kg in 7 min and lowest frequency of dropping (1) at 50 mg/kg. However, the number of fecal droppings was not linearly correlated with dose progression. Fluidity of the dropping increased with dose. This effect may be due to the stimulation or sedation of gastrointestinal tract. At 5 mg/kg, the animals were calm, but at 15 mg/kg of fluphenazine, there was severe torticollis as the dose increased. In conclusion, fluphenazine has hormetic dose response of gastrointestinal stimulation and inhibition as well as central nervous system depression and stimulation, respectively.

Key words: Fluphenazine, purgation, extrapyramidal effect, acetylcholine, sedation.

INTRODUCTION

Hormesis is a phenomenon of biphasic dose response characterized by exhibiting stimulatory or beneficial effects at low doses and inhibitory or toxic effects at high doses (Bao et al., 2015). Fluphenazine, a phenothiazine, was one of the first drugs to be classified as an antipsychotic and was approved by the Food and Drug Administration in 1959. In Britain, it was first used for the relief of anxiety (Matar et al., 2013). It causes elevated liver enzymes during management of delirium in infants (Turkel et al., 2013). Fluphenazine was on the World Health Organization (WHO) list of Essential Medicines of 2009 (Cieslik-Boczula et al., 2014). It reduces proteotoxicity in *C. elegans* and mammalian models of alpha-I-antitrypsin deficiency (Li et al., 2014). Fluphenazine can also be used for the treatment of Tourette syndrome (Wijemanne et al., 2014). Effectiveness trials and cost-effective analysis have caused first-generation antipsychotics to be re-examined regarding place in therapy. Plasma level of fluphenazine could be detected 4 months after the last administration with severe extrapyramidal symptoms, which is resolved by titrating 4 mg/day of benztropine within 72 h. This suggests complex pharmacokinetic properties of long-term fluphenazine decanoate treatment and the adverse

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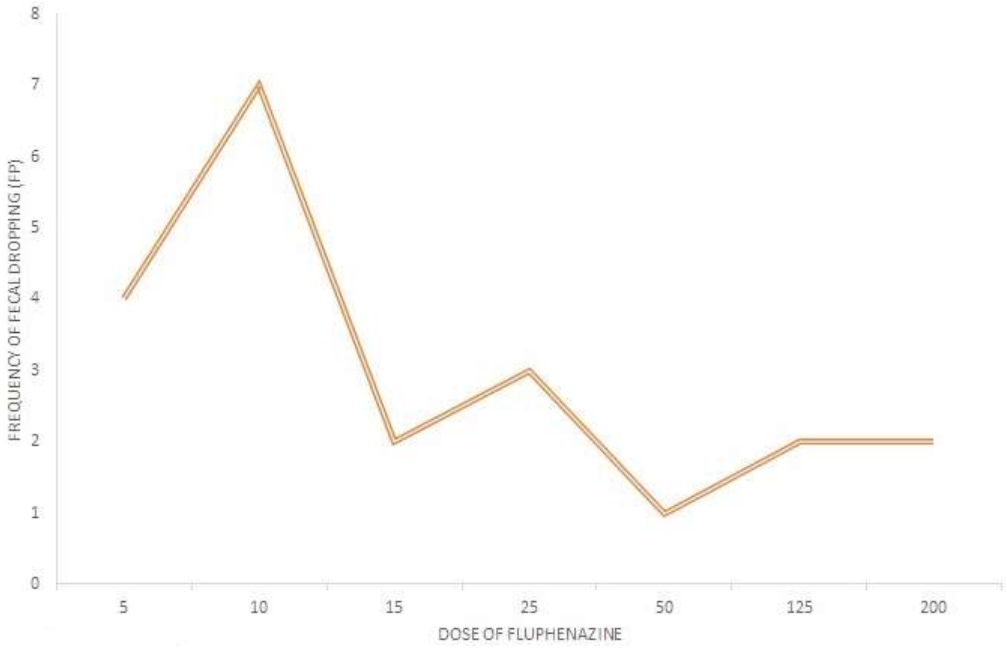

Figure 1. Frequencies of fecal dropping per milligram of fluphenazine.

effect resulting from dopamine D_2-receptor antagonism (Purvis et al., 2012).

Many of the problems that occur when patients are changed from oral depot fluphenazine are caused by high dosages; therefore low-dose treatment strategies are required in schizophrenia (Levine, 1980). Fluphenazine hydrochloride induces the formation of a small population of rod-like fibrils that differ from the characteristic ribbon-like fibrils normally observed for APOC-II (Zlatic et al., 2015). It is used in race horses as performance-enhancing drug, and for that reason, it has been banned by the Association of Racing Commissioners International (Costello et al., 2013). Hence, intramuscular fluphenazine was investigated in healthy turkeys.

MATERIALS AND METHODS

Seven female turkeys of 12 weeks old and weighing 2.1±0.5 kg were purchased from a commercial turkey raiser in Makurdi, Nigeria and kept in the laboratory of the Department of Veterinary Physiology, Pharmacology and Biochemistry, University of Agriculture, Makurdi, Nigeria. The turkeys were acclimatized for two weeks and thereafter intramuscularly administered 5, 10, 15, 25, 50, 125 and 200 mg/kg body weight of fluphenazine; it was observed for a period of 30 min and thereafter for 24 h. Neurological signs and number of fecal droppings from the turkeys were recorded. Feed and water were provided *ad libitum*. All the animals were handled according to the international guiding principles for biomedical research involving animals (CIOMS, 1985) as certified by University of Agriculture Makurdi Ethical Committee on the use of laboratory animals. All the turkeys were fed a commercial feed (Grower®) produced by Grand Cereals and Oils Limited (GCOML) Jos, Nigeria. Clean water was provided *ad libitum*.

