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Research Article

Breast cancer educational program and breast self-examination in Sana'a, Yemen

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ABSTRACT

Breast cancer is one of the most common cancer in women, constituting 22% of all cancer cases worldwide. One of the methods of breast cancer screening is breast self-examination. This study aimed to assess the impact of education on breast cancer and breast self-examination on female workers' knowledge, attitude, and practice. The study concerned to 103 females as a control group and 103 females as a case group. Intervention health education program was administrated to individual females of the case group. The results showed an improvement in knowledge of breast cancer risk factors, between control and case groups of participant females. Females' knowledge and attitude about breast self-examination factors clarify a high improvement in all answers of case group participants. In the control group 83.5% of women not practiced breast self-examination compared to 66% practicing it in the case group after the intervention. Radio and TV showed the highest percentage (41.7%) in the control group participants as a source of knowledge about breast cancer and breast self-examination. While health education program, represents the highest percentage (46.6%) in the case group participants as a source of knowledge for both breast cancer and breast self-examination. Correlation tests revealed a significant difference between knowledge and behavior scores in the control group participants (P = 0.019). This study results concluded that education can significantly improve the level of females' knowledge, attitude, and behavior of breast cancer and breast self-examination. This study recommended that educational programs used to improve females' knowledge of breast cancer and advantages of breast self-examination are an effective method for early recognition of this disease.

Keywords: Breast cancer; Breast self-examination; Knowledge; Attitude; Practice.

1. INTRODUCTION

Cancer is one of the most important diseases which threaten human health nowadays [1]. Because of abnormal cell growth, malignant cells can invade and spread to adjacent tissues and even distant organs. While the tumor reaches advanced stages, it can lead to patient death [2]. Among different types of cancers, breast cancer is one of the most common cancer in women worldwide; constituting 22% of all cases worldwide [3-5]. In Yemen, in 2008, the prevalence rate of breast cancer between years 2001-2005 estimated to be 42.4 per 100,000 women. At 2009, an average of breast incidence rate in Yemen was 20.9 (1261 cases) according to Worldwide Breast Cancer 2011 [6]. The most common age group affected in Yemen was women aged 41-50 years, with (35%) of cases occurring in this age [7]. Yemen's patients with late inoperable stages represented 67.0% of the total staged breast cancer patients, indicating the need for increased

community awareness and early detection of the disease [8]. In Arabic countries in general, women currently face a significant risk of high mortality rate from breast cancer due to late diagnosis [9]. So early detection must be considered the best second choice for reducing mortality [11]. The breast self-examination has a major role in early detection of breast cancer (48%), compared with annual mammography and clinical breast examination (41% and 11%, respectively) [12]. Breast self-examination is recommended to be performed routinely on a monthly basis in all the women aged above 20 years and the importance of raising awareness on breast cancer via BSE is noted [1]. In addition, breast self-examination is easy and can be done by anyone without any special equipment. Furthermore, it is also an economic, secure and noninvasive process [13]. This study aimed to evaluate the effect of the educational program regarding breast cancer and breast self-examination on knowledge, attitude, and behavior of female workers in Sana'a city institutes.

2. MATERIALS AND METHODS

2.1. Research design

This study was an interventional educational program and was conducted on a sample of 103 female workers as a case group who are attended educational program regarding breast cancer and breast self-examination, under coverage of the National Oncology Centers' health team in Sana'a city. And a sample of 103 female workers as a control group who have not attended the educational program. All individuals of the both groups were selected among female workers from the different institutes of government and private sectors in Sana'a city, Yemen, through systematic sampling on the list of their working institutes. All females (age 20-70 years) were considered the target population. Data collection for the study was carried out in the period from March to June 2011.

2.2. Tools of the study

Data gathering tool was a standard questionnaire developed by the investigator based on the related literature to assess the level of knowledge, attitude, and practice of female workers about breast cancer and breast self-examination. The validity of the research tool was ensured through a review by 3 experts who hold a Ph.D. in Oncology and the necessary modification was made. A pilot study was conducted on 20 females to ensure the visibility of the tool.

The questionnaire consisted of two parts: demographic characteristics (4 questions), namely are; age, marital status, educational level and monthly income. And questions about individuals' knowledge (14 questions) to identify the knowledge regarding breast cancer and breast self-examination, included items regarding general knowledge of cancer, causes, symptoms, examination, treatment, prevention of breast cancer and knowledge of breast self-examination. An attitude and behavior (13 questions) for assessing females practice about breast self-examination. Included items related to breast self-examination practice. Both groups' individuals filled in the questionnaire.

The study tool was used for individuals of the control group to get a baseline data of participants' knowledge of breast cancer and their level of breast self-examination practice. The same tool was used for individuals of the case group to test if there is any difference in participants' knowledge, attitude, and practice, compare to the control group.

The awareness program was offered for a case group. The methods of teaching used were a lecture followed by focus group discussion and demonstration for practices. Posters were also used to provide and view more information and each female is provided a copy. The post-test questionnaire was given to the respondents after a period of three months of the program was offered. This period of time was given for them to familiarize themselves with the feel and appearance of their breasts so that they can notice any difference or change in the way their breasts looked and felt.

2.3. Data analysis

All data