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Role of Suvarna Bhasma with ghee and honey (Suvarnaprashan) for the anti-oxidative potential

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Reactive oxygen species (ROS) play a crucial role in regulating cell signaling mechanisms and redox balance of the cells, while their imbalance contributes to cellular damage and the pathogenesis of diseases. Redox status is very important for its immune homeostasis, and the dysregulation of which is involved in many diseases. Suvarnaprashan, an Ayurvedic formulation consisting of Suvarna Bhasma, Ghee, and Honey, has been known since ancient times for its use as an immunity booster, for general well-being, and enhancing cognition and memory in adolescents. However, underlying molecular mechanisms, which are modulated by Suvarna Bhasma, are still not explored. The present study aimed to prepare Suvarnaprashan, assess its cytotoxicity, and modulate redox balance inside the cells. Firstly, Suvarna Bhasma was characterized via various techniques, including heavy metal analysis, scanning electron microscopy, elemental analysis, Fourier-transform infrared (FTIR) spectroscopy, and X-ray diffraction (XRD) analysis, to confirm its physicochemical properties. Then, Suvarnaprashan was prepared with Suvarna Bhasma, ghee, and honey as per Ayurvedic literatures. Further, cell cytotoxicity was evaluated using the MTT assay for 24 h with Suvarna Bhasma and Suvarnaprashan (100, 50, 25, 12.5, 6.25, and 0 µg/mL), ghee (100, 50, 25, 12.5, 6.25, and 0 mg/mL), and honey (5, 2.5, 1.25, 0.625, 0.3125, and 0%). Intracellular ROS levels were quantified using a DCFH-DA assay to evaluate antioxidant activity. The results demonstrated that Suvarna Bhasma exhibited structural and elemental similarity to pure gold. No significant toxicity of Suvarna Bhasma was observed at different concentrations ($p>0.05$), while Suvarnaprashan, ghee and honey showed little toxicity. Suvarnaprashan and its ingredients significantly reduced intracellular ROS levels compared to untreated controls ($p<0.05$), indicating notable antioxidant activity. In conclusion, Suvarnaprashan is low-toxic and exerts significant antioxidant effects by modulating intracellular ROS levels, suggesting its potential role in maintaining redox homeostasis and supporting immune function. Further studies are required to elucidate its detailed molecular mechanisms.

Keywords: Antioxidant, ROS, Suvarna bhasma, Suvarnaprashan

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Ayurveda is one of the ancient traditional systems of medicine, widely practiced in Southeast Asia. *Kaumarabhritya* is a branch of Ayurveda that specializes in pediatric medicine. Ayurveda recommends using electuaries, commonly known as Prashan (lickables), a popular practice from the neonatal period. Licking *Suvarna Bhasma* (*incinerated gold particles, IGP*) along with *Madhu* (honey) and Cow *Ghrita* (clarified butter oil, CBO) is known as *Suvarnaprashan* (SP)[1]. The practice of *SP* dates back to the *Vaidik* period (before 2nd B.C.) from *Paraskar Grhihyasutra*[2] to *Bhaisajya Ratnavali*[3]. During the Vedic period, Suvarnaprashan was administered as a combination of Suvarna Bhasma (pure raw very fine gold particles), honey, and CBO, accompanied. It has been described that pure gold should be triturated along with honey and Ghrita on a pre-washed and clean stone, facing the eastern direction, and the mixture should be licked by the child. With *Rasa Shastra*'s (Indian Alchemy) development and improvement, Suvarna Bhasma (IGP) was used instead of pure raw gold. The main aim of SP was to prevent postpartum complications, improve immunity and lifespan, and enhance cognition and memory in neonates and growing children[1,4-6]. The dose of SP ranges from 1 to 15 mg/day. As a specific outcome, it is mentioned that