RESULTS AND DISCUSSION

The number of fecal droppings at various doses were 4 (5 mg/kg), 7 (10 mg/kg), 2 (15 mg/kg), 3 (25 mg/kg), 1 (50 mg/kg), 2 (125 mg/kg) and 2 (200 mg/kg) body weight, respectively (Figure 1). The purgative effect stopped at the end of 10 min of observation. Pearson correlation of -0.4 showed that the number of fecal droppings was not linear with dose progression (that is the number of droppings was loosely scattered away from the line). But, there was a weak correlation of 16% (r^2 = 0.16) between dose of fluphenazine and frequency of fecal droppings, signifying that it may be used to remove ingested toxicant within a very short period of time. The purgative effect may be due to intestinal stimulation associated with fluphenazine (Peryriere et al., 2009). However, the probability of one fecal dropping from healthy turkey in 10 min was $^1/_7$. The 10 mg/kg of fluphenazine caused 7 fecal droppings in 7 min but at 15, 125 and 200 mg/kg, the frequency of fecal droppings was 2, at 50 mg/kg there was one frequency of fecal dropping in 7 min. Other signs observed were standing still, opisthotonos, calmness, torticollis and hyperventilation (Figures 2 to 8). The findings agree with the report of Calabrese (2006) indicating that anxiolytics have predominantly hormetic dose response and represent the most fundamental and common dose-response model in

Figure 2. Standing still (5mg/kg) torticollis (15 mg/kg).

Figure 4. Recumbent and torticollis (15 mg/kg).

Figure 3. Calm (10 mg/kg).

Figure 5. Torticollis (25 mg/kg).

the biomedical and toxicological sciences. They have important implications for the process of drug discovery development, clinical evaluation, and quantitative expectation of drug treatment effects (Calabrese, 2006).

A common feature of these drugs is that they act via inverted U-shaped dose response, consistent with the hormetic dose response model at described window (Calabrese, 2008). The depression at 5 mg/kg and torticollis and opisthotonos from 15 to 200 mg/kg show that as the dose is reduced, the response is increased, therefore having two distinct phases-biphasic and non-monotonic (Hayes, 2006). But, the idea that low dose effects may be different is accepted and questionable (Mattson and Cheng, 2005). Fluphenazine can increase action potential duration and induce QT prolongation in several animal models and humans, as the block of cardiac human ether-a-go-go-related gene (hERG) channels is one of the leading causes of acquired long

Figure 6. Recumbent and torticollis (125 mg/kg).

Figure 7. Hyperventilation and Opisthotonos (50 mg/kg).

Figure 8. Severe torticollis (200 mg/kg).

QT syndrome (Hong et al., 2013).

Levinson (1990) reported that antipsychotic effects of fluphenazine are in graded fashion and doses greater than 0.2 mg/kg per day were associated with greater clinical improvement, but also with a high incidence of extrapyramidal symptoms. But doses over 0.3 mg/kg per day were associated with more severe extrapyramidal symptoms, suggesting a linear relationship between fluphenazine dosage and acute outcome, and this relationship is observed in patients whose conditions improve to a criterion level (Levinson et al., 1990). CNS agents may have both excitatory and sedative effects (Saganuwan, 2017a) which may be dependent on metabolite (Saganuwan, 2017b), functional group of the compound (Saganuwan, 2017c), polymeric carriers of the CNS agents (Saganuwan,2017d) and their physicochemical and structure activity properties (Saganuwan, 2016). Fluphenazine decanoate produces fewer movement disorder effects than fluphenazine enanthate (Maaxam et al., 2015). Both are effective antipsychotics for treating schizophrenia. The benefits gained by long acting preparations may be offset by a higher incidence of adverse effects . Though the use of depot fluphenazine continues to be based on clinical judgement rather than evidence from methodical evaluation within trials (Adams et al., 2006), fluphenazine decanoate and enanthate may be associated with equal or more side effects than oral fluphenazine (Zhornitsky and Stip, 2012).

Intramuscular injections offer an advantage over oral medications for treating schizophrenia by reducing poor compliance (Maaxam et al., 2015). Fluphenazine plasma levels above 1.0 ng/ml and doses above 0.2 to 0.25 mg/kg per day showed better activity (Levinson et al., 1990).

Fluphenazine decanoate is a long-acting phenothiazine neuroleptic that attenuates the stress response and may be useful during intensive handling for reproductive procedures in non-domestic ungulates and elevate serum prolactin, which can suppress fertility in some species (Weiss et al., 2014). But, the first dropping at 125 and

200 mg/kg was soft,and the second dropping was watery. The volume of excreta increased with the dose, but the frequency of dropping decreased with the increased doses, a typical phenomenon of hormetic dose response.

In this study, the most effective purgative dose was 10 mg/kg which caused 7 fecal droppings. At 15 mg/kg, the number of fecal droppings reduced to 2. This finding agrees with the report of Maaxam et al. (2015) indicating that fluphenazine (12.5 mg/day) treatment was discontinued due to lack of efficacy and adverse effect (Conley et al., 2005).

Conclusion

Fluphenazine has extrapyramidal and purgative effects in turkeys. The purgative action may be by stimulation or sedation of gastrointestinal tract activity. Therefore, fluphenazine may be used to remove toxic ingesta from the gastrointestinal tract of turkeys in a very short possible time.

CONFLICT OF INTERESTS

The author has no conflict of interest whatsoever.

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Full Length Research Paper

Anti-ulcer and hematological properties of virgin coconut oil (VCO) against indomethacin-induced gastric ulcer in experimental rats

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Previous studies have reported various health benefits of virgin coconut oil (VCO) such as weight management, treatment of burns, infections, improve phagocytic activity, etc. The present study was conducted to investigate the anti-ulcer and hematological properties of VCO against indomethacin-induced gastric ulcers using rat model. Twenty-four Wistar albino rats were used for the study and were divided into 6 groups of 4 rats each. Group 1 rats served as the normal control; group 2 (positive control) rats were administered indomethacin only, at a dose of 100 mg/kg body weight. Group 3 rats were treated with a standard drug (cimetidine) at a dose of 100 mg/kg body weight. Groups 4 (3 ml/kg body weight VCO), 5 (6 ml/kg body weight VCO) and 6 (9 ml/kg body weight VCO) rats were treated as stated. The gastric juice volume of group 6 showed a statistically significant reduction ($p < 0.05$) in the gastric juice volume when compared with the positive control group. There was a significant increase ($p < 0.05$) in the packed cell volume (PCV), hemoglobin concentration and red blood cell count of VCO treated groups 4, 5 and 6 when compared with the positive control. Nonetheless, there was a significant decrease in the HDL-cholesterol, TAG concentration and total cholesterol level of the tests groups 4, 5 and 6 when compared with the positive control. Histological findings revealed that stomach sections of rats in groups 4 and 5 showed moderate widespread mucosal necrosis and ulceration, while that of group 6 rats showed focal area of mucosal ulceration with evidence of healing by fibrosis when compared with the positive control. The findings of this research revealed that VCO possesses ulcer ameliorative properties and could therefore be used for the treatment of gastric ulcers.

