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Antimicrobial and Biodegradable Sanitary Pads with Nanomaterials Fused Polymers

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ABSTRACT

Background and Objective: Sanitary pads and diapers are routinely used nowadays to manage menstrual and toilet secretions. The commercially available menstrual pads contain harmful chemicals sodium polyacrylate and dioxins. Biodegradable pads with antimicrobial properties are a novel concept. This work highlights sanitary pads and diapers containing lignocellulose based, chemical-free, nanomaterial, pectinosone and chitinase-coated pads with long-lasting antimicrobial activity. The prepared pad was evaluated for its water-holding capacity, biodegradability test, antimicrobial activity, pH, inlet time and toxicity. **Materials and Methods:** Considering the findings of the current novel study, nanoparticles such as copper, silver and zinc were synthesized and characterized, pectinosone and chitinosone were prepared and coated on agarose. Further, the novel pad was examined for biodegradability, water-holding capacity, antimicrobial activity, pH, inlet time and toxicity tests. **Results:** The novel pad showed a high absorbance (37-40 mL), an inlet time of 3.6 sec, a neutral pH, anti-microbial activity and no toxicity. The complete biodegradability of the novel pad was achieved in 3 months. **Conclusion:** The prepared novel sanitary pad is having a high-water holding capacity, long duration anti-microbial activity with a neutral pH. The pad is completely biodegradable and non-toxic and is prescribed for routine purposes.

KEYWORDS

Antimicrobial, biodegradable, sanitary pad, nanoparticles, diaper

INTRODUCTION

It has become very important for human beings to live in a world of hygiene and freshness. Menarche is a biological milestone and symbolizes the beginning of women's menstrual phase[1]. There seems to be a lack of knowledge on menstruation preparedness and management in many developing countries making the situation worse for girls[2,3]. Menstruation being a natural process is still taboo in Indian society. Menstrual fluid may or may not have an unpleasant odor especially when it comes in contact with air[4]. Women have developed their strategies which vary based on their personal preferences, availability of resources, economic/cultural status and knowledge education/knowledge about menstruation. The medical literature showed that most girls are unaware and unprepared for menarche or such diseases due to the unwariness of menstrual management[5]. Menstrual hygiene practices are of the utmost significance since they affect health, if unkempt, they can cause toxic shock syndrome, RTIs (Respiratory tract infection) and other vaginal medical conditions, including certain malignancies[6,7]. Menstrual wastes have created a

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serious dent in the environment across the globe, India notwithstanding[8,9]. Menstrual pads for menstrual conditions and diapers for hospitalized geriatric patients and children are used for their daily routine. In either case, poor genital hygiene negatively affects health[10]. The chemicals and microbes are also a concern when considering sanitary pads/ diapers. Although synthetic plastic materials are known to release volatile organic compounds and hormone-disrupting toxins, they have been used as liquid absorbents to boost efficiency[11,12]. The fabrics used in daily sanitary pads were characterized thoroughly to focus on the development of future products[13]. The FTIR (fourier transform infrared)/ATR (attenuated total reflectance) spectrum demonstrates that the samples were made of polypropylene. While the Indian Government is struggling to ensure that all women get sanitary pads to manage menstruation, managing menstrual waste is also a major concern. Current advances in the healthcare industry have seen the integration of biomaterials improve the calibre of life for both normal and critically ill hospitalized patients[14]. Finally, the material if not biodegradable leads to the accumulation of waste and thus the infectious spread of disease. The neglect in the management of sanitary pads, adult diapers and similar material is a universally reported issue[14-16]. Thus, an antimicrobial, bio-degradable and efficient holding capacity pad/diaper is the need of the hour. The current study aimed to prepare a biodegradable, antimicrobial, high water-absorbing sanitary pad that can be used during menstrual periods or as a diaper for children and adults.

MATERIALS AND METHODS

Study area: The study's execution was done in 2019 from February to September in the Department of Microbiology Laboratory at Nizam College, Osmania University, Hyderabad, India. In 8 months, every aspect of the study had concluded.

Materials: Microbial growth medium (beef extract, peptone, agar agar, NaCl, etc.), Chemical of research grade obtained from Hi Media laboratories were used.