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Abbreviations
ROS: Reactive Oxygen Species, DCFDA: 2'-7'-Dichlorodihydrofluorescein diacetate, ICP-AES: Inductively Coupled Plasma Atomic Emission Spectroscopy, SP: *Suvarnaprashan*, SB: *Suvarna Bhasma*, IGP: Incinerated gold particles, SEM: Scanning electron microscopy, EDX: Energy-dispersive X-ray spectrometer FTIR: Fourier-transform infrared, XRD: X-ray diffractometer

SP can cure diseases within one month of administration in children[7]. Swarnaprashan also boosts immunity, supports growth and development, and improves overall health in children[8]. While a specific form of immunity is boosted by the presence of cellular oxidants, an increase in cellular oxidants causes damage to the immune cells and may contribute to the lowering of the immunity[9], leading to a number of immunological disorders such as infections[9]. Increased cellular growth and cellular metabolism can also lead to an increase in cellular ROS. Hence, antioxidants may play a vital role in maintaining strong immunity. Due to accelerated growth and cellular proliferation, faulty dietary habits and lifestyles, children are more likely to have increased amounts of ROS and might require supplementation with antioxidants for counterbalancing the detrimental effect of free radicals. The immune system in children is immature and keeps on developing by antigen exposure with age. Hence, in adolescents, an excess of ROS might cause redox imbalance contributing to the immune dysfunction and raising the risk of infectious diseases[10]. So, antioxidant therapy might be a good strategy for preventing and progressing pandemics or other infectious diseases of unknown origin[8]. SP has been used in routine practice for generations by Ayurvedic pediatricians with fruitful outcomes though its exact mechanism remains unknown. Few studies investigate the antioxidant effects of Suvarna bhasma (IGP)[11]. This led to the present study as a preliminary attempt to assess the antioxidant activity of Suvarnaprashana which contains incinerated gold along with Ghrita and honey. This study aimed to examine the size, shape, and antioxidant potential of IGP in SP along with the safety and efficacy of SP. To achieve this, we developed a comprehensive framework, including Suvarna Bhasma characterization, ROS procedures, and advanced analytical techniques.

Materials and Methods

Procurement of raw materials

Premium quality Suvarna Bhasma (incinerated gold particles) weighing 100 mg, Batch No. P22110056, was purchased from Shree Dhootapapeshwar Limited (SDL), India (a reputed pharmaceutical company). Honey, Lot No. BT01212, certified under GMP, was obtained from Dabur Private Limited (DPL), India. Homemade traditionally processed A2 Desi cow Bilona ghee (clarified butter) was utilized for Suvarnaprashan preparation. Every component was evaluated following the guidelines of the Ayurvedic Pharmacopoeia of India[12].

Preparation of Suvarnaprashan

Samples were prepared according to classical Ayurveda literature[12,13]. A hundred milligrams of Suvarnabhasma were mixed in 10 mL of clarified butter to make a concentration of 10 mg/mL or 0.5 mg per drop (SPG). The mixture was triturated for 3 h in a clockwise direction with the help of Mortar and Pestle, till the homogeneous mixture was formed. The mixture was stored at room temperature until further use. Two times of SPG, honey was added at the time of oral administration to the patients.

Characterization of raw materials

Heavy metal analysis

Heavy metal (Pb, Cd, Hg, As) screening was done to ascertain the sample's metal toxicity. The presence of heavy metals was ascertained using ICPAES (Inductively Coupled Plasma Atomic Emission Spectroscopy). The sample was prepared in dilute aqua regia and processed in an ICP machine (machine model), at the Indian Institute of Technology, Bombay.

Scanning electron microscopy (*SEM*)

A scanning electron microscope (Zeiss model), Department of Geology, BHU, was used to analyze the morphology of Suvarna Bhasma. The sample was sprinkled on a double-sided carbon tape and fixed to an aluminum stub. Then, the sample was examined at different magnifications, using 20kV voltage.

Elemental analysis

EDX was used to anticipate the moiety and chemical composition of the sample/drug, allowing faster sample examination. Elemental analysis of Suvarna Bhasma was performed by an energy-dispersive X-ray spectrometer (EDX) of the Department of Geology, BHU.