Key words: Virgin coconut oil (VCO), ulcer, cholesterol, hematology, gastric juice.

INTRODUCTION

Gastric ulcer refers to sores in the stomach lining or the duodenum. It is one of the most common ailments suffered by people especially in the developing countries (Balogun et al., 2013). In Nigeria, at least one member of

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each family has suffered from, or may still suffer from stomach ulcers. Gastric ulcers are caused by *Helicobacter pylori* infection and/or the use of non-steroidal anti-inflammatory drugs (NSAIDs). More than 50% of the world population is believed to be infected with *H. pylori*, the infection usually occurring during childhood days, although adults are not immune. The Gram-negative bacterium is responsible for about 90% of duodenal ulcers and more than 60% of gastric ulcers (Kogilavani et al., 2014). *H. pylori* produces the enzyme urease which breaks down urea to ammonia and carbon dioxide (Minkara et al., 2014). The ammonia reacts with the stomach acid (HCL), producing ammonium chloride, increasing the stomach pH and facilitating the survival of the bacterium. In developing countries, there is little or no effective regulation of medications by the appropriate government bodies.

Current treatment for stomach ulcers includes: anti-secretory drugs (Proton pump inhibitors, antihistaminic (H2) and anticholinergic (M1)) and cyto-protective (sucralfate and prostaglandin analogues) agents (Reham et al., 2017). In addition to being expensive, treatment of ulcer with these drugs produces undesirable side effects or resistant strains of *H*. *pylori* (Balogun et al., 2013). This has stimulated the interest of scientists towards finding ulcers treatments using natural plant products, which would produce no resistant bacteria strains, and negligible contraindications; this interest has opened the field of “nutraceuticals”. Coined by Stephen De Felice defined nutraceuticals as foods or components of food which provide medical/health benefits including the prevention and treatment of diseases (Karla,2003). Examples of nutraceuticals include lycopene in tomatoes, omega 3 fatty acids in salmon, and medium chain saturated fatty acids in coconut oil (Loomba and Jothi, 2013).

Virgin coconut oil (VCO) may be defined as oil obtained from fresh mature coconut (*Cocos nucifera*) kernel by mechanical or natural means with or without the use of heat and without subjecting the oil to any chemical refining or bleaching (Mohammad et al., 2014). Dietary oils capable of lowering low density lipoprotein (LDL) cholesterol level and increasing high density Lipoprotein (HDL) cholesterol level are known to be beneficial to health (Nevin and Rajamohan, 2004). However, saturated oils are believed to aggregate and cause cardiovascular diseases; unsaturated oils on the other hand do not clog blood vessels and therefore cause fewer of such diseases. It then follows that dietary oils with high concentrations of polyunsaturated fats are better for the health than those with higher saturated fats content (Nevin and Rajamohan, 2008). Virgin coconut oil is an exception; it contains saturated fatty acids yet very beneficial to health (Mouna et al., 2012). This is because VCO contains mostly saturated medium chain fatty acids (MCFA) which are easily metabolized by the body. VCO also contains antioxidants like polyphenols, tocopherols, and antioxidants vitamins (Ahmad et al., 2015). Based on the aforementioned premise, this study has been designed to investigate the anti-ulcer effect of VCO and its effect on hematology and lipid profiles.

Significance statement

The riddle of the etiopathogenesis of gastric ulcer remains unresolved. This is a current pursuit for a highly efficient anti-ulcer drug from natural source. Thus, this research is geared towards investigating the anti-ulcer activity of VCO on Wistar albino rats.

MATERIALS AND METHODS

Plant

Fresh matured nuts (12-13 months old) of *Cocos nucifera*, with no haustoria were obtained from Awgu Local Government Area, Enugu State of Nigeria and were identified at International Center for Ethnomedicine and Drug development (InterCEDD) with herbarium voucher no InterCEDD/16287. The nuts were de-husked, cracked mechanically to obtain the coconut meat, and the coconut meat was ground using a motorized grinder.

Animals

The animals used for the study were albino mice (21-33 g), for LD_{50}, and adult male Wistar albino rats (120±137 g), for the animal model. Animals were obtained from the Animal House of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The animals were acclimatized for 1 week before the experiment and were fed with standard rat’s pellets (Pfizer Livestock feeds Plc, Enugu, Nigeria) and tap water was given *ad libitum*. Animal experimental studies were conducted according to the guidelines of Institutional Animal Ethical Committee of Faculty of Biological Sciences, University of Nigeria, Nsukka.

Experimental design

Twenty-four adult male Wistar albino rats were acclimatized for 1 week at the same conditions of temperature and pressure, and the same animal feeds were used for all the rats. The rats were divided randomly into six groups of four animals each and treated as follows:

Group 1: Normal control (No indomethacin + No VCO)
Group 2: Positive control (Untreated group): (Indomethacin)
Group 3: Standard control (Indomethacin + Cimetidine/Standard Drug)
Group 4: Low-dose treatment (Indomethacin + 3 ml/kg body weight VCO)
Group 5: Mid-dose treatment (Indomethacin + 6 ml/kg body weight VCO)
Group 6: High-dose treatment: (Indomethacin + 9 ml/kg body weight VCO)
Rats were fasted overnight prior to the commencement of treatment

Cimetidine (standard drug) and VCO were administered orally for 4 days. On the 5th day, after an overnight fasting, gastric ulcer was induced in the rats of groups 2 to 6 with 100 mg/kg body weight (p.o) indomethacin using standard procedures. Four hours after induction, blood was collected through ocular puncture and the rats were sacrificed. Blood samples collected were used for biochemical analysis. Stomachs were removed and opened along the greater curvature to remove gastric contents. Gastric ulcers were viewed and counted with the aid of a magnifying hand lens (×10). The experimental design is a modified method of Reham et al. (2017).

Preparation of indomethacin sample

The stock concentration of indomethacin was prepared by adopting the method of Hiroshini et al. (1987). A known weight (500 mg) of the standard drug was dissolved in 2 ml of distilled water and water was added to bring the stock concentration to 25 mg/ml. The dose used was 100 mg/kg body weight.

Preparation of cimetidine (Standard drug)

The stock concentration of cimetidine was prepared by adopting the method of Hiroshini et al. (1987). 800 mg of the standard drug was dissolved in 10 ml of distilled water to get a stock concentration of 80 mg/ml. The dose used was 100 mg/kg body weight.

