

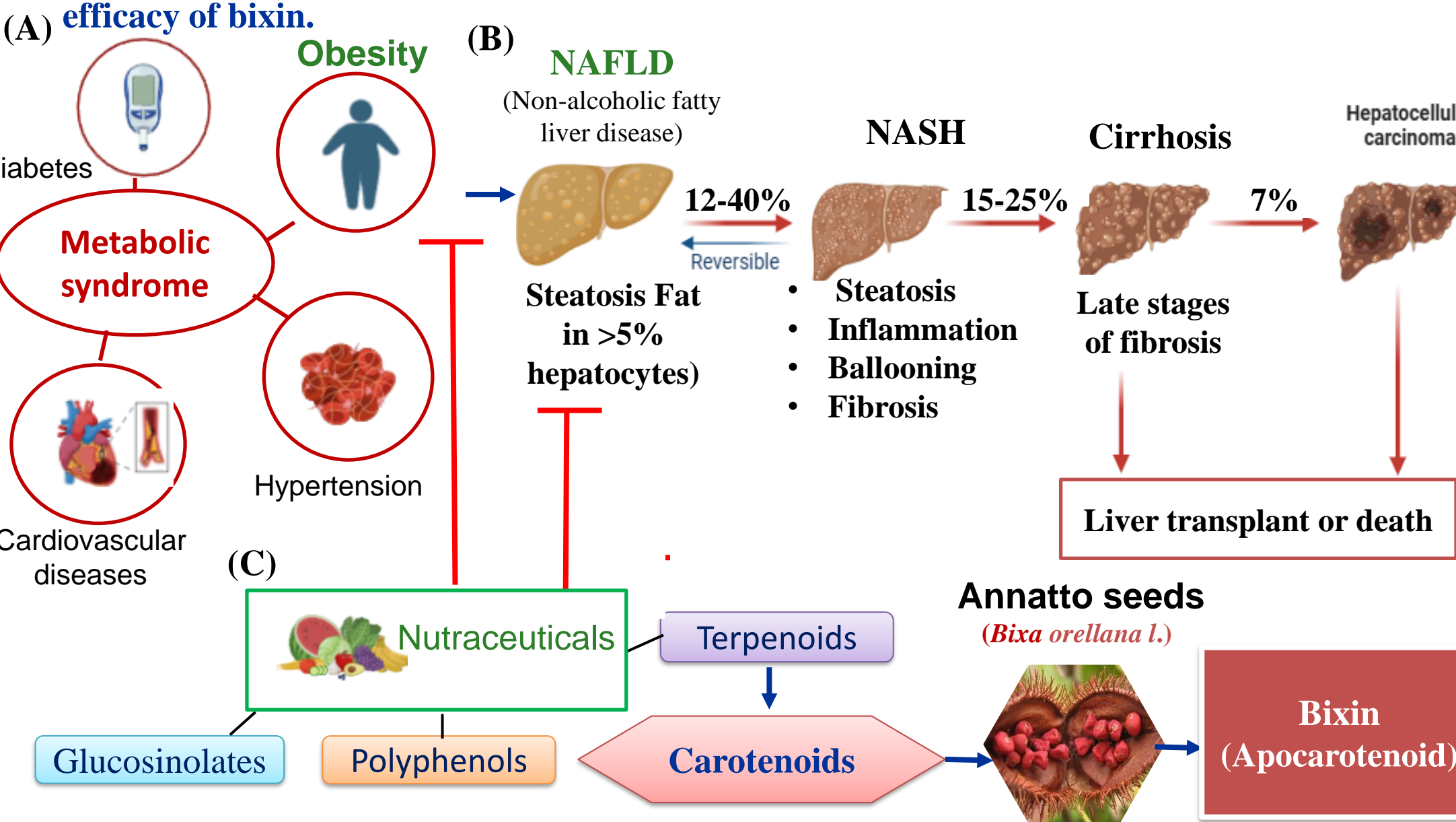
Anti-obesity and anti-steatotic effects of Bixin (apocarotenoid from *Bixa orellana* L. seeds): Dose-dependent correlations with bioactivity

