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Antiulcerogenic activity of Yttrium and Copper oxide nanoparticles

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ABSTRACT

Gastric ulcer is an excavation of mucosa and extending to submucosal layers due to increased gastric secretion, generation of free radicals and imbalance between protective factors and aggravating factors. The present study aimed to screen yttrium and copper oxide nanoparticles for antiulcerogenic activity. Metal oxide nanoparticles were evaluated for various biological activities considering their high surface area to volume ratio, different oxidative states, cell permeability etc. Various studies have proved metal oxide nanoparticles to be beneficial in various ailments due to their antioxidant, antibacterial, and other pharmacological activities. Nanoparticles prepared using biogenic methods were relatively more biocompatible and less toxic compared to conventional methods of synthesis. In the present study, green synthesized yttrium and copper oxide nanoparticles were evaluated for acute toxicity, antioxidant, proton pump inhibition and antiulcerogenic activity. *In vitro* antioxidant activity by DPPH, nitric oxide scavenging methods and H^+K^+ATPase assay was performed to evaluate the mechanism of action. Synthesized nanoparticles have shown no signs of acute toxicity as per OECD 423. Pyloric ligation method was performed to evaluate gastric volume, pH and ulcer severity. The present study revealed the dose-dependent antiulcer potential of yttrium oxide nanoparticles at doses of 2mg/kg and 20mg/kg. Antisecretory action of yttrium and copper oxides were evident from *in vitro* H+K+ATPase activity and inhibition of gastric volume. Antioxidant activities of yttrium oxide nanoparticles indicate gastric mucosal protection by free radical scavenging action. Copper oxide nanoparticles produced antisecretory activity but produced mucosal damage and hemorrhage at a dose of 20mg/kg.

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INTRODUCTION

Metal oxide nanoparticles have gained importance in biomedical research due to their unique physicochemical and biological properties. The use of biogenic methods for nanoparticle synthesis proved to be effective, eco-friendly, and less toxic.

Yttrium oxide nanoparticles were screened for various pharmacological activities like anti-inflammatory, antioxidant, antidiabetic (Tang, 2021), anticancer (Nagajyothi *et al.*, 2018), hepatoprotective (Song *et al.*, 2019), neuroprotective (Schubert *et al.*, 2006) and found to be effective. Yttrium oxide nanoparticles have shown antibacte-

rial activity against *Escherichia coli*, *Staphylococcus aureus*, Pseudomonas aeruginosa (Kannan and Sundararajan, 2015) .

Copper oxide nanoparticles were evaluated for antibacterial, anticancer, and other pharmacological properties (Das *et al.*, 2016).

Owing to the literature on the biological activities of Yttrium and Copper oxide nanoparticles, the present experimentation aimed to screen the antiulcerogenic activity of the synthesized nanomaterial.

MATERIALS AND METHODS

The material used in the study, Sodium Nitroprusside, Sulphanilamide, Trichloroacetic acid, Tris buffer, and DPPH were Procured from Loba Chemie, Mumbai.

Green synthesis mediated Yttrium and Copper oxide nanoparticles were synthesized and used for the study.

Animals

Male albino rats, Wistar strain (140-200gms) were procured from CPCSEA approved facility, Hyderabad. The animals were acclimatized for 7 days, under standard 12h light cycles, and at a temperature of 25^0c.

Standard pellet diet and RO purified drinking water were made available. The study was approved by IAEC (10/IAEC/SVCP/2018-19).

In vivo antiulcerogenic activity

Pyloric ligation induced Gastric ulcer model (Shay, 1945)

Experimental animals were divided into six groups of six each. Segregated into Control, standard, and four test groups. Animals were deprived of food 48h before ligation.

They were allowed to recover and sacrificed after 8hrs. Stomachs were isolated, cut along the greater curvature, washed with normal saline, and fixed using 10% buffered formalin.