Preparation of VCO

VCO was prepared using the method of Divina and Keith (2006) as described:

(1) Fresh matured nuts were de-husked manually.
(2) The de-husked nuts were de-shelled manually and the coconut meat and testa fed into a motorized coconut shredder.
(3) The milk from the grated coconut meat was extracted by hand. The milk obtained was set aside, while the coconut milk residue (sapal) was prepared for the second extraction.
(4) The sapal was mixed with distilled water at a ratio of 2 sapal: 1 water. The mixture was pressed to obtain the second milk extraction.
(5) The first and second milk extracts were mixed by stirring vigorously for about 10 min.
(6) The resulting mixture was kept in a refrigerator for 3 h and allowed to stand.
(7) The coconut cream (oily part) was separated from the coco skim milk (watery part) by scooping the cream from the top. The coco skim milk was discarded, while the coconut cream was transferred into a beaker and used for subsequent procedures.
(8) The coconut cream was placed in a water bath and heated slowly at 50°C for 2.5 h to separate the coconut protein (latik) from the coconut oil. The coconut oil was separated from the latik by straining the mixture through a muslin cloth. The latik was discarded while the coconut oil was retained.
(9) The coconut oil was dried by incubating at 50°C for 12 h to remove all residual moisture.
(10) The oil was filtered and stored
(11) The resulting oil was called VCO.

Acute toxicity studies

Acute toxicity studies were conducted by a modification of Loke (1983) method as described by Mouna et al. (2012). Twenty-four albino mice were divided into two phases I and II, with each group subdivided into four groups made up of three animals each.

Experimental protocol for acute toxicity studies

Phase I

Group 1: Mice were administered with 2 ml/kg body weight of VCO.
Group 2: Mice were administered with 4 ml/kg body weight of VCO.
Group 3: Mice were administered with 6 ml/kg body weight of VCO.
Group 4: Mice were administered with 6 ml/kg body weight of distilled water.

Phase II

Group 1: Mice were administered with 8 ml/kg body weight of VCO.
Group 2: Mice were administered with 10 ml/kg body weight of VCO.
Group 3: Mice were administered with 12 ml/kg body weight of VCO.
Group 4: Mice were administered with 12 ml/kg body weight of distilled water.

The mice were monitored closely for 24 h for signs of toxicity and lethality. The median lethal dose (LD_{50}) was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

where D_0 = highest dose that caused no mortality and D_{100} = lowest dose that produced mortality.

Methods for biochemical examination

Determination of gastric juice volume

Gastric juice volume was determined according to the method of Balogun et al. (2013). The gastric content was centrifuged at 3000 rpm for 10 min, then separated and the volume measured using a graduated cylinder.

Total cholesterol concentration

The total cholesterol concentration was determined using QCA commercial enzyme kit as described by Allain et al. (1976).

Determination of low density lipoprotein-cholesterol concentration

Low density lipoprotein-cholesterol (LDL-cholesterol) concentration was determined using QCA Commercial Kits as described by Assmann et al. (1984).

Estimation of triacylglycerol concentration

Triacylglycerol concentration was determined using Randox Enzyme Kit as described by Albers et al. (1978).

Table 1. Percentage yield of virgin coconut oil (VCO).

Weight of VCO (g)	Weight of coconut meat (g)	Percentage yield
228	1900	12

Table 2. Acute Toxicity Results.

Phase	Groups of mice	Doses of VCO (ml/kg)	Mortality
Phase I	Group 1	2	0/3
	Group 2	4	0/3
	Group 3	6	0/3
Phase II	Group 1	8	0/3
	Group 2	10	0/3
	Group 3	12	0/3

n=3.

Determination of packed cell volume (PCV)

This was done using the procedure described by Ochei and Kolharta (2008).

Determination of total white blood cell (WBC) count

Total WBC concentration was determined using by the method of Ramnik (2003).

Determination of red blood cell (RBC) count

RBC concentration was determined using the method described by Cheesbrough (2000).

Determination of haemoglobin concentration

This was done using the method described by Ochei and Kolhaktar (2008).

Assay for aspartate aminotransferase activity

A Randox Commercial Enzyme kit according to the methods of Reitman and Frankel (1957) and Schmidit and Schmidit (1963) was used.

Assay for alanine aminotransferase (ALT) activity

A Randox Commercial Enzyme Kit based on the methods of Reitman and Frankel (1957) and Schmidt and Schmidt (1963) was used.

Assay for alkaline phosphatase (ALP) activity

This was done using a Commercial Enzyme Kit according to the method of Klein et al. (1960).

Histopathological examination

The histological examination of the tissues of the pancreas of Wistar albino rats was done using the method of Drury et al. (1967).

Statistical analysis

Results from the experimental study were expressed as mean ± standard deviation (SD) and test of statistical significance was carried out using one-way analysis of variance (ANOVA). The means were separated using Duncan Multiple Test. The statistical packaged used was the statistical product and service solutions (SPSS), version 20. Differences at $p<0.05$ were considered statistically significant.

RESULTS

Percentage yield of VCO

As presented in Table 1, the percentage yield of VCO was found to be 12% with respect to the starting material.

Acute toxicity results

The extract was found to be non-toxic, although there was furring and decreased activity/movement following the administration of a high dose (12 ml/kg body weight VCO) in the phase II. The LD_{50} was calculated to be more than 12 ml/kg body weight (Table 2).

Table 3. Effects of VCO on the ulcer parameters of experimental rats.

Group	Gastric juice volume (ml)
Group 1	0.13 ± 0.05^{a}
Group 2	0.46 ± 0.15^{b}
Group 3	0.45 ± 0.31^{b}
Group 4	0.21 ± 0.10^{ab}
Group 5	0.26 ± 0.13^{ab}
Group 6	0.14 ± 0.05^{a}

Data are expressed as mean ± SD (n = 4). Values with different alphabet in the superscript has a statistical significant difference at $p < 0.05$ while same alphabet has no significant difference. Group 1=Normal Control (No Indomethacin + No treatment). Group 2=Positive Control (Indomethacin-Induced + Untreated Rats). Group 3=Standard Control (Indomethacin-Induced Rats + 100mg/kg body weight of cimetidine). Group 4=Low Dose Treatment (Indomethacin-Induced Rats + 3 ml/kg body weight of VCO). Group 5=Mid Dose Treatment (Indomethacin-Induced Rats + 6 ml/kg body weight of VCO). Group 6=High Dose Treatment (Indomethacin-Induced Rats + 9 ml/kg body weight of VCO).