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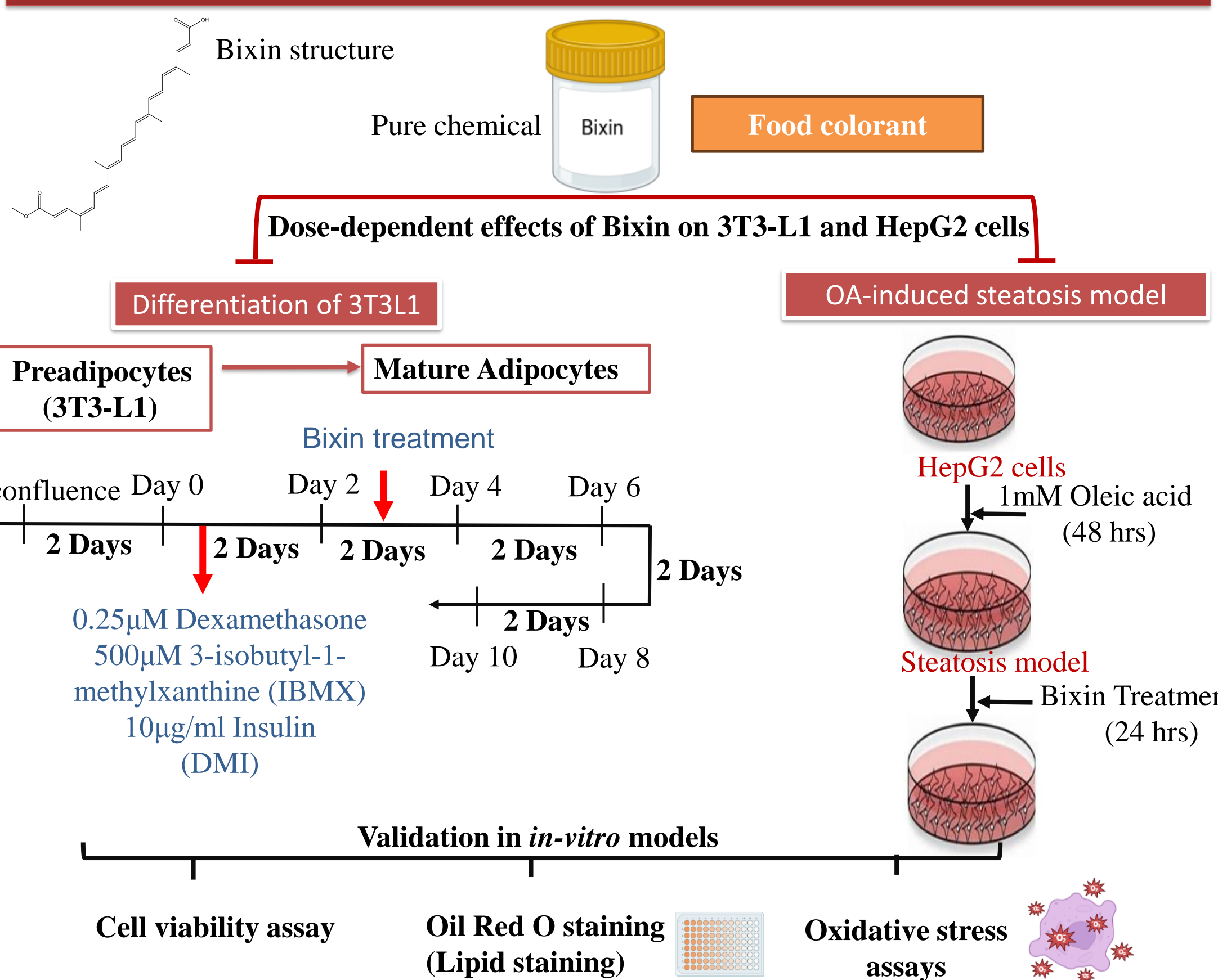
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INTRODUCTION & AIM

- MetS (Metabolic syndrome) - Constellation of diseases: Diabetes, Obesity, Hypertension, Cardiovascular diseases. Hepatic complication of MetS: NAFLD
- Globally, over 1 billion people are overweight¹, 25-35% suffer from NAFLD².
- The global prevalence of NAFLD among the obese is 75.54%¹.
- The present study was aimed to evaluate dose-dependent anti-obesity and anti-NAFLD efficacy of bixin.