Fourier-transform infrared (*FTIR*) *spectroscopy*

FTIR spectra of Suvarna bhasma were captured at room temperature, using PerkinElmer Spectrum 100, Department of Chemistry, BHU. Suvarna bhasma pellets were prepared using KBr powder. Spectra were acquired between 4000 to 400 cm^{-} wavenumber[14].

X-ray diffraction (*XRD*) *analysis*

X-ray diffractometer machine, Department of Physics, BHU, was used for the XRD study. XRD pattern of Suvarna Bhasma was obtained by using Cu-

Kα radiation, filtered by nickel foil on Empyrean, X-ray diffractometer in the range of 10°2θ -80°2θ.

Suvarnaprashan preparation for cell culture studies

We added to DMEM Triton-X100 in 0.01% concentration and DMSO in 0.02% concentration to solubilize and emulsify the lipid content so that it can be evenly mixed with cells for cell culture studies to enhance the bioavailability of the SP. For our experiments, a working medium was prepared with DMEM containing Triton-X100 (0.01%) and DMSO (0.02%) for all cell culture experiments. Samples were thoroughly mixed before performing the cell culture experiments.

Cell culture

HEK-239 (Human Embryonic Kidney) cells were obtained from the National Cell Line repository, National Center for Cell Science (NCCS), Pune, Maharashtra, India. The cells were cultured in high-glucose Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum, 2 mM glutamine, and 1% antibiotics in a humidified 5% CO_2 environment at 37°C. Upon reaching 80% confluency, cells were passaged.

Cell viability assay

MTT assay was performed to determine the cell viability as described previously[15]. HEK cells were seeded in a 96-well plate, at a density of 5000 cells/well in 100 µL of DMEM, and allowed to grow overnight in the CO_2 incubator. Following overnight growth, cells were incubated with various concentrations of the Suvarna bhasma (100, 50, 25, 12.5, 6.25, and 0 µg/mL), Suvarnaprashan (100, 50, 25, 12.5, 6.25, and 0 µg/mL), ghee (100, 50, 25, 12.5, 6.25, and 0 mg/mL) and honey (5, 2.5, 1.25, 0.625, 0.3125, 0 %) for 24 h. Cell viability at different concentrations was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Cells were incubated with 10 µL of the MTT (5 mg/mL stock) for 2 h at 37°C in a CO_2 incubator. Media was discarded, and 100 µL of DMSO was used to dissolve the formazan crystals. The absorbance was measured at 570 nm using a microplate reader (Biotek Synergy HT).

Intracellular ROS determination using 2',7'-Dichlorofluorescein diacetate (DCFDA)

2',7'-Dichlorofluorescein diacetate was used to measure intracellular ROS in accordance with a previous protocol[15]. HEK cells were seeded in a 96-well plate at a density of 10,000 cells per well in 100 µL volume. Following an overnight growth, cells were treated with SB, SP, ghee, and honey in four replicates for 24 h. Cells were treated with H_2O_2 for 3 h, then washed with pre-warmed DMEM, and 10 µM DCFDA (Sigma Aldrich) was added to each well for 30 min in a humidified CO_2 incubator at 37°C in the dark. Following staining, the cells were washed with DMEM once and with pre-warmed 1X PBS twice. The plate was read using a Microplate Reader (Biotek Synergy HT) in the green channel (excitation 485 nm and emission 530 nm).

Data analysis

The data analysis was done using Excel and the GraphPad software.

Statistical analysis

The difference between the groups was calculated using one-way ANOVA followed by Dunnett's post hoc test.

Result

Heavy metal analysis

The heavy metal content in Suvarna Bhasma was found according to WHO guidelines for drug safety (Table 1), indicating that it is safe for clinical study.

SEM

Scanning electron microscopy indicates the presence of aggregated particles of Suvarna with rough surfaces and sizes ranging from various nm to µm (Fig. 1).