Table 4. Effects of VCO on haematological parameters of experimental rats.

Group	PCV (%)	Hb (g/dl)	RBC ($\times 10^{6}$ mm^{-3})	WBC (mm^{-3})
Group 1	47.00 ± 3.65^{c}	13.08 ± 0.66^{b}	12.50 ± 0.68^{bc}	4700 ± 258.20^{bc}
Group 2	38.75 ± 4.11^{a}	9.12 ± 0.59^{a}	7.30 ± 0.60^{a}	3800 ± 516.40^{a}
Group 3	45.00 ± 3.74^{bc}	13.19 ± 0.93^{b}	14.33 ± 1.56^{c}	6150 ± 191.49^{d}
Group 4	44.25 ± 5.38^{b}	13.60 ± 0.50^{b}	12.30 ± 2.32^{bc}	5050 ± 660.81^{c}
Group 5	45.25 ± 2.22^{bc}	13.38 ± 0.97^{b}	11.20 ± 1.13^{b}	4100 ± 258.20^{ab}
Group 6	47.75 ± 5.74^{c}	13.43 ± 0.61^{b}	12.78 ± 1.83^{bc}	4300 ± 476.10^{ab}

Data are expressed as mean ± SD (n = 4). Values with different alphabet in the superscript has a statistical significant difference at $p < 0.05$ while same alphabet has no significant difference. Group 1=Normal Control (No Indomethacin + No treatment). Group 2=Positive Control (Indomethacin-Induced + Untreated Rats). Group 3=Standard Control (Indomethacin-Induced Rats + 100mg/kg body weight of cimetidine). Group 4=Low Dose Treatment (Indomethacin-Induced Rats + 3 ml/kg body weight of VCO). Group 5=Mid Dose Treatment (Indomethacin-Induced Rats + 6 ml/kg body weight of VCO). Group 6=High Dose Treatment (Indomethacin-Induced Rats + 9 ml/kg body weight of VCO).

Effect of VCO on the gastric juice volume of indomethacin-challenged rats

There was a non-significant ($p > 0.05$) increase in the gastric juice volume of rats in the treatment groups (4, 5 and 6) as compared to the normal control (Table 3). However, such an increase was found to be non-dose dependent. Group 5 rats (0.26 ml ± 0.13 ml) were found to have the highest juice volume when compared with groups 4 (0.21 ± 0.10 ml) and 6 (0.14 ± 0.05 ml).

From Table 4, a non-significantly ($p > 0.05$) difference was observed in the PCV of VCO treated groups 5 and 6 when compared with the negative control; nevertheless; group 4 showed a significant decrease in the PCV when compared with the negative control. There was a significant increase ($p < 0.05$) in the PCV of VCO treated groups when compared with the positive control. The haemoglobin (Hb) concentration of groups 4, 5 and 6 rats were found to be non-significantly ($p > 0.05$) higher as compared to the negative control; notwithstanding the Hb concentration of the test groups 4, 5 and 6 rats was found to be significantly ($p < 0.05$) higher than the positive control. The RBC concentration of groups 4, 5 and 6 rats treated with VCO was found to be significantly ($p < 0.05$) higher than the positive control, while the RBC concentration of groups 4, 5 and 6 rats was observed to be non-significant ($p > 0.05$) when compared with the negative control. The WBC of groups 4, 5 and 6 rats was found to be significantly ($p < 0.05$) higher when compared with the positive control. The total WBC count of the test groups were however found to be non-significantly ($p > 0.05$) different when compared with the negative control.

From Table 5, a dose-dependent non-significant ($p > 0.05$) decrease in the HDL-cholesterol concentration of

Table 5. Effects of VCO on lipid parameters of experimental rats.

Group	LDL (mmol/L)	TAG (mmol/L)	T.Chol. (mmol/L)
Group 1	2.35 ± 0.80[a]	1.14 ± 0.08[a]	3.98 ± 0.80[ab]
Group 2	2.67 ± 0.37[a]	1.75 ± 0.10[b]	5.10 ± 0.77[b]
Group 3	2.45 ± 0.84[a]	1.18 ± 0.24[a]	4.14 ± 0.74[ab]
Group 4	2.35 ± 0.76[a]	1.17 ± 0.09[a]	3.94 ± 0.65[ab]
Group 5	2.21 ± 0.97[a]	1.28 ± 0.13[a]	3.80 ± 0.95[a]
Group 6	2.36 ± 0.34[a]	1.19 ± 0.21[a]	3.83 ± 0.45[a]

Data are expressed as mean ± SD (n = 4). Values with different alphabet in the superscript has a statistical significant difference at $p < 0.05$ while same alphabet has no significant difference. Group 1=Normal Control (No Indomethacin + No treatment). Group 2=Positive Control (Indomethacin-Induced + Untreated Rats). Group 3=Standard Control (Indomethacin-Induced Rats + 100mg/kg body weight of cimetidine). Group 4=Low Dose Treatment (Indomethacin-Induced Rats + 3 ml/kg body weight of VCO). Group 5=Mid Dose Treatment (Indomethacin-Induced Rats + 6 ml/kg body weight of VCO). Group 6=High Dose Treatment (Indomethacin-Induced Rats + 9 ml/kg body weight of VCO).

Table 6. Effects of VCO on the activities of liver marker enzymes of experimental rats.