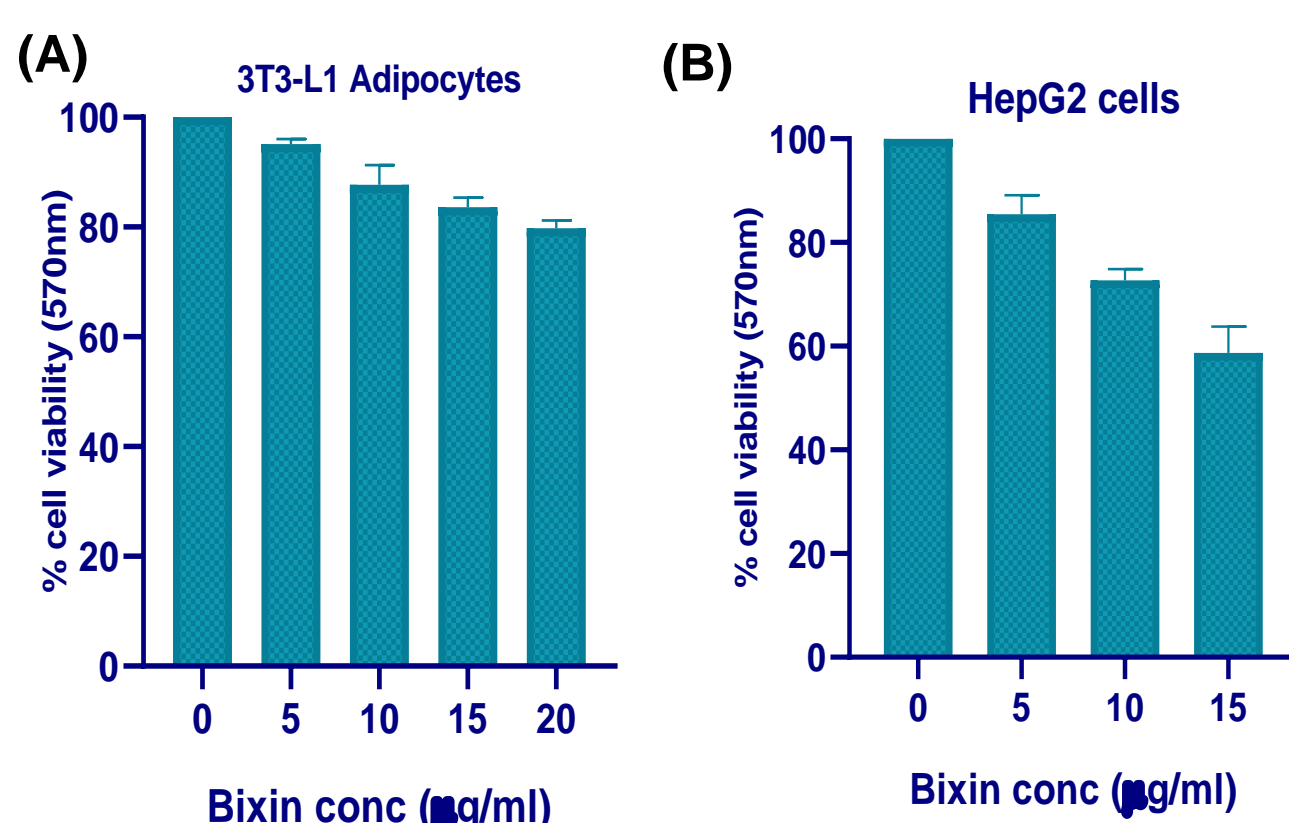


METHOD



RESULTS & DISCUSSION

Dose-dependent cytotoxicity assessment by MTT assay



Selection of non-toxic doses for anti-obesity and anti-NAFLD effect

Figure 1: Concentration-dependent effects of bixin on cell viability. (A) cell viability in 3T3-L1 cells (B) cell viability in HepG2 cells. Values are expressed as mean ± SD of three independent experiments performed in triplicates.