Ulcers were evaluated using the following score (Khadeerunnisa *et al.*, 2020)

1. Normal stomach - 0
2. Red coloration - 0.5
3. Spot ulcers - 1
4. Hemorrhagic streaks - 2
5. Perforation - 3

Ulcer index was measured using the formula:

% inhibition of ulceration=

$$\frac{Control\ mean\ ulcer\ index\ -\ Test\ mean\ ulcer\ index}{Control\ mean\ ulcer\ index} \times 100$$

pH and Total acidity (Parmar and Hennings, 1984)

pH is tested using LI-120 table-top pH meter.

Total acidity was estimated using 0.01 N NaOH and phenolphthalein indicator.

In vitro assays

Nitric oxide scavenging assay

4mL of test suspension and 1mL of Sodium Nitroprusside solution were mixed and incubated at 37^oc for 3h. A fraction of incubation solution (0.5ml) was taken and 0.3mL of Griess reagent was added. The absorbance of the chromophore developed was measured immediately at 570 nm. A control was prepared using 0.1 ml of the vehicle in the place of the test sample. (Marcocci *et al.*, 1994)

DPPH scavenging assay

The free radical scavenging activity of the nanoparticles on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was determined. In the present study, 0.002% of the DPPH solution is used. Different concentrations of nanopowder suspension in distilled water are used as a test. The inhibition of the DPPH content in the suspension is measured using a UV spectrophotometer. (Brand-Williams *et al.*, 1995)The absorbance was determined at 517 nm and from these values, the corresponding percentage of inhibitions were calculated by using the following equation:

$$\%\ inhibition\ =\ \frac{(ABS\ control - ABS\ sample)}{ABS\ control} \times 100$$

H^+K^+ATPase assay

H^+K^+ATPase was derived from Goat stomach mucosal Scrapings. The mucosa was homogenized in Tris–HCl (20 mM). The contents were centrifuged for 10 min at 10000 RPM and the resulting supernatant was subsequently centrifuged for 20 min at 10000 RPM. The extracted enzyme of 0.1 ml was added to different concentrations of Nanoparticles in distilled water and incubated at 37 ^{0}C for 60 min. After incubation, 0.2 ml Tris–HCl (20 mM, pH 7.4); 0.2 ml $MgCl_2$ (2 mM); 0.2 ml of KCl (2 mM); 0.2 ml of ATP (2 mM) and incubated at 37 C for 30 min. The reaction was terminated by the addition of 1 ml of 10% TCA, followed by centrifugation. The amount of inorganic phosphorus liberated from ATP was determined at 640 nm. The assay was performed in triplicates and the results were averaged. (Reyes-Chilpa *et al.*, 2006)

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Table 1: Evaluation of antiulcerogenic activity by pyloric ligation method

Group	Gastric Volume (mL)	pH	Acidity (meq/L)	Mean Ulcer index	% Inhibition of Ulcer
Control	5.15±0.06	2.5±0.30	115.6±0.6	2.83±0.16	–
Ranitidine (50mg/kg)	1.58±0.09	4.6±0.12	57.3±0.6	0.58±0.08	79.38
Y2O3 (20mg/kg)	2.40±0.17	3.5±0.10	67.3±0.8	1.08±0.08	61.71
Y2O3 (2mg/kg)	3.18±0.14	3.4±0.02	70.5±0.8	1.33±0.10	52.88
CuO (20mg/kg)	1.52±0.11	3.0±0.08	65.8±0.5	2.33±0.21*	17.55
CuO (2mg/kg)	2.56±0.06	3.1±0.05	63.6±0.3	2.16±0.27*	23.43

Table 2: Antioxidant activity

Sample	Concentration (μg/ml)	Inhibition of NO (%)	Inhibition of DPPH (%)
Ascorbic acid	50	80.1	73.1
	100	89.6	84.9
Yttrium oxide	50	53.9	59.8
	100	56.2	63.9
Copper oxide	50	23.6	42.1
	100	31.3	43.8

Values are represented in percentages compared with control absorbance. Triplet absorbances were considered for each sample.