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Group 1	37.08 ± 2.88[a]	34.60 ± 3.30[a]	47.13 ± 5.45[a]
Group 2	41.71 ± 9.65[a]	35.73 ± 2.14[a]	51.20 ± 8.73[a]
Group 3	36.21 ± 6.05[a]	32.80 ± 2.59[a]	46.25 ± 6.40[a]
Group 4	44.50 ± 10.07[a]	38.10 ± 9.41[a]	47.75 ± 10.69[a]
Group 5	37.67 ± 7.22[a]	42.53 ± 1.76[a]	41.00 ± 11.22[a]
Group 6	41.33 ± 8.22[a]	40.38 ± 10.26[a]	48.25 ± 5.74[a]

Data are expressed as mean ± SD (n = 4). Values with different alphabet in the superscript has a statistical significant difference at $p < 0.05$ while same alphabet has no significant difference. Group 1=Normal Control (No Indomethacin + No treatment). Group 2=Positive Control (Indomethacin-Induced + Untreated Rats). Group 3=Standard Control (Indomethacin-Induced Rats + 100mg/kg body weight of cimetidine). Group 4=Low Dose Treatment (Indomethacin-Induced Rats + 3 ml/kg body weight of VCO). Group 5=Mid Dose Treatment (Indomethacin-Induced Rats + 6 ml/kg body weight of VCO). Group 6=High Dose Treatment (Indomethacin-Induced Rats + 9 ml/kg body weight of VCO).

the test groups (4, 5 and 6) as compared to the normal control was observed. There was a significant decrease in the HDL-cholesterol concentration of the tests groups 4, 5 and 6 when compared with the positive control. The LDL-cholesterol assay revealed a non-significant ($p > 0.05$) difference in groups 4, 5 and 6 treated with VCO when compared with the positive control and the negative control group. There was a significant ($p < 0.05$) decrease in the triacylglyceride (TAG) concentration of the VCO treated groups (4 to 6) when compared with the positive control. Also, as can be observed in Table 5, there was a significant decrease ($p < 0.05$) in the total cholesterol level of treatment groups 5 and 6 when compared with the positive control group; however, group 4 showed no significant difference ($p < 0.05$) in the total cholesterol concentration when compared with the positive control.

From Table 6, there was no significant ($p > 0.05$) increase or decrease in the activities of ALT, AST and ALP of the treatment groups (4, 5 and 6) as compared to the positive control and the negative control.

Histological

Plate 1 shows the stomach tissues of normal control (group 1) rats. Sections of the stomach collected from the animals in group 1 showed the normal gastric histomorphology for laboratory rats. Normal structures of the mucosa (M), sub-mucosa (SM) and muscularis layers (ML) were observed. Normal gastric pits (white arrow) are lined by low columnar epithelial cells and opening into the mucosa were observed. Normal lamina propria of mucosa containing abundant inflammatory and immune cells was observed. The submucosa and muscularis mucosa both showed the normal histomorphology: Luminal side (LS); H&E ×40.

As shown in Plate 2, sections of the stomach collected

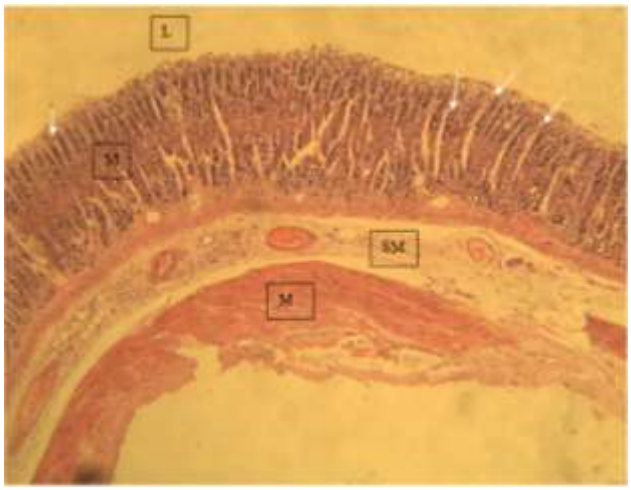

Plate 1. Photomicrograph of stomach tissues of normal control (group 1) rats.

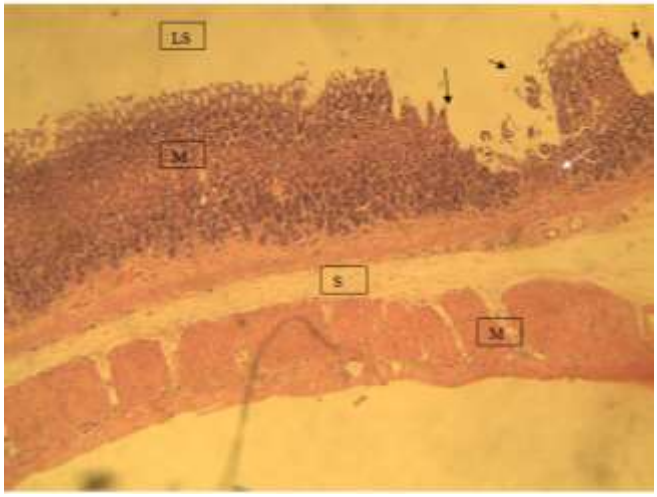

Plate 3. Photomicrograph of stomach tissues of standard control (group 3) rats.

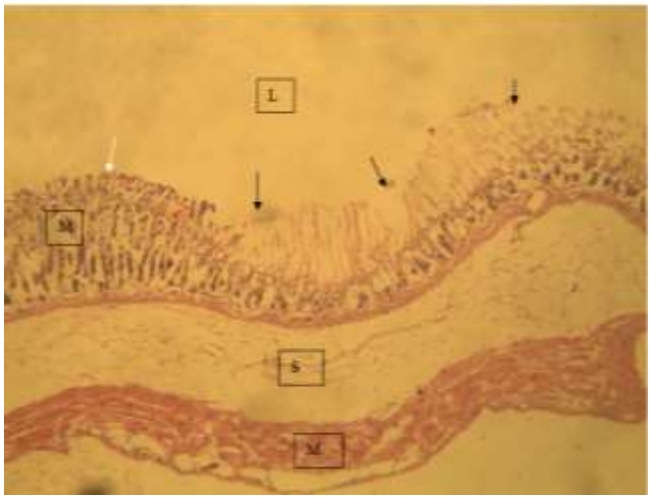

Plate 2. Photomicrograph of stomach tissues of positive control (group 2) rats

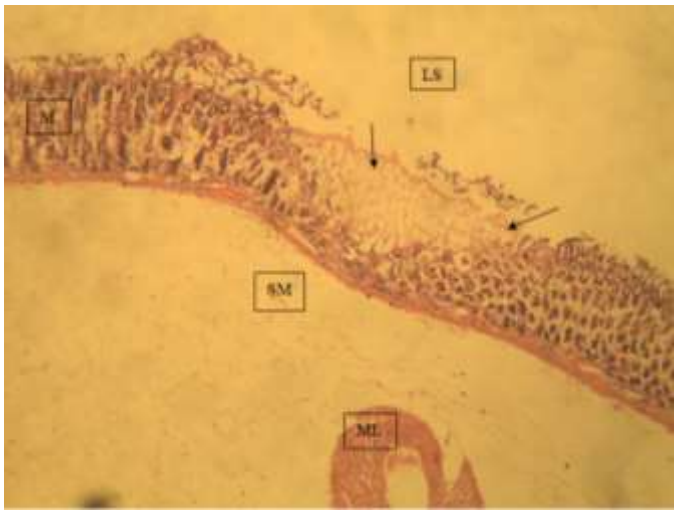

Plate 4. Photomicrograph of stomach tissues of low-dose VCO treatment (group 4) rats.

