Mechanism underlying the colcemid inhibition of rejoining of nucleotide excision repair

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Abstract

Colcemid, a drug initially for arresting cells at mitotic stage, was found to inhibit the rejoining of nucleotide excision repair (NER) in cells exposed to UV irradiation. Subsequent studies reveal not only colcemid but the chemicals which cause oxidative stress have the similar inhibitory effect on gap filling of NER. The inhibitory effect has been correlated to the base excision repair (BER) of oxidative DNA damage and was proposed to result from the competition between BER and NER for common components such as PCNA in the gap filling step. The proposition was supported by the observation that overexpression of PCNA attenuates the oxidative stress-induced inhibition of gap filling of NER. Considering the roles of PCNA in both repairing of oxidative DNA damage and translesion DNA synthesis, a model is proposed. Lastly, the chemistry of colcemid in causing oxidative stress is briefly reviewed.

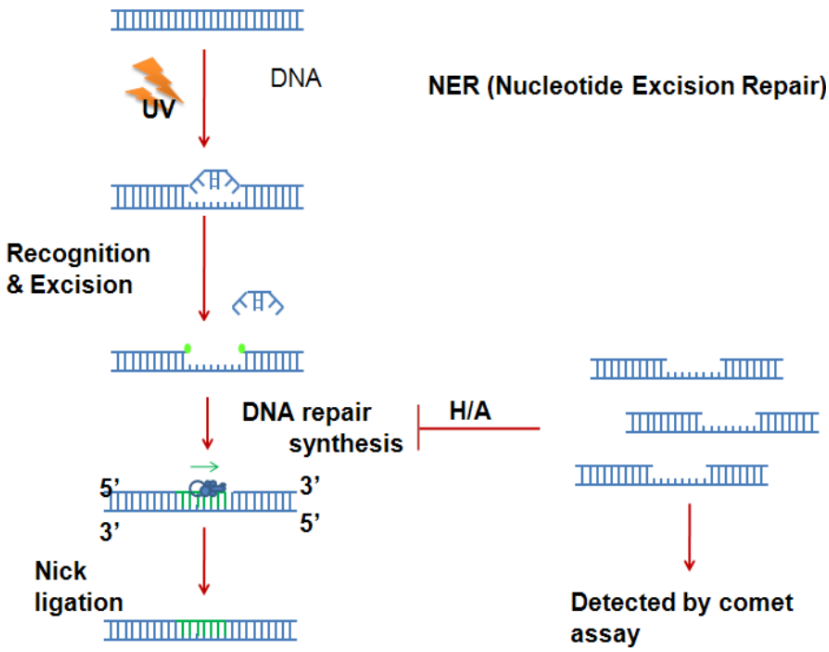
Keywords: Colcemid, Nucleotide excision repair, Rejoining, Oxidative DNA damage, Base excision repair, PCNA

1. Introduction

1.1 Unexpected feature of colcemid

In our early study, colcemid was found to inhibit the rejoining of nucleotide excision repair [1]. Initially, we tried to use colcemid to arrest Chinese hamster ovary cells, CHO-K1 at mitosis stage while studying the function of the cellular p53 tumor suppressor gene. Since p53 is activated to induce cell cycle arrest at G1 stage when the cellular genome is damaged, we used UV irradiation at 254 nm as source of DNA damaging agent. Thus, the CHO-K1 cells were UV irradiated and recovered in the presence of colcemid. Unexpectedly, the additional treatment of colcemid caused a moderate increase of cell death as compared to UV irradiation alone [2]. To understand the underlying mechanism, we found the presence of colcemid does not affect the excision of photo-induced DNA adducts i.e. CPD and 6,4 PP, however, it inhibits rejoining of the intermediates following excision in NER [1]. Since the rejoining process consists of gap filling and ligation, two consecutive steps [for review of NER, see [3]], it is desirable to know if the effect of colcemid is on which of the two steps. We were unable to answer the question unequivocally until the following experimental scheme [4, 5] was adopted for allowing the investigation of effect on gap filling. In the experiment, cells were treated with UV irradiation and recovered in the presence of hydoxyurea and Ara-C, the chemicals to perturb respectively the nucleotide metabolism and inhibit DNA polymerase activity, for a period of time e.g. 4 hours. As the result, the gap filling is inhibited and the repair intermediates are accumulated. Then, the chemicals hydoxyurea and Ara-C are removed to allow the gap filling to resume and the completion of the rejoining is observed by the single cell alkaline electrophoresis or commonly called comet assay (see Fig 1 for illustration of the experimental scheme). We found colcemid inhibits the gap filling.