SP and SKM are recipients of Institute Fellowships from BITS, Pilani. Research grant (52/13/2022-BIO/BMS) from the Indian Council of Medical Research(ICMR), New Delhi, is gratefully acknowledged.

Assessment of lipid accumulation by Oil Red O staining

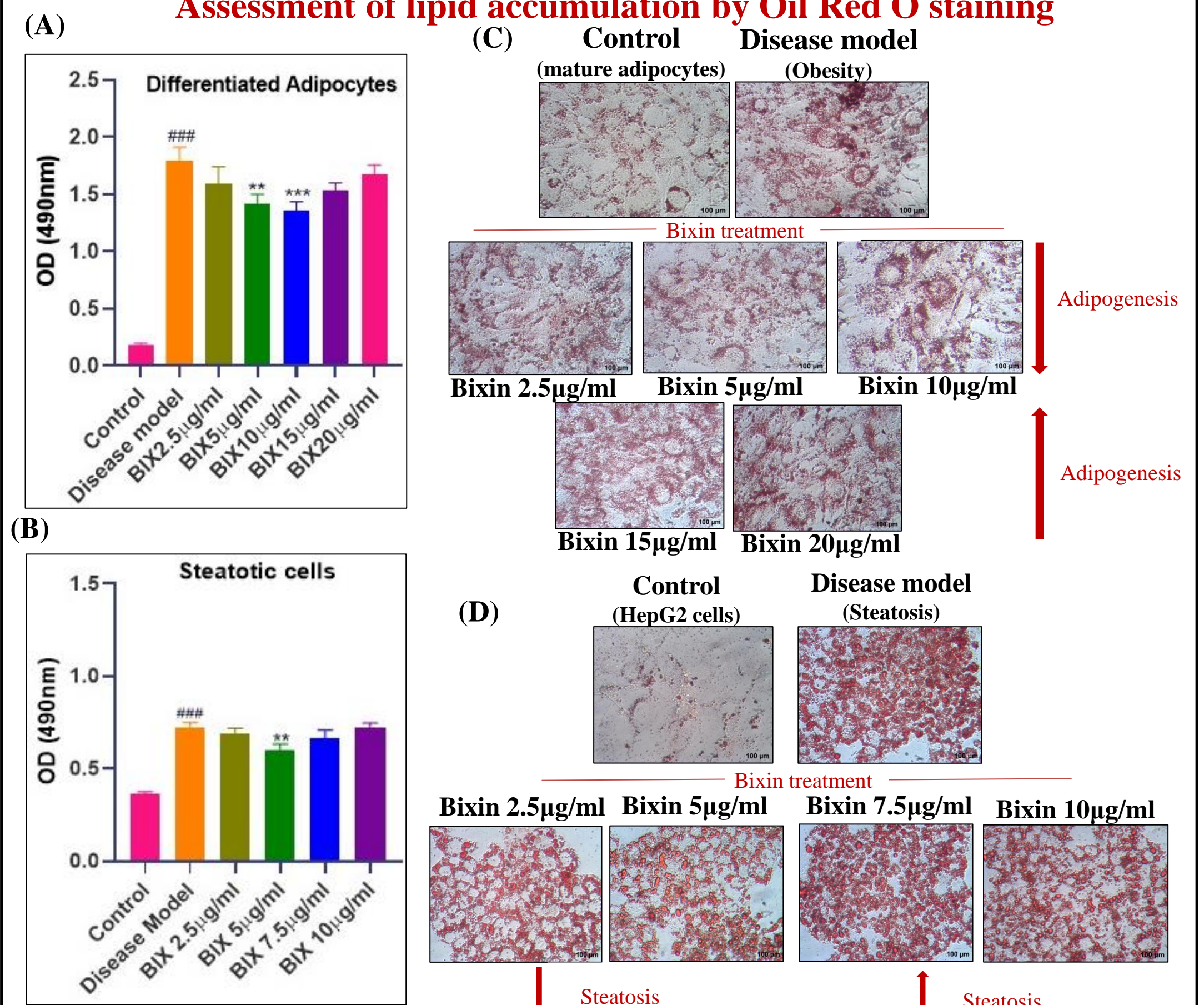


Figure 3. Inhibitory effects of bixin on lipid accumulation in *in vitro* disease models. (i) (A-B) spectrophotometric quantification of lipid accumulation (ii) (C-D) Representative photomicrographs of Oil Red O staining.