from the animals in group 2 showed multiple, widespread, and mucosal ulceration (arrow) when compared with normal gastric mucosa (white arrow): Luminal surface (LS); Submucosa (SM); Mucosa (M); Muscularis layer (ML); H&E ×40

As shown in Plate 3, sections of the stomach collected from animals in group 3 (standard control) showed multifocal mucosal ulcerations (arrow). However, the affected areas showed evidence of healing by fibrosis (white arrow): Luminal surface (LS); Mucosa (M); Submucosa (SM); Muscularis layer (ML); H&E ×40.

In Plate 4, sections of the stomach collected from group 4 rats treated with 3 ml/kg body weight VCO showed moderate widespread mucosal necrosis and ulceration (arrow): Luminal surface (LS); Mucosa (M); Submucosa (SM); Muscularis layer (ML); H&E ×40.

Plate 5 shows sections of the stomach collected from the animals in group 5 administered with 100 mg/kg body weight indomethacin and treated with a mid-dose of 6 ml/kg body weight VCO. There was a wide area of mucosal necrosis and ulceration (black arrow) when compared with the relatively normal mucosa (white arrow): Luminal surface (LS); Mucosa (M); Submucosa (SM); H&E ×100.

In Plate 6, stomach tissue sections collected from the animals in group 6 showed focal area of mucosal

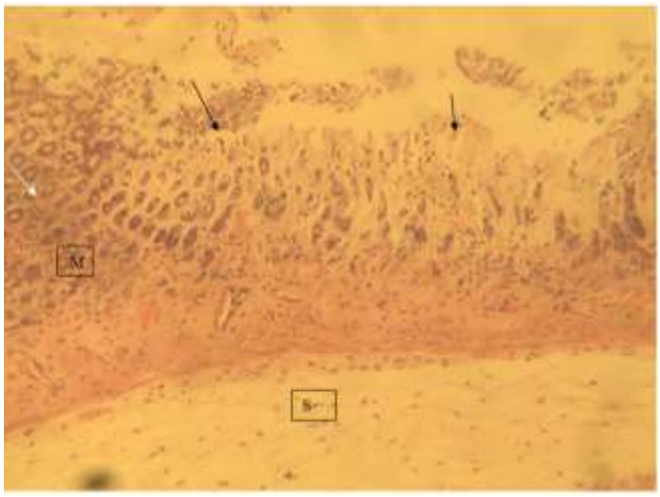

Plate 5. Photomicrograph of stomach tissues of mid-dose VCO treatment (group 5) rats.

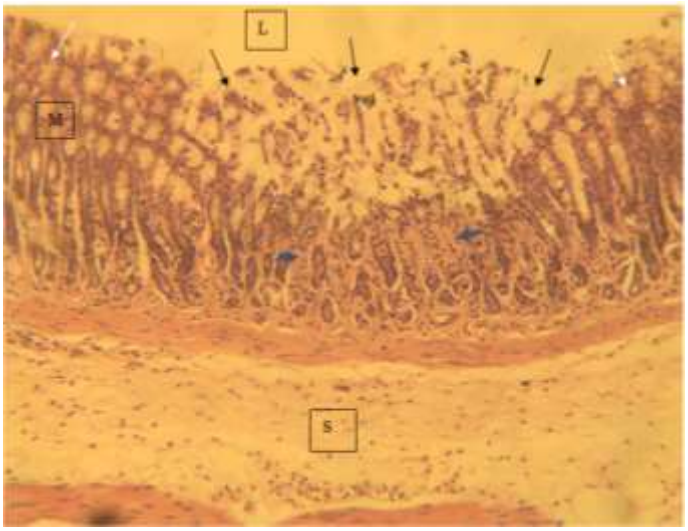

Plate 6. Photomicrograph of stomach tissues of high-dose VCO treatment (group 6) rats.

ulceration (black arrow) with evidence of healing by fibrosis (blue arrow) when compared with the relatively normal gastric mucosa (white arrow): Luminal surface (LS); Mucosa (M); Submucosa (SM); H&E ×100.

DISCUSSION

Gastric ulcers are sores in the intestinal mucosa caused by the use of non-steroidal anti-inflammatory drugs (NSAIDs) or the Gram-negative bacterium, *H. pylori*. VCO contains predominantly medium chain fatty acids (MCFA) which are potent bactericidal and antimicrobial agents (Fabian, 2014). VCO also stimulate prostaglandin synthesis, platelet aggregation and wound healing, and can therefore be used to treat NSAIDs-induced ulceration. It also contains antioxidant vitamins and stimulates the activities of enzymatic antioxidants thereby minimizing damage caused by peroxidation and free radicals generation (Nevin and Rajamhan, 2005; Ahmad et al., 2015).

From Table 2, it can be deduced that VCO is safe for consumption. Results from Table 3 shows that there was a non-significant ($p > 0.05$) increase in the gastric juice volume of rats in the treatment groups (4, 5 and 6) as compared to the normal control. However, the gastric juice volume of the treatment groups was found to be lower when compared with the positive control however only group 6 showed a statistically significant reduction in the gastric juice volume when compared with the positive control group. VCO stimulates prostaglandin secretion thereby reducing the volume of gastric acid secreted into the lumen, leading to a consequent decrease in gastric juice volume. The results obtained are in agreement with the works or Ikwebe et al. (2017) where the effect of ethanolic extracts of selected dietary spices on gastric acid secretion in Wistar rats was evaluated.