Methods

Synthesis and characterization of copper nanoparticles: As 100 mL, 0.01 M of copper sulphate and 0.1 M of ascorbic acid suspension were made in deionized water in 250 mL of the conical flask. This mixture was heated in a water bath at 85-90°C. During this process, the blue-colored mixture turns into brick red. Now copper nanoparticles are filtered and centrifuged and washed to extract the desired copper nanoparticles. The nanoparticles were placed on a copper grid with carbon-coated formvar film for 10 min at room temperature. The 4% aqueous phosphotungstic acid was used for staining at pH 7. The sample was air-dried overnight and examined with a Zeiss TEM 900 electron microscope (Piscataway, New Jersey, United States), Carl Zeiss AG it was operated at 50 kV. The nanoparticles were visualized using the Image SP software V2.5 SYSPROG under the guidance of TRS, Duncelbuch, Moorenweis, Germany and a CCD (charge-coupled device) camera Horiba instruments, Piscataway, New Jersey, United States.

Synthesis and characterization of silver nanoparticles: As 100 mL, 0.01 M of silver nitrate and 0.1 M of ascorbic acid suspension were made in deionized water in 250 mL of the conical flask. This mixture was heated in a water bath at 85-90°C. During this process, the solution turns from pale to an ash color. Now, silver nanoparticles were filtered and centrifuged and washed to extract the desired silver nanoparticles. Zeiss TEM 900 electron microscope (Piscataway, New Jersey, United States) was used for the characterization of nanoparticles.

Synthesis and characterization of zinc oxide nanoparticles: As 100 mL, 0.01 M of zinc nitrate and 0.1 M of ascorbic acid suspension were made of deionized water in 250 mL of the conical flask. This mixture was heated in a water bath at 85-90°C. During this process, the solution precipitates into white nanomaterials. Now, zinc oxide nanoparticles are filtered and centrifuged and washed to extract the desired zinc oxide nanoparticles. The prepared ZnO nanoparticles were characterized by the LabX XRD-6100 XRD machine (Shimadzu Corporation, India).

Pectinosone preparation: For Pectinosone preparation orange peels were used. The preparation was as follows. As 25 g of orange peels were collected and placed in 1000 mL of distilled water and homogenized in the blender. The pH-7.5 was adjusted with inorganic acid HCl and NaOH and heated at 85-90°C with constant stirring. The pH was adjusted to 4.5 every 15 min and was replaced with lost water. The material was filtered with a moist cheesecloth and heated to remove maximum water. It is cooled rapidly to 4°C in an ice bath. The filtrate was transferred to another steel container with an equal amount of 95% ethanol. Stirred for 10 min and filtered through cheesecloth. Washed with 95% ethanol (30 mL) and acetone to obtain pectin. Pectin is suspended in a 40% KOH aqueous solution and boiled for 1 hr. Once boiling is done, cooled and washed to get neutral pH. The material was filtrated through cheesecloth and dried at 60-70°C, overnight in the hot air oven. Ground and stored in an airtight container for further use.

Chitinosone preparation: Chitinosone was obtained from prawn shells. As 5% ethanol (w/w) was incorporated into the prawn shells before they were heated in a water bath for an hour at 70-75°C. It is rinsed to remove the protein. Deproteinized repeatedly employing the same procedure until no protein is left. Following deproteinization, the sample was meticulously rinsed with tap water and dried for 8 hrs at 65°C. The material was crushed up, cleaned and dried for a brief period. The material was then thoroughly covered with a 40% KOH aqueous solution and it was heated at 100°C for 5 hrs. After draining the KOH solution, the product had been rinsed extensively in cool potable water and dried for 8 hrs in a 65°C oven. The substance was then ground into a powder and bottled as chitinosone.

Agarose surface coating and characterization of nanomaterials, pectinosone and chitinosone: In double distilled water 0.1% agarose and 1% nanomaterials were added and boiled at 100°C for 10 min. The suspension was cooled and dried at 60°C for 8 hrs. The solid suspension formed was ground to get a fine powder. These coated, non-coated nanomaterials, pectinosone and chitinosone were characterized using a Zeiss TEM 900 electron microscope (Piscataway, New Jersey, United States). Coated, non-coated nanomaterials, pectinosone and chitinosone matrix and prepared pad were used for anti-microbial activity studies at various time intervals from day 0 to 4 months.