Table 3: H^+K^+ATPase assay

Sample	Concentration (μg/ml)	Inhibition (%)
Omeprazole	50	80.1
	100	89.6
Yttrium oxide	50	52.1
	100	58.3
Copper oxide	50	64.8
	100	70.1

Values are represented in percentages compared with control absorbance. Triplet absorbances were considered for each sample.

Statistical analysis

The *in vivo study* results were analyzed using Graph pad prism 8. Obtained values were compared with the control applying the Student t-test.

RESULTS AND DISCUSSION

Acute toxicity study and selection of dose

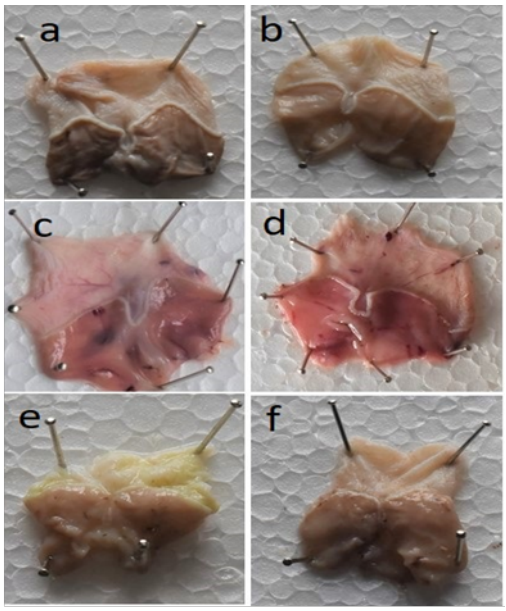
OECD 423 was followed for Acute toxicity evaluation. Yttrium oxide and Copper oxide nanoparticles at a dose of 2000mg/kg produced no mortality or toxic effects. Animals were observed for 2 hours continuously for changes in behavior, lacrimation, salivation, diarrhea, and neurological symptoms. They were examined every 24hrs for 14 days. Two doses (2mg/kg and 20mg/kg) were selected below $1/10^{th}$ of acute toxic dose, pertaining to the high surface area to volume ratio in nanoparticles.

The values in the table are expressed as Mean ± SEM. Compared values are significantly different from control at P<0.001* indicates values that are not significantly different from control. Other values are significantly different (P<0.05).

Effect of Nanoceria on *in vivo* ulcerogenic parameters

From Table 1, it is observed that Ranitidine (50mg/kg) treated and Yttrium oxide nanoparticles showed significant (P<0.001) cytoprotection and

inhibition of ulcer index compared to the control. Copper oxide nanoparticles deteriorated the mucosal layer in a dose-dependent manner which is evident from Figure 1. Other aggravating factors like pH and gastric volume were inhibited by both nanoparticles. Copper oxide showed a significant reduction in gastric volume but increased mucosal damage.



a) Control
b) Standard
c) Treated with Y_2O_3 (2mg/kg)
d) Treated with Y_2O_3 (20mg/kg)
e) Treated with CuO (2mg/kg)
f) Treated with CuO (20mg/kg)

Figure 1: Images showing ulceration

Free radical scavenging activity

Nitric oxide was studied to play a vital role in cell damage. Values in Table 2 indicates more than 50% inhibition of free radicals was observed with yttrium oxide nanoparticles. Copper oxide produced less effect on free radicals compared to yttrium.

Observing the values from Table 3, Yttrium oxide and copper oxide nanoparticles produced considerable H+K+ATPase inhibition activity and can be attributed to the antisecretory and cytoprotective action of the particles.

CONCLUSION

Yttrium oxide nanoparticles showed a dose-dependent and significant reduction in aggravating factors evaluated in the study. Antioxidant and H^+K^+ATPase inhibition assays revealed mucosal protection and antisecretory effects of Yttrium oxide. Copper oxide inhibited proton pump but produced Mucosal deterioration and severe ulcer index. Further studies on dose-dependent activity and cytotoxicity assays are needed to arrive at the cellular mechanisms of the activity produced.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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