Elemental analysis

EDAX of Suvarna Bhasma indicates the presence of gold, as the major portion, 91.87%, and oxygen, 8.13% (Fig. 2, Table 2).

Table 1 — Heavy metal content in the sample

Sample	Hg	Pb	Cd	As
Suvarna bhasma	<0.01 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm

Table 2 — Elemental analysis of Suvarna Bhasma

Element	Weight %	Atomic %	Net Int.	Error %	K ratio	Z	A	F
O K	8.13	52.14	40.94	14.83	0.0226	1.5734	0.1771	1.0000
Au L	91.87	47.86	128.46	12.41	0.8591	0.9295	1.0059	1.0001

FTIR study

FTIR spectra of Suvarna Bhasma indicate the peaks at wavelengths of 3450.06, 2933.78, 2378.93, 1632.83, 1372.64, 678.24, and 615.04 (Fig. 3). These peaks match with the peak in the FTIR spectra of pure gold as well as the reported FTIR spectra of Suvarna Bhasma[16,17].

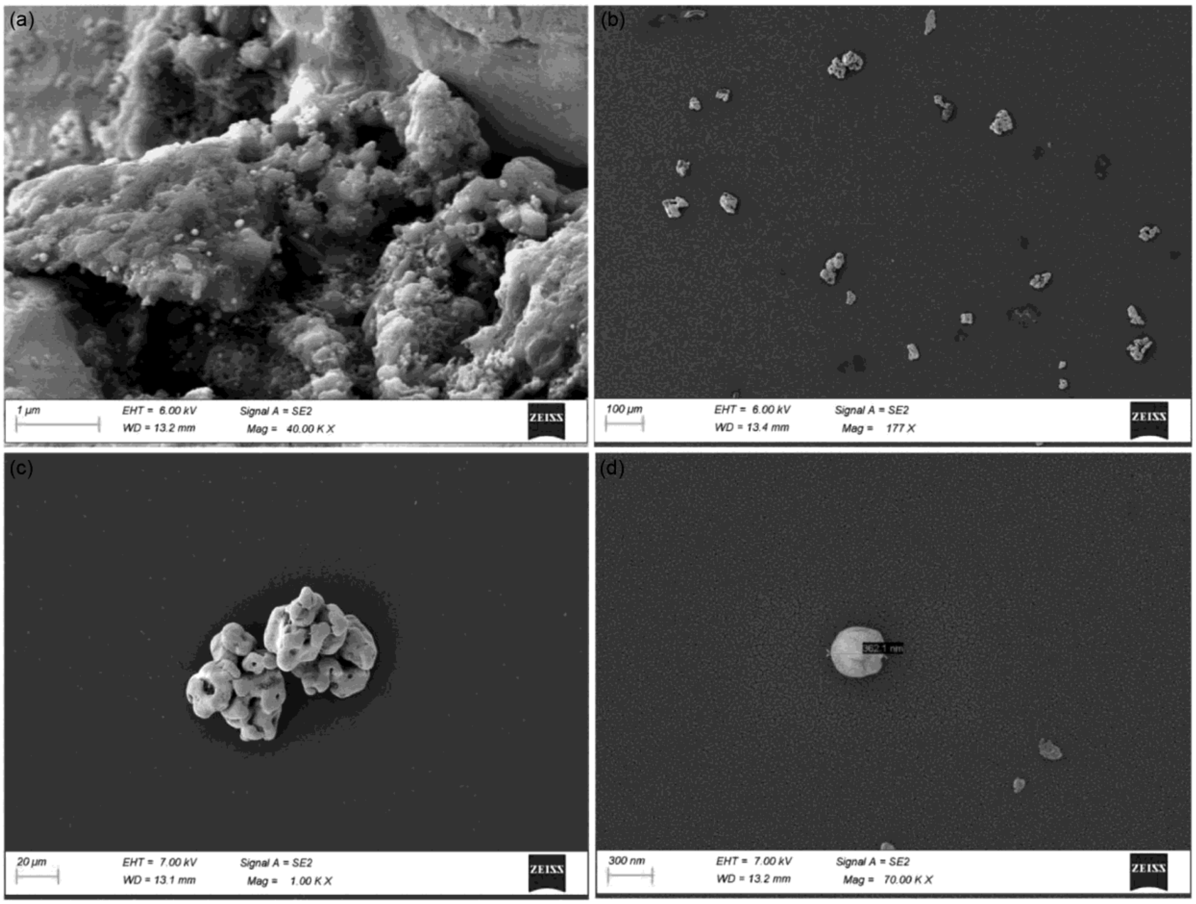

Fig. 1 — SEM of Suvarna Bhasma showing (a) rough surface, (b) heterogeneous distributions of particles, (c) aggregate particles with (d) size ranges from various nm to µm