(A)



(B)

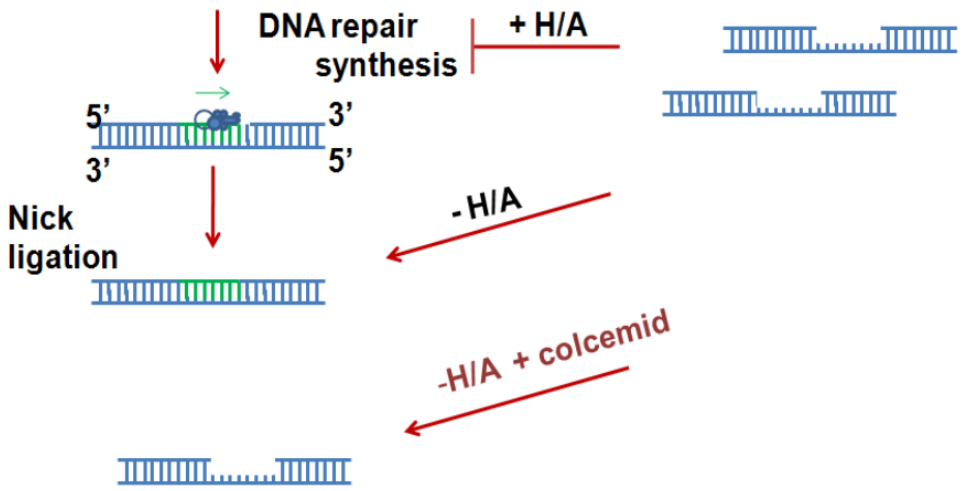


Fig. 1. Schematic illustration of experimental procedure involving chemicals hdyroxyurea and Ara-C (H/A) to block DNA repair synthesis for building up intermediates which can be detected by comet assay (A). Removal of H/A allow the resume of DNA repair synthesis; however, presence of colcemid inhibits the resumption of DNA repair synthesis (B).

1.2 Linkage to oxidative stress

In addition to colcemid, amoxicillin, a penicillin like compound also inhibits gap filling. Moreover, amoxicillin was found by us and others [6, 7] to induce oxidative stress and cause DNA damage. This prompted us to wonder if the oxidative DNA damage is associated with the inhibition of gap filling. First, like amoxicillin, colcemid is an oxidative stress inducer and causes oxidative DNA damage [8]. Second, the oxidative DNA damage and inhibition of gap filling could be correlated in a quantitative manner [9]. Oxidative stress induced DNA lesions are known to be repaired by base excision repair (BER) pathway [10, 11]. Consistent with the correlation aforementioned, we found that the inhibition or delay of gap filling by oxidants such as hydrogen peroxide, amoxicillin and propolis is absent in EM9 cells [9], a BER deficient line of Chinese hamster ovary [12]. Also, antioxidants such as beta-carotene and glutathione attenuate the oxidative stress induced delay of gap filling [9].

2. Explanation

Taking together, the inhibition of rejoining of NER by colcemid is because of the BER triggered by the colcemid induced-oxidative DNA damage. For further understanding, we proposed that the gap filling of NER may be delayed by BER as result of competition between the two repair pathways for common machinery. As shown in Fig. 2, the two excision repair pathway may share the components for rejoining including DNA polymerase and accessory factors such as PCNA.

To repair oxidative DNA damage, the glycosylases of BER namely Ogg1 and Fpg recognize and cleave the oxidized purines or pyrimidines and generate abasic sites which are removed and processed by either short patch pathway or long patch pathway depending on the nature of the targets [10]. While examining the common components

aforementioned, we found PCNA is essential for cells to repair oxidative DNA damage. Knockdown of PCNA greatly reduces the repair of hydrogen peroxide caused DNA adducts [8]. Overexpression of PCNA attenuates the oxidative stress induced delay of gap filling of NER, however, overexpression of ligase l dose not [8]. These suggest that PCNA but not ligase I is the common component during the gap filling of NER and also during the repair of oxidative DNA damage by BER. Ligase I and PCNA are known to be involved in gap filling of NER and long patch pathway of BER. Our study suggests that repair of oxidative DNA damage is not long patch BER. This was supported by the observation that knock down of Fen I did not impair the repair of oxidative DNA damage [8]. In summary, the competition for common machinery such as PCNA by BER may delay the gap filling of NER.