Dose-dependent effects of Bixin on Oxidative Stress

Lipid peroxidation (MDA) assay

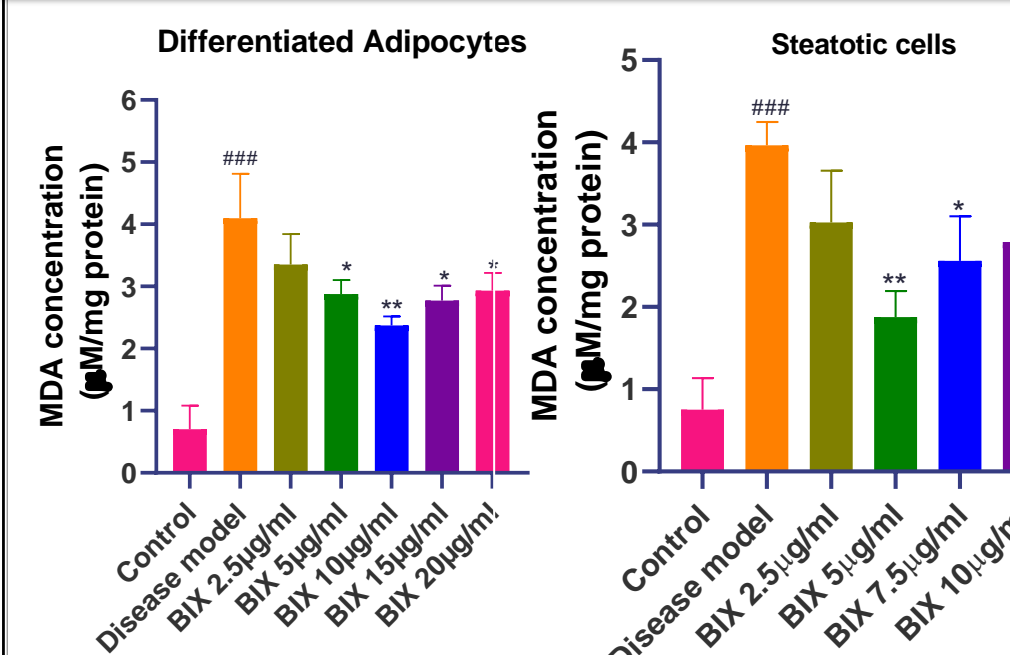


Figure 4: MDA concentration (marker of lipid peroxidation) assessed spectrophotometrically by TBARS assay.