From Table 4, there was a significant increase ($p < 0.05$) in the PCV of VCO treated groups when compared with the positive control. PCV is a measure of the amount of space (volume) occupied by the red blood cells. VCO contains antioxidants which could stabilize the erythrocyte membrane, preventing erythrocyte depletion and thereby leading to an increase in PCV. This result supports the work of Penner et al. (2005) who reported an increase in the PCV of rats treated with VCO. The haemoglobin (Hb) concentration of groups 4, 5 and 6 rats were found to be non-significantly ($p > 0.05$) higher as compared to the negative control; notwithstanding, the Hb concentration of the test groups 4, 5 and 6 rats was found to be significantly ($p < 0.05$) higher than the positive control. Haemoglobin is spilt following haemolysis of the red blood cells caused by free radicals. Free radical scavenging potentials of VCO is probably responsible for the observed increase in the Hb concentration. This finding supports the research of Penner et al. (2005) who observed a decrease in Hb concentration of rats treated with VCO as compared to the normal control. The RBC concentration of groups 4, 5 and 6 rats treated with VCO was found to be significantly ($p < 0.05$) higher than the positive control, while the RBC concentration of group 4, 5 and 6 rats was observed to be non-significantly ($p > 0.05$) when compared with the negative control. This is probably due to the antioxidant vitamin components of VCO which could protect against RBC membrane oxidation by free redicals thereby reducing the breakdown of RBC thus increasing RBC count. This finding supports the reports of Anosike et al.

(2010) who reported the anti-ulcerogenic and membrane stabilization effect of ethanol extract of coconut (*Cocos nucifera*).

The WBC of groups 4, 5 and 6 rats was found to be significantly ($p < 0.05$) higher when compared with the positive control. This is probably due to the antioxidant components of VCO stimulating the immune system to produce more WBC to fight the disease condition thereby increasing WBC count. Coconut (*Cocos nucifera*) was reported to protect several organs against oxidative damage activity as an antioxidant due to high content of L-arginine and vitamin C (Ahmad et al., 2015), thus boosting the immune system and reducing lipid peroxidation.

The LDL-cholesterol assay revealed a visible decrease but was statistical non-significant ($p > 0.05$) in groups 4, 5 and 6 treated with VCO when compared with the positive. LDL-cholesterol (the so called “bad cholesterol”) transports lipid particles (e.g. cholesterol, phospholipids and tri-acyglycerols) to extra-hepatic tissues. When LDL particles are oxidized in arterial walls, they are retained by proteoglycans ultimately leading to atherosclerosis and cardiovascular diseases. Lipid peroxidation in arterial cells; therefore, causes an increase in the release of retained LDL cholesterol resulting in an increase in LDL concentration. VCO contains antioxidants which prevent such oxidation; hence, the decrease in LDL concentration. This finding supports the research of Moshira et al. (2016) who reported a significant ($p < 0.05$) decrease in the LDL-cholesterol concentration of rats treated with virgin coconut oil after copper-induced LDL oxidation. There was a significant ($p < 0.05$) decrease in the TAG concentration of the VCO treated groups (4 to 6) when compared with the positive control. This observation of this work is in tandem with the works of Hayatullina et al. (2012) who observed a decrease in TAG concentration after treatment with VCO. Also, as can be observed in Table 5, there was a significant decrease ($p < 0.05$) in the total cholesterol level of treatment groups 5 and 6 when compared with the positive control group; however, group 4 showed no significant difference ($p > 0.05$) in the total cholesterol concentration when compared with the positive control. Nevertheless, these results suggest that the mid and the high doses are more effective when compared with the low dose. Notwithstanding, it is most probable that VCO modulates the removal of cholesterol from the system maybe by stimulating the activity of lecithin-cholesterol acyltransferase (LCAT), which attaches cholesterol molecules in the blood to either VLDL/LDL (α-LCAT activity) or to the HDL (β-LCAT activity), thereby removing cholesterol and leading to a decrease of the amount of cholesterol in the sera. This result is in agreement with the work of Nevin and Rajamohan (2009) who reported a decrease in cholesterol concentration following VCO treatment.

From Table 6, there was a non-significant ($p > 0.05$) increase or decrease in the activities of ALT, AST and ALP of the treatment groups (4, 5 and 6) as compared to the positive control and the negative control. The gradation in the AST activity of the VCO treated groups was observed to be dose-dependent. This is probably as a result of the MCFA contents of VCO which decrease the stomach pH towards acidity. Alkaline phosphatase functions maximally in alkaline medium and therefore, its activity reduces following VCO treatment. This finding supports the research of Kabara (2014) who observed a non-significant ($p > 0.05$) decrease in the ALP activity of rats treated with VCO after oxidative stress has been induced with cyclophosphamide.

Histological findings revealed that stomach sections of rats in groups 4 and 5 showed moderate widespread mucosal necrosis and ulceration, while that of group 6 rats showed focal area of mucosal ulceration with evidence of healing by fibrosis when compared with the positive control.

The stomach of the animals in the positive control group showed severe ulceration with haemorrhages (H) and inflammatory cells when compared with the normal and standard controls with intact epithelium, gastric glands, and the lamina propria (LP). Treatments with VCO showed some visible effect from the photomicrograph against ulceration showing the decreasing trend of mucosal congestion. The results from this study is in tandem with the work of Amjad and Tahir (2017) where the effect of ethanolic extract of coconut (*C. nucifera)* on aspirin-induced gastric ulcer in albino rats was evaluated.

Conclusion

The findings of this research revealed that VCO possesses nutraceutical properties and could therefore be used for the treatment of gastric ulcers. However, there is need for more work to be done to ascertain the actual mechanism of action and bioactive compound responsible for its medicinal properties.

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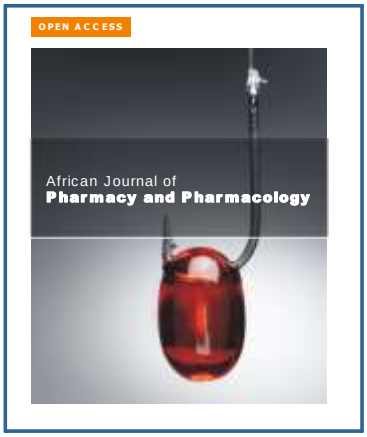

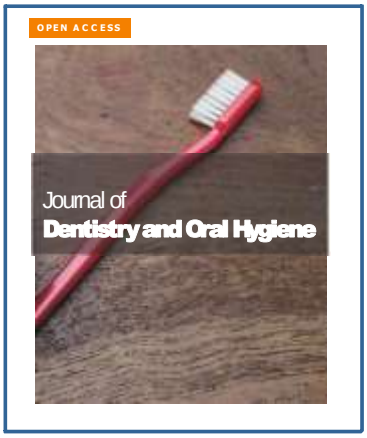

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