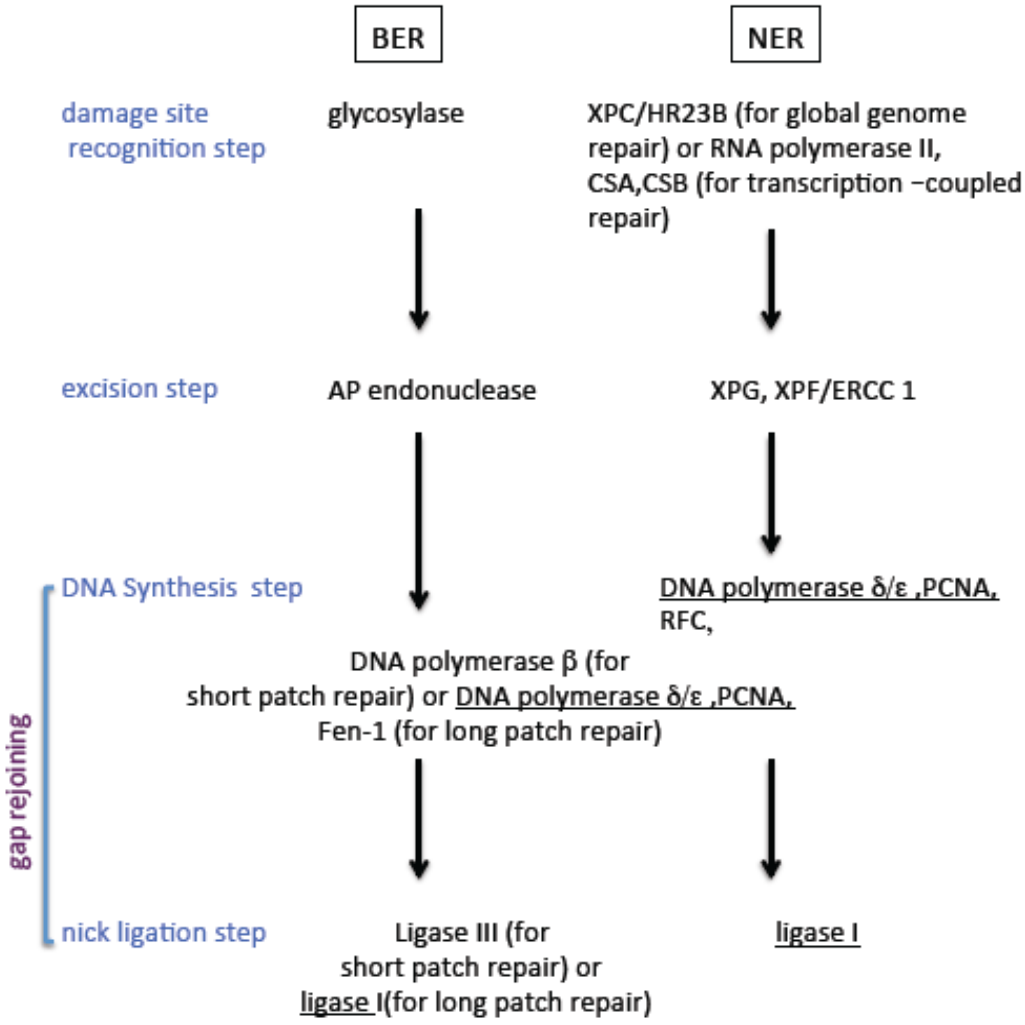


Fig. 2 Cellar components (underlined) shared by both NER and BER.

3. Questions to be solved

3.1. Why BER is dominant over gap filling of NER?

Our study indicates that repair of oxidative DNA damage by BER competes components such as PCNA and delays the completion of NER. If the competition is equal, the repair of oxidative DNA damage by BER should also be delayed. Interestingly, the repair of oxidative DNA damage by BER is not affected by the treatment of UV irradiation, suggesting that BER is dominant over gap filling of NER as shown in the illustration (Fig 3). The underlying mechanism is unclear. We speculate that repair of oxidative DNA damage is a more urgent job for cells to avoid mutagenesis and/ or the intermediates subject to gap filling of NER are relatively stable and can withstand until the job of BER is completed. Our previous study indicates that colcemid does not inhibit the excision of UV induced photo products, only inhibits the rejoining step. This is consistent with the notion that NER and BER rely on distinct machineries for the initial recognition and excision. In NER, the photo induced DNA lesions are initially recognized by XPC-HR23B or RNA pol II, and then excised by the co-work of XPG and XPF-ERCC1[3]. In BER, the oxidized bases are identified and removed by bifunctional glycolsylases such as OGG1, NEIL1 and NTH1 in human [13].