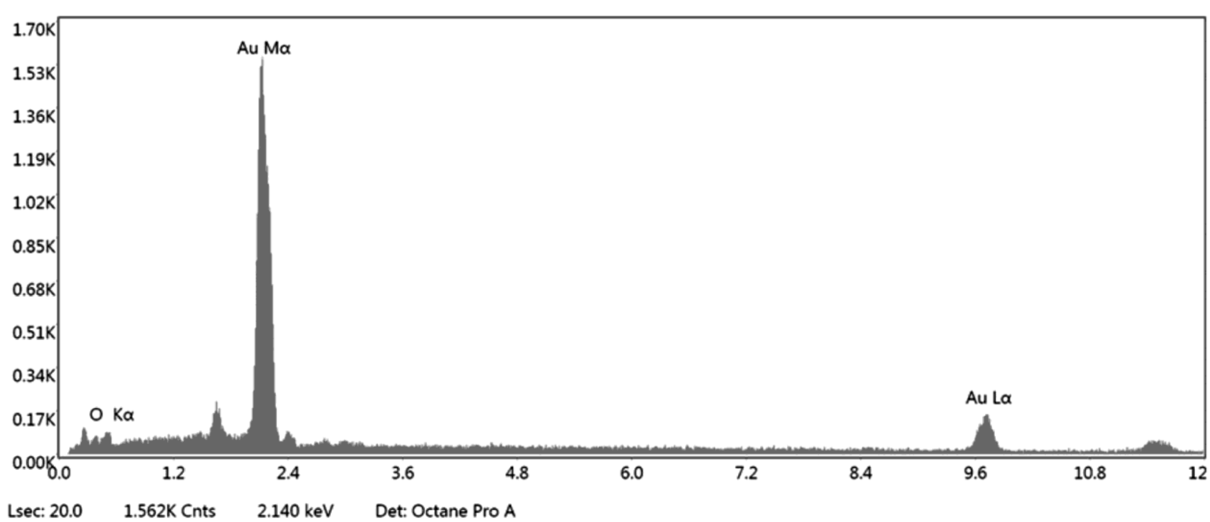

Fig. 2 — EDAX of Suvarna bhasma showing the presence of gold as a major portion

XRD study

XRD pattern of Suvarna Bhasma indicates four peaks at 2θ of 38.35°, 44.46°, 64.67, and 77.68° (Fig. 4), similar to the XRD peaks of pure gold. The high intensity of peaks indicates the crystalline nature of Suvarna Bhasma. Also, the absence of other peaks indicates that Suvarna Bhasma is mainly composed of gold particles[18].

Suvarna Bhasma (SB), Suvarnaprashan (SP), Ghee, and Honey toxicity to HEK cells

Cell viability analysis using the MTT assay revealed that Suvarna Bhasma did not exhibit significant cytotoxicity across the tested concentrations ($p>0.05$), indicating good biocompatibility (Fig. 5 a). Honey, ghee and Suvarnaprashan showed a little significant cytotoxicity ($p<0.05$) as compared with control (0 µg/mL) (Fig. 5, b-d).