Antimicrobial activity of nanomaterials, pectinosone and chitinosone: Well, the diffusion method was followed for the anti-microbial study. Sterile Mueller Hinton Agar was prepared and poured into sterile plates. Wells were created on plates using a 5 mm sterile dweller. Active-growing *E. coli* and *Staphylococcus aureus* were spread on separate plates. As 10 mg agarose coated and uncoated copper, silver, zinc oxide nanomaterials, pectinosone and chitinosone were taken individually and all in one combination were taken in wells and incubated at 37°C for 24 hrs. Positive controls of 100 µg of ampicillin and triclosan were also tested and negative control of normal saline was used.

Examining the biodegradability of the novel pad: The novel pads were buried in the soil to check their biodegradability one at a place. A comparative biodegradable study was done for novel pads and commercial pads. Every month one pad is collected and observed for decomposition. After an observation of 3 months, explicit results were considered.

Holocellulose extraction by chlorination method: Holocellulose is a polymer that consists of pentoses and hexoses. The extraction is done by getting rid of lignin by the chlorination method. A 2.5 g of powdered dry sample of sugarcane bagasse was put in a 250 mL flask. Then 80 mL of hot distilled water, 0.5 mL acetone and 1 g of sodium chlorite were added to the 250 mL flask. The mixture was heated in a water bath at 70°C for 1 hr. After this another dose of 0.5 mL acetone and 1 g of sodium chlorite were added and heated further for 1 hr. This process is repeated for 3-5 times until lignin is completely removed. The mixture was left in a hot air oven to dry at 80°C for 24 hrs. The solid was a whitish residue left on the filtrate giving the weight of lignin-free holocellulose.

Blending of coated materials of holocellulose, cellulose, lignocellulose-absorbent materials and characterization: In a conical flask to 100 mL water, added 2 g of holocellulose, 0.1 g of coated nanomaterials such as copper, zinc and silver, pectinosone, chitinosone and 2 g of agar, homogenized the mixture and boiled. After boiling, cooled the mixture to room temperature and allowed it to solidify. Later placed in the hot air oven at 80°C for one day and ground into fine powder. The adsorbent materials were further tested for water adsorbing capacity, biodegradability test, pH, anti-microbial activity and inlet time.

Pad preparation: The sanitary pad was fabricated in six layers, blanketed with a self-adhesive tape that binds to the underwear. This was further sealed with paraffin-coated cotton fabric to prevent water leakage. On top of the paraffin-coated hydrophobic cotton fabric, a dried bamboo leaf was placed. Above the dried leaf, adsorbent material was placed with a composition of 2% finely powdered holocellulose, 0.1% agarose, 0.1% of coated nano copper, zinc, silver, chitinosone, pectinosone and 0-0.1% of tea tree oil and 0-0.1% of fragrance. The adsorbent material is pressed to a thin layer and laid on non-absorbent cotton which is in turn covered and pressed by a thin cotton layer that provides coaching and comfort.

The cotton fabric was placed on top as a covering agent and aided in preliminary water absorption. Henceforth, the pad is chemical-free and is further evaluated for water holding capacity, biodegradability test, anti-microbial activity, pH, inlet time and toxicity as per standards.

Pad design: The inner matrix layer along with non-absorbent cotton was fixed in a cup-like paraffin-coated thin cotton cloth which was closed at the bottom and vertical sides but, opened on the top. At the opening side thin layer of cotton is added and at the topmost, cotton fabric is placed. It is designed to get side wedges and stitched for stability and design.

Comparative study of novel pad with commercial pad with different parameters: The novel pad prepared was compared with the commercial pad for water holding capacity, antimicrobial activity, pH, Inlet time and toxicity studies.

Water holding capacity tests: Novel pads and commercial pads were placed in the center of the respected test area where the water holding capacity test has to be performed. Water is dropped with burette on stand by moving to dry sites. The water is dropped till unabsorbed water is lost from the pads.

Biodegradability test: Commercial and novel pads were dug in the soil to examine their biodegradability rate. Pads are kept under observation for 3 months. Bleaching of the pads explains the potency of biodegradability.

Anti-microbial activity tests: Well, the diffusion method was followed for the anti-microbial study of pads. Sterile Mueller Hinton Agar was prepared and poured into sterile plates. Active growing *E. coli* and *Staphylococcus aureus* were spread on separate plates and a pad of 0.1 cm^3 was placed on wells of inoculated plate and incubated at 37°C for 24 hrs. Positive controls of ampicillin and triclosan were also tested.

Acidity tests (pH): The prepared matrix and commercial matrix were suspended in water with 1% concentration separately and checked pH through the pH meter (Labcare pH meter, LB-901 model, Hyderabad, Telangana, India).

Inlet-time estimate: A mixture of 49.5% glycerin, 1.00% phenoxyethanol and 49.50% of a 0.9% (w/w) sodium chloride solution constituted the test liquid to make it less complicated to see the liquid dispersion during testing, a little amount of blue tint was additionally included in the test liquid. Onto the top sheet, 4.0 mL of test fluid was pipetted from a height of 3 cm. For estimating the inlet time, the test liquid for stay-free pads endures an identical technique.

Toxicity tests: The cell toxicity was carried out by an MTT method[17]. The A549 cells (lung cancer) and A375 (melanoma cells) cells were taken in 96-well microtiter plates. After 24 hrs of incubation, the cells were treated with the prepared matrix. After incubation, the media were replaced with 20 μL of MTT reagent (5 mg mL^{-1}) and incubated in 5% carbon dioxide at 37°C for 4 hrs. The second set of cells was treated with stay free pad matrix and control was maintained with normal saline. After incubation, optical density was measured at 570 nm.

Statistical analysis: Experiments were conducted thrice in triplicates and average values with standard deviation are presented.

RESULTS

Synthesis of copper nanoparticles: A black-brown colored compound was formed. The TEM images confirmed that copper nanoparticles were spherical in shape. The average particle size was 20±5 nm (Fig. 1).

Synthesis of silver nanoparticles: An ash-colored compound was formed. The TEM images confirmed that silver nanoparticles were octagonal in shape. The average size was 18.7±4 nm (Fig. 2).

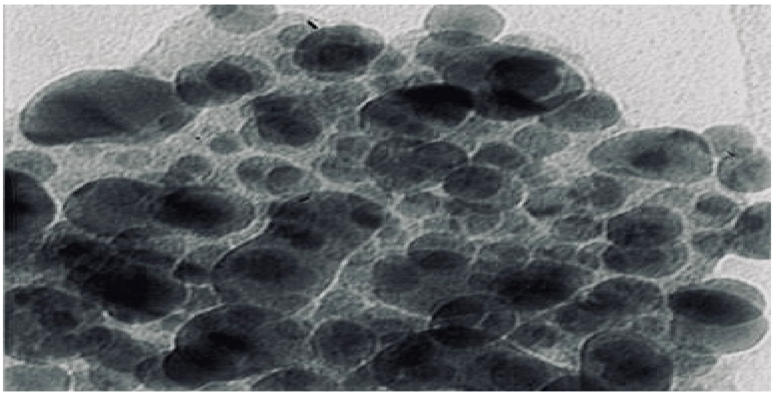

Fig. 1: TEM images of copper nanoparticles (size 20±5 nm)

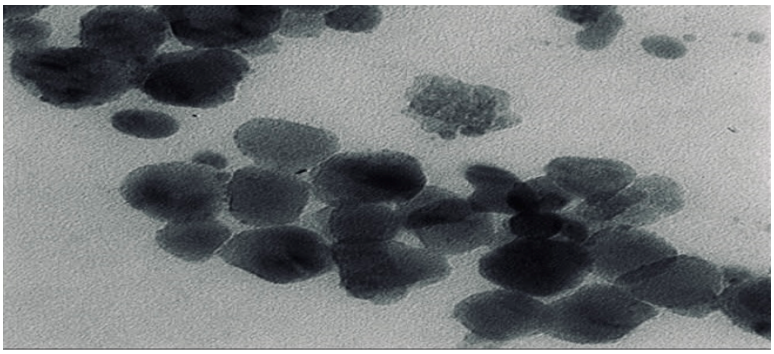

Fig. 2: Characterization of silver nanoparticles through SEM (size 18±3 nm)

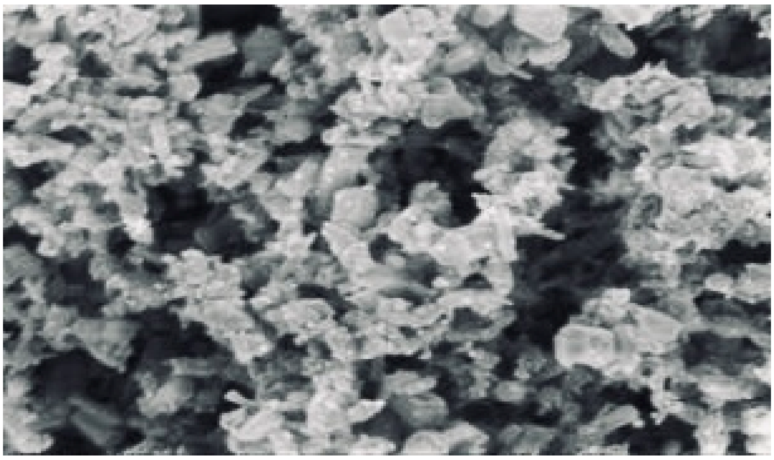

Fig. 3: X-ray diffractometry image of zinc nanoparticle (size 30±2 nm), Cluster form

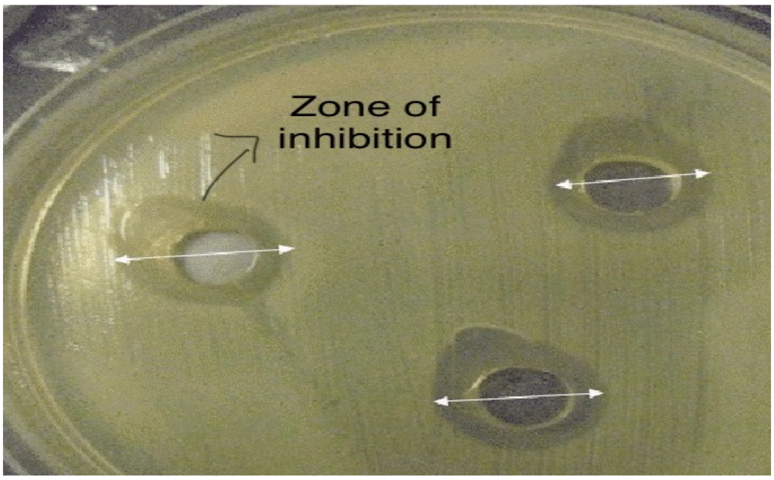

Fig. 4: Antimicrobial activity through well diffusion method (occurrence of the high zone of inhibition resulting in high anti-microbial activity)

Synthesis of zinc oxide nanoparticles: A white precipitate is formed. The X-ray diffractometer images confirmed that zinc oxide nanoparticles were star-like and isometric in shape. Particles were present in clusters. The average particle size was 30±2 nm (Fig. 3).

Pectinosone and chitinosone: The extracted pectinosone and chitinosone colors were dark brown and grey, respectively.

Synthesis of coated nanoparticles with agarose: The SEM images confirmed that coated nanoparticles with agarose possess an average size of 22±5 nm for copper, 21±5 nm for silver and 30±3 nm for zinc oxide. There is little increase in size observed with copper and silver coated nanomaterials in comparison to non-coated ones, whereas size change in zinc oxide before and after coating was not noted. This may be the TEM used for copper and silver and XRD used for zinc oxide.

Antimicrobial activity of nanomaterials, pectinosone and chitinosone: The zone of inhibition for coated particles was quite higher than the uncoated matrix of the novel pad. Whereas, the commercial pad had even less zone of inhibition (Fig. 4). Hence, the anti-microbial activity for the novel pad was high in comparison to the commercial pad (Table 1).

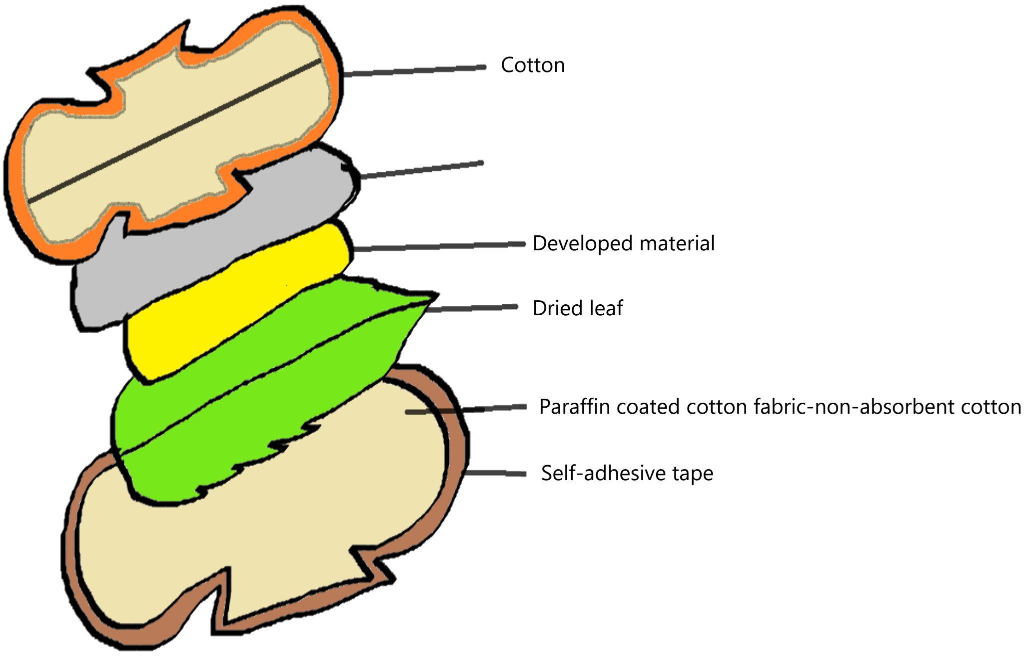

Fig. 5: Design of novel pad

Table 1: Anti-microbial activity of coated and uncoated silver, copper zinc nanoparticles, pectinosone and chitinosone over 4 month intervals

	Zone of inhibition (mm)									
	Staphylococcus aureus (Gram positive)					*Escherichia coli* (Gram negative)				
Materials	0 month	1 month	2 months	3 months	4 months	0 month	1 month	2 months	3 months	4 months
Coated zinc oxide	19.4	14.3	13.8	9.4	5.2	29.7	26.6	23.4	20.3	17.1
Coated silver	16.6	12.4	11.5	7.6	4.5	21.7	20.6	17.6	15.3	11.4
Coated copper	14.8	11.7	9.9	7.3	5.1	18.4	16.6	14.7	12.7	9.9
Coated chitinosone	10.7	8.8	7.1	6.2	4.5	14.3	12.6	11.1	10.6	8.7
Coated pectinosone	9.2	8.4	6.9	5.6	4.2	13.7	12.1	10.3	9.7	7.5
Uncoated Zinc oxide	23.6	22.4	19.4	16.7	11.6	30.1	27.4	24.4	22.5	20.3
Uncoated silver	17.7	15.3	12.5	10.2	9.5	24.7	22.1	19.3	17.8	13.5
Uncoated copper	17.1	14.7	11.5	9.7	7.3	23.7	21.8	17.2	15.2	11.7
Uncoated chitinosone	13.7	11.3	9.1	7.8	6.7	19.5	14.3	12.7	11.9	9.8
Uncoated pectinosone	12.6	10.7	7.7	6.3	5.2	15.8	13.4	12.9	10.1	8.8
Positive control 1 (ampicillin)	18.6	18.6	18.6	18.6	18.6	28.3	28.3	28.3	28.3	28.3
Positive control 2 (triclosan)	10.2	10.2	10.2	10.2	10.2	19.3	19.3	19.3	19.3	19.3
Matrix (material infused in pad)	9.4	7.1	6.8	6.3	5.5	11.9	9.6	7.3	6.1	5.6
Novel pad	8.8	6.8	5.6	5.1	4.3	10.5	9.3	7.1	5.7	4.2
Commercial pad	4.1	4.1	4.1	4.1	4.1	5.7	5.7	5.7	5.7	5.7

Pad design and preparation: Six-layer design was made (Fig. 5) and prepared a biodegradable and antimicrobial sanitary pad (Fig. 6).

Comparative study of novel pad with commercial pad with different parameters: Certain parameters are considered to prove that the novel pad is safe and efficient for usage in comparison with the commercial pad. The absorption of water is one of the major parameters in sanitary pads. Commercial pads have quite less water absorption capacity of about 15 mL with an inlet time of 10 sec. Whereas, the novel pad has a high-water absorption capacity of about 37 mL with an inlet time of just 3.8 sec. The biodegradability of the novel pad is quick in comparison to the commercial pad. Novel pad completely degrades within 3 months whereas, the commercial pad takes years to degrade as it contains an excess amount of chemicals (dioxins) and plastics. The pH of the novel pad is neutral as it is chemical-free. Commercial pads are highly alkaline with a pH of 8-9 making them highly toxic and unsafe to use. Pads

Fig. 6: Novel pad

Table 2: Comparative study of the novel pad with a commercial pad with different parameters

Properties	Novel pad	Commercial pad
Water absorption/gram	37 mL	15 mL
Biodegradability test	4-5 weeks	More than a year
Antimicrobial property	High (zone of inhibition 25±5 mm)	Medium (zone of inhibition 05±5 mm)
pH	7	9.5
Inlet time	3.8 sec	10 sec
Toxicity	Non-toxic	Toxic

should provide such an environment that they inhibit the growth of several bacteria. The novel pad is infused with nanomaterials that exhibit high anti-microbial activity. In the case of commercial pads which are devoid of nanomaterials (Table 2).

DISCUSSION

In the current study, the leaf surface was layered with a hollo cellulose blend of 0.1% each coated nano copper, silver, zinc oxide, chitinosone and pectinosone membrane to prevent oxidation, enhance the absorption and water retention of the sanitary pad. The novel pad exhibits high anti-microbial activity and meritorious biodegradability.

The management of menstruation presents significant challenges for women in lower-income settings. Poor menstrual hygiene management (MHM) can affect the reproductive tract with specific infections. The reproductive phase of women has natural complications needing routine management with menstrual pads and hygiene maintenance[18]. Menstruation or menstrual fluid, adults with urinary incontinence and children need diapers/ pads with good holding capacity and ideal antimicrobial properties[19]. Multiple diseases and some cancers are linked to poor genital health and maintenance[20]. Synthetic plastic materials with volatile organic compounds[21] and dioxins[22] are well-reported as hazardous. Nanomaterials of copper, silver and zinc oxide are known for antimicrobial activities[23-25] but the antimicrobial activity is lost due to oxidation and nanometals pose toxicity on human tissues[26]. In the present study, nanomaterials were coated with agarose to prevent oxidation[27] and retain antimicrobial activity for a long period without toxicity to humans. The neglect in the management of sanitary pads and adult diapers was a serious issue all over the world calling for eco-friendly materials[28]. The concerns with the management of menstruating women and hygiene awareness are needed in the coming times as well[29]. The novel pad has an apt application here as it is completely biodegraded in 3 months. Development and characterization of sanitary napkins made from regenerated cellulose fibers[30]. The natural cotton fibres were used in layers to allow no adverse reactions. In the present study, the holocellulose, leaf and coated nano materials-based sanitary pads are prepared[31]. The strength of this model lies in its properties i.e., biodegradable, anti-microbial, high-water absorbing. There are other reports also where scientists have worked on eco-friendly sanitary pads[32,33]. A similar attempt to manufacture paper towels using sustainable biosynthetic silver nanoparticles and *Ocimum sanctum* leaf extract was shown to have a bactericidal activity[34]. The anti-microbial activity was examined through the good diffusion method by measuring the zone of inhibition[35]. Zinc oxide nanoparticles exhibit a wide range of anti-microbial activities[36]. Chitinosone is highly biodegradable and exhibits adequate anti-microbial activity[37]. The usage of hollo cellulose extract decreases the duration of biodegradation and is less hazardous to the environment[38]. The market for biodegradable pads is flourishing massively. The current study mainly implied the use of biodegradable and anti-microbial sanitary pads which are environment-friendly and plastic free. Hence, they are devoid of hazardous chemicals and are no threat to our health and are made to provide comfort and safety equally. The infusion of dried leaves in the pad acts as a hydrophobic agent to avoid leakages. With a neutral, this pad is bereft of alkaline substitutes.

CONCLUSION

Commercial pads which are available in the market comprise plastics and carcinogenic chemicals such as dioxins and sodium polyacrylate. To overcome this major problem, the novel sanitary pad design is eco-friendly with high antimicrobial properties with a neutral pH, a high-water holding capacity, is completely biodegradable and non-toxic and is suggested for routine purposes.

SIGNIFICANCE STATEMENT

The motto of this novel study is to deduce the number of harmful chemicals which are usually infused in commercial pads. The materials used in these pads are eco-friendly hence, making them cost-effective and equally biodegradable and anti-microbial. The prominent outcomes of this study were the infusion of nanoparticles, pectinosone and chitinosone with agar yielding high anti-bacterial and anti-fungal results.

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