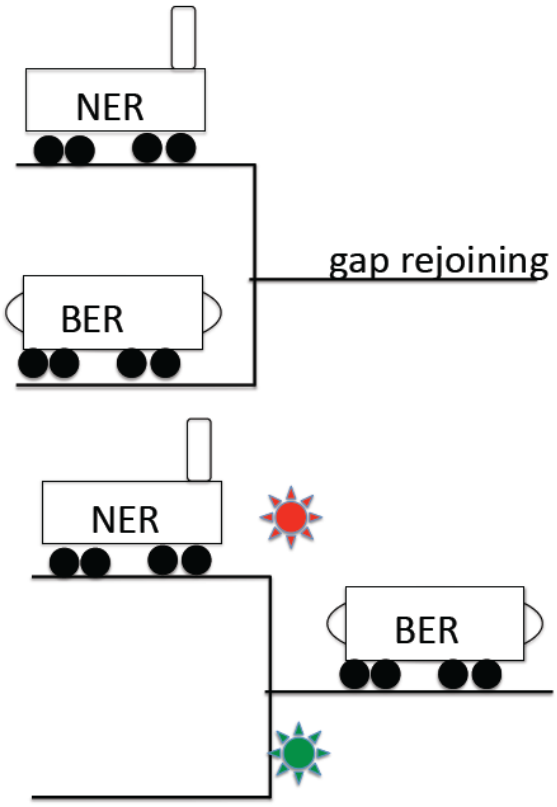


Fig. 3. Model for delay of gap-filling of NER by BER. NER and BER are illustrated as trains sharing the common track for proceeding gap rejoining. Red and green for traffic red light and green light, respectively. NER must yield the right of traffic for BER if BER is also present.

3.2. How PCNA play role in repairing oxidative DNA damage?

Our study indicates that repair of oxidative DNA damage requires PCNA and is not of long patch BER. How PCNA is involved in the repair of oxidative DNA damage is unclear. Previous study has indicated that repair of oxidative DNA damage is replication coupled [14]. Indeed, we found that the removal of 8-OHdG was greatly reduced by DNA replication inhibitory drug such as aphidicolin. Similarly, the repair of hydrogen peroxide induced DNA damage was slower in serum deprived cultured cells as compared to those grown in normal medium (our unpublished results). Also, it has been known that translesion, i.e. bypassing the damage may take place to avoid occurring of double strand break in the replication fork [15, 16]. In the situation of UV damage, monoubiquinated PCNA may play role in the DNA polymerase switching during translesion [17]. To know if translesion synthesis (TLS) is involved in repair of oxidative DNA damage, we performed the following experiment: Our preliminary study showed that a mutant PCNA, which is unable to be ubiquitinated at amino acid residue 164 exhibited lower repair rate as compared to those complemented with wild type PCNA, suggesting that TLS may be involved in repair of oxidative DNA damage.

3.2.1 Experimental procedures for the unpublished results just mentioned

3.2.1.1

For studying the effect of aphidicolin on the repair of 8-OHdG:
Human gastric adenocarcinoma AGS cells in logarithmic phase were treated with 15 μM aphidicolin for 2 h before being exposed to H_2O_2. The cells were harvested at 1 or 4 h after H_2O_2 treatment for detecting 8-OHdG by method of immunostaining or ELISA analysis, respectively. *For immunostaining*: cells seeded for 1 day were treated with 1 mM H_2O_2 for the indicated time points and harvested for immunostaining. Cells after fixation were treated with 4N HCl to denature DNA. The antibody to 8-OHdG (1:500) and a secondary antibody conjugated with Hilyte Flour 488 (1:200) was used for detecting 8-OHdG. Nuclei were counterstained with 4',6-diamidino-2-phenylindole. Fluorescence images were captured by a digital camera on a Fluorescence Microscope.