Griess assay

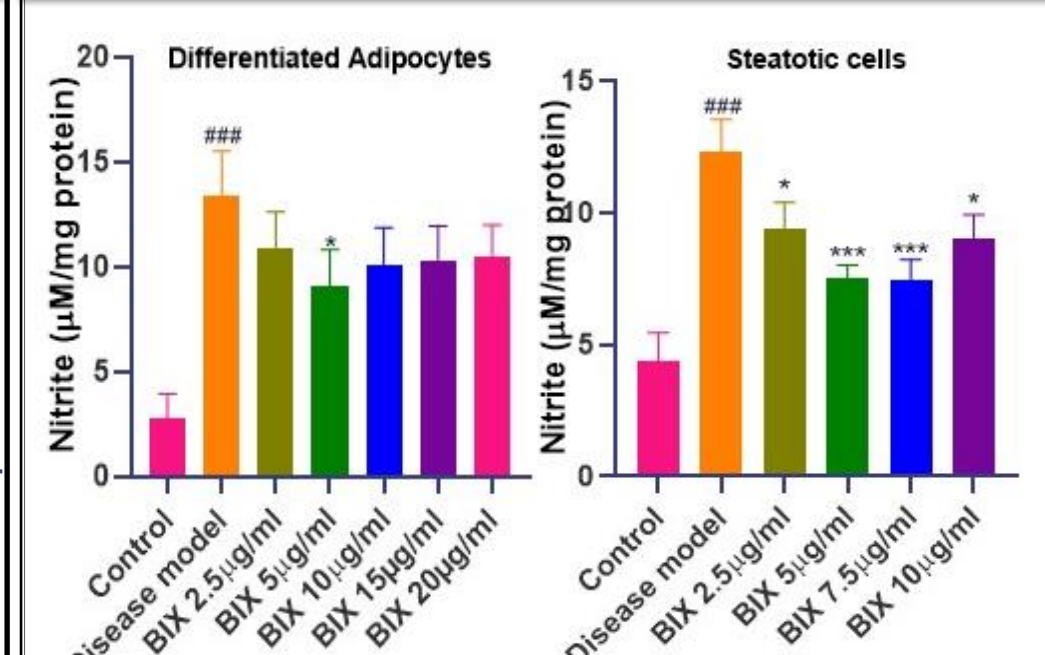


Figure 5: Reactive Nitrogen Species (RNS) levels in disease and treated groups

DCFDA assay

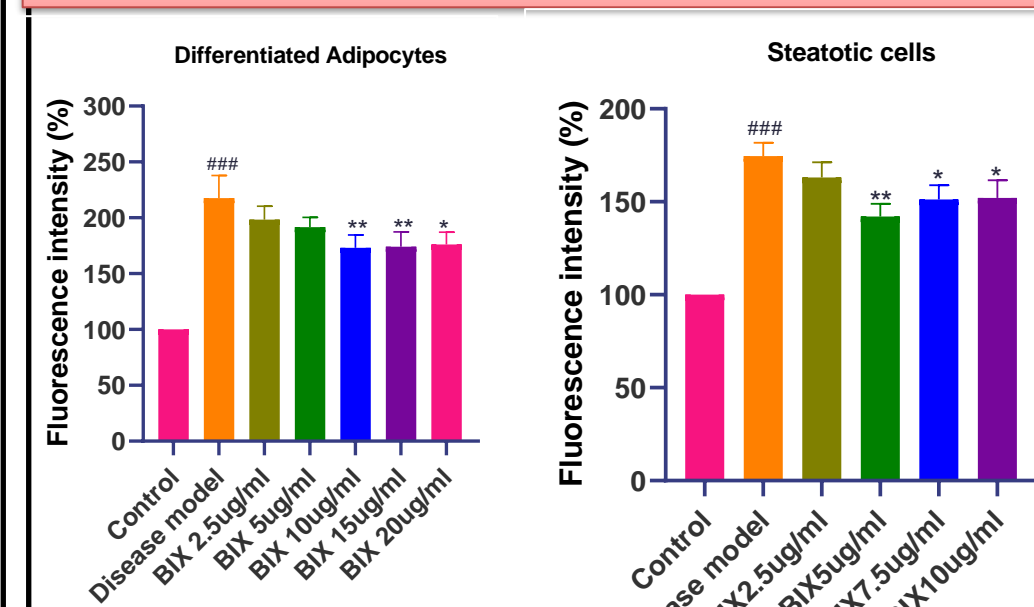


Figure 6: Quantification of total ROS/RNS levels by DCFDA assay.

Values are expressed as mean ± S.D. of a minimum of three independent experiments. ### P < 0.001, relative to the control group. *P < 0.05, **P < 0.01, and ***P < 0.001 relative to the disease model.

CONCLUSION

- Bioactivity of the apocarotenoid bixin was found to be **dose-dependent**.
- Bixin exhibited anti-obesity and anti-NAFLD effects at **10µg/ml** and **5µg/ml** respectively.
- However, at **higher concentrations**, that is, above 10µg/ml in differentiated adipocytes and 5µg/ml in steatotic cells, it ceased to inhibit lipid accumulation. This adipogenic/steatotic response was accompanied by elevated oxidative stress/lipid peroxidation, indicating a **pro-oxidant effect at higher doses**, which implicates its anti-cancer potential.
- Evaluation of dose-dependent pharmacological efficacy of natural products is emphasized.

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