Suvarna bhasma (SB), Suvarnaprashan (SP), Ghee, and Honey ameliorate ROS levels

To determine the effect of SB, SP, ghee, and honey on intracellular ROS levels, HEK cells were treated with 50 µg/mL of SB, 50 mg/mL of ghee, 1% of honey, and 50 µg/mL of SP. Statistical analysis using one-way ANOVA followed by Dunnett's post hoc test revealed that SB, Ghee, honey, and SP in the presence of H_2O_2 showed a statistically significant reduction in ROS levels compared to the H_2O_2-treated control

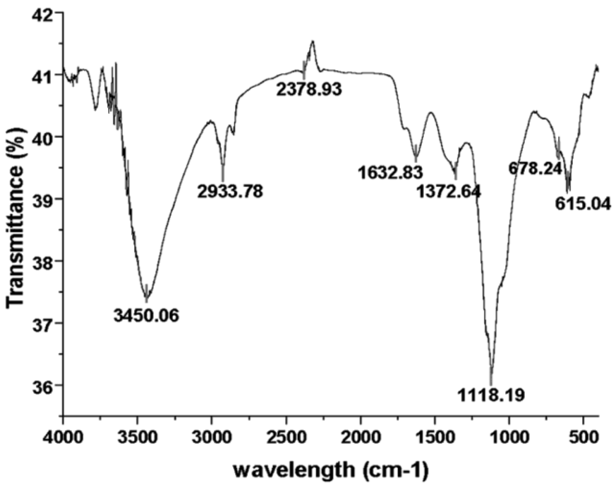

Fig. 3 — FTIR spectra of Suvarna bhasma in between the wavelength of 4000 to 400 cm-1

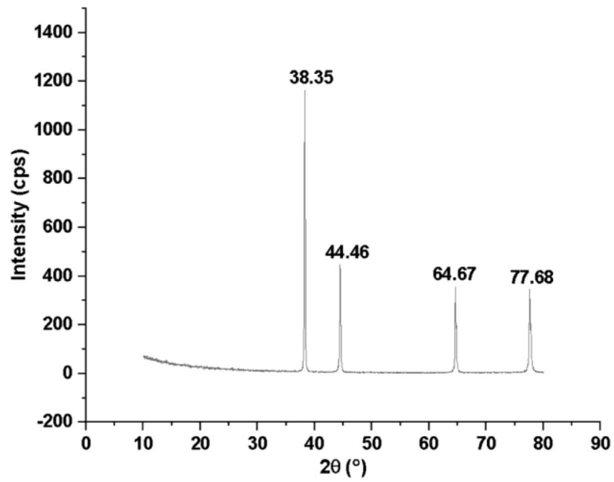

Fig. — 4 XRD pattern of Suvarna bhasma

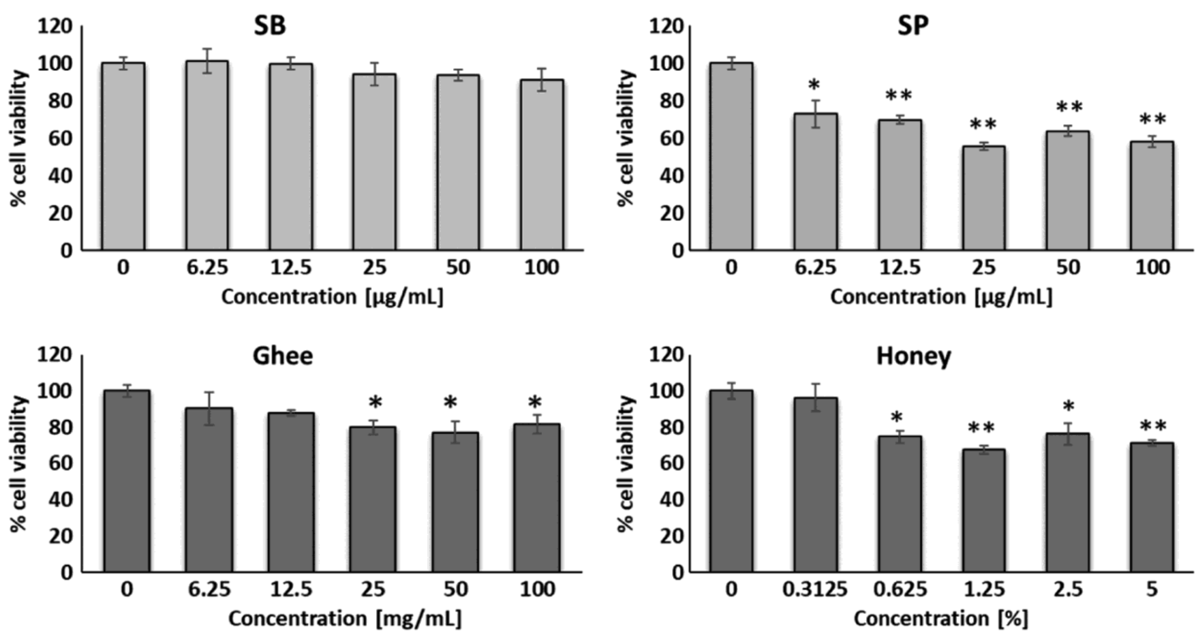

Fig. 5 — Cell viability in HEK cells, (a) Suvarna bhasma (SB), (b) Suvarnaprashan (SP), (c) ghee and (d) honey *p<0.05, **p<0.01

group ($p<0.05$), demonstrating their protective antioxidant effect (Fig. 6). SP shows the strongest effect with $p=0.003$.No significant difference was observed in all the groups in the absence of H_2O_2 compared to control group without H_2O_2.

Discussion

Nano particle-based therapeutics have transformed modern medicine through modulation of biological properties by fine-tuning of the physicochemical properties, bioavailability and molecular interaction. While the nanotherapy concept is newer to modern medicine, Ayurveda is practicing nano formulations since ancient times in the form of Bhasmas[11]. The growing body of research indicates that gold nanoparticles interact with the components of the innate immune system, particularly macrophages and dendritic cells, influencing antigen uptake, cytokine signaling, and antigen presentation[19]. Rather than acting as immune stimulants, gold nanoparticles are increasingly recognized as immune modulators, which are capable of shaping immune response towards homeostasis. Several studies have demonstrated that gold nanoparticles can attenuate excessive inflammatory signaling and oxidative stress, thereby preserving immune cell viability and function[20].

Reactive oxygen species function as critical second messengers in immune activation. However, excessive oxidative stress compromises immune cell signaling, proliferation, and survival[9]. Gold nanoparticles have been reported to exhibit antioxidant and redox buffering properties either by quenching ROS directly or modulating redox-sensitive signaling pathways such as NF-κB and NRF2[21]. In this context, antioxidant activity may directly enhance immune competence by maintaining cellular redox homeostasis required for optimal immune responses[19].

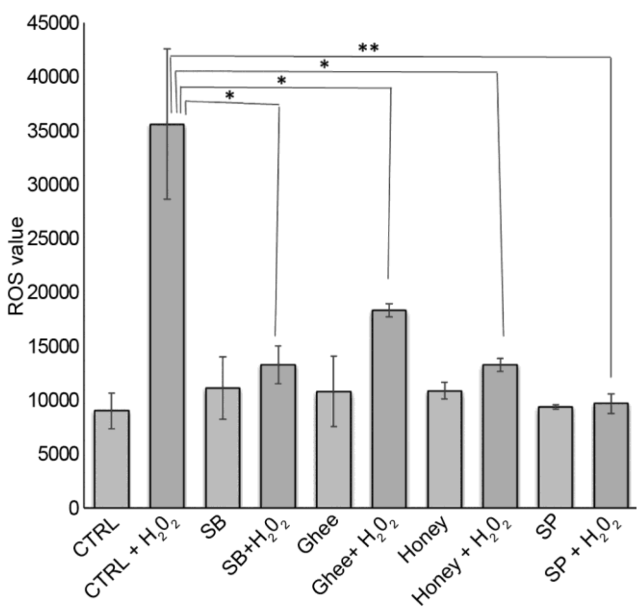

Fig. 6 — Effect of Suvarna Bhasma (SB), Suvarnaprashan (SP), Ghee, and Honey on ROS levels $^*p<0.05$, $^{**}p<0.01$

In the present study, administration of gold particles/gold nanoparticles along with the ghee and honey (Suvarnaprashan) demonstrated redox modulatory activity by reducing hydrogen peroxide–induced ROS levels. Honey, a rich source of polyphenols and bioactive compounds, is known for its antioxidant, antimicrobial, and immunoregulatory properties[22]. Ghee, being a lipid-rich matrix, may enhance nanoparticle stability, intestinal absorption, and cellular uptake, consistent with previous reports on lipid-mediated nano-delivery systems[23,24]. This synergistic formulation likely contributes to the improved bioavailability and controlled immune engagement.

Cell viability assays of Suvarna Bhasma (SB), indicated no significant toxicity across most tested concentrations. However, mild cytotoxicity was observed with SP, ghee, and honey. Interestingly, ghee- and honey-treated cells did not exhibit a dose-dependent response; instead, cell viability increased at higher concentrations, suggesting that these components are not inherently toxic and may even support cell survival[25]. The slight cytotoxicity observed with Suvarnaprashan could be attributed to factors such as the type of cell line used, the extent of cellular uptake, intracellular processing, and metabolism of gold nanoparticles. Although not experimentally validated in this study, it is plausible that ghee-coated gold nanoparticles facilitate enhanced cellular uptake due to their lipid nature, consistent with previous reports on lipid-mediated bioactive delivery[26]. SP toxicity may be due to the presence of honey, which is reported to be cytotoxic in previous study[27]. Nonetheless, as these mechanisms were not directly investigated, it would be premature to draw definitive conclusions regarding the exact cause of the observed effects.

From a developmental immunology perspective, these observations are particularly useful or relevant in pediatric and early life pediatric contexts, where immune competence is shaped progressively through antigen exposure and redox-sensitive immune education. The postnatal immune system is functionally immature and undergoes gradual maturation as innate and adaptive immune compartments are trained through environmental and microbial encounters[10]. Maintaining redox balance during this critical window is essential as excessive oxidative stress can impair immune development. Nanoparticle-based interventions that support redox homeostasis may therefore contribute to immune resilience and functional maturation rather than direct immune activation.

Our present study contributes to the establishment of Suvarnaprashan as a redox-active immunomodulator, offering a scientifically grounded framework for supporting immune function through maintenance of oxidative homeostasis.

Conclusion

From the result of the present study, we can conclude that characteristics of SB are similar to pure gold. From the *in-vitro* study, we can speculate that SP and its raw ingredients are low/non-toxic to cells and boost immunity by decreasing ROS levels. However, further *in-vivo* and clinical studies are needed to confirm the results of the present study.

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Author Contributions

KP: Project administration, Conceptualization, Formal analysis, Writing - review & editing; KS: Methodology, Formal analysis, Writing - original draft; PK: Methodology, Formal analysis, Writing - review & editing; NP: review & editing, NG: Conceptualization, Formal analysis, Resources, Supervision, Writing - review & editing.

Conflict of Interest

The authors declare no conflict of interest.

Informed Consent

Not applicable

Ethical Approval

Not applicable

Use of Artificial Intelligence (AI)

No Artificial Intelligence (AI) tools were used in this study.

Data Availability

Data included within the article. Processed data and analysis files are available from the corresponding author upon reasonable request.

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