For ELISA:

AGS cells were treated with 1 mM H_2O_2 and harvested at the indicated time points after treatment for genomic DNA extraction. Each DNA sample was denatured at 95^{o}C for 5 min and was then chilled on ice followed by incubation with 2 units of alkaline phosphatase and 5 units of DNase I at 37 ^{o}C for 2 h. 96-well plates were first coated with 0.003% protamine sulfate and then with 100 ng 8-OHdG. Coated wells were added in a series of concentrations of pure 8-OHdG or DNA samples. The antibody to 8-OHdG, biotin goat anti-mouse IgG and peroxidase-streptavidin were used sequentially for the detection of 8-OHdG. O-phenylene diamine dissolved in citrate phosphate buffer was used as a substrate for peroxidase. The absorbance was read at 492 nm with a microplate reader.

3.2.1.2

For studying the effect of serum starvation on the repair of H_2O_2 induced-DNA adducts. Cells in logarithmic phase were re-cultured in 10% or 0.5% FBS containing medium for 24 h before being treated with 20 μM H_2O_2. Kinetic changes in levels of H_2O_2 induced-DNA adducts were monitored by comet-Fpg/EndoIII assay.

3.2.1.3.

For studying the involvement of TLS in the repair of H_2O_2 induced-DNA adducts. PCNA expression of the cells was knock downed for 24 h, then the knockdown cells were transfected with pGFP-PCNA (wild type) or pGFP- PCNA(K164R) for 24 h, respectively before being treated with 20 μM H_2O_2. The kinetic changes in levels of H_2O_2 induced-DNA adducts were monitored by the comet-Fpg/EndoIII assay.

Thus, based on the above arguments, we propose that PCNA, by coupling with DNA replication, may act as a scaffold for BER repair complex to reach the oxidative DNA damage sites of the entire genome (see the model illustrated in Fig. 4). Repair of the oxidative DNA damage sites is done after the sites are bypassed by the replication machinery which requires PCNA for polymerase switch.

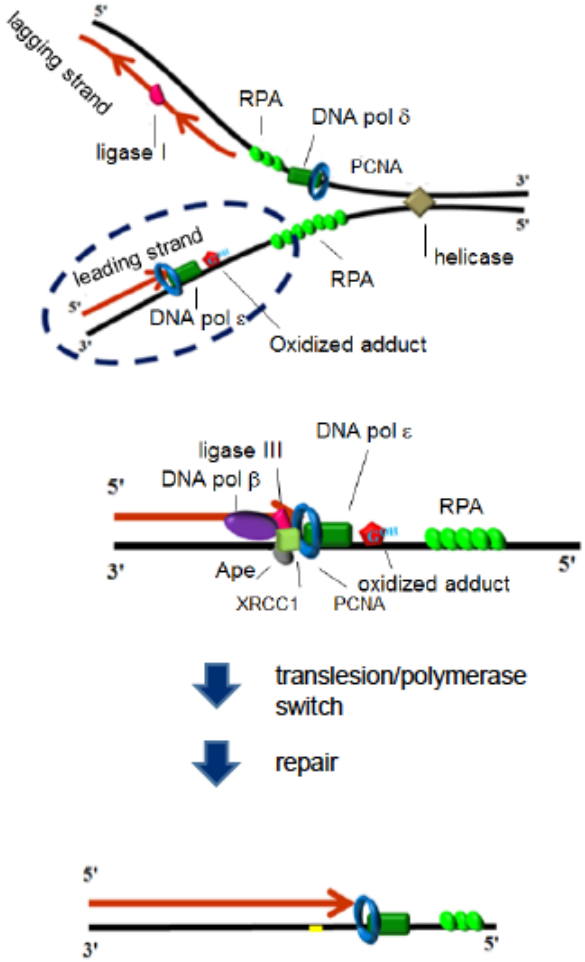


Fig. 4. Model for the roles of PCNA in both translesion DNA synthesis and repair of oxidative DNA damage. DNA is replicated in both lagging strand and leading strand fashions. Coupled to DNA synthesis, BER is able to identify the oxidized adducts of the entire genome. PCNA serves dual roles both in translesion DNA synthesis for DNA polymerase switch and in repair of oxidative lesions.

3.3. Chemistry of colcemid induction of the oxidative stress

Colcemid has been used as mitotic inhibitor and had not been described as oxidative stress inducer until our report. We relied on comet-fpg/endoIII method (Fig. 5) [18] to detect the oxidative DNA lesions caused by colcemid and other chemicals such as amoxicillin. In comparing with other methods for detecting oxidant capacity such as TEAC [19], the method is more sensitive as the signals can be detected in single cells. Our previous study suggests that the polyphenol groups in these chemicals (See Fig. 6) are involved in producing the reactive oxygen species (ROS) in the presence of divalent metallic ions e.g. iron which are present ubiquitously in the ordinary reaction mixtures [20]. ROS particularly the hydroxyl radicals are responsible for formation of oxidized bases.

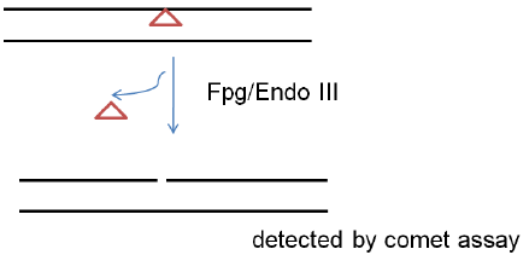


Fig. 5. Illustration of comet-fpg/endoIII assay. Fpg and endoIII are bacterial enzymes recognizing oxidized purines and pyrimidines, respectively.

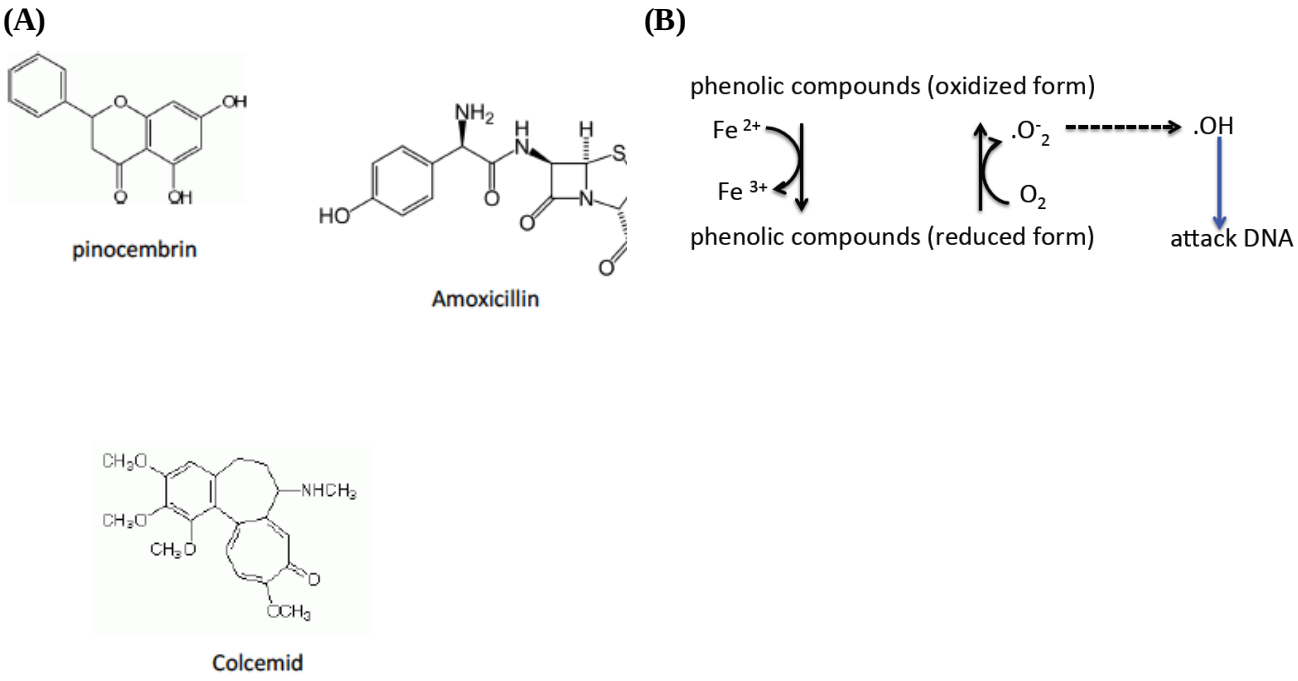


Fig. 6. (A) Chemical structures of oxidative stress inducers e.g. pinocembrin (a major flavonoid compounds of propolis) , amoxicillin and colcemid which contain the polyphenolic groups. (B) Model for the phenolic compounds to cause reactive hydroxyl radicals to damage DNA

4. Acknowledgement

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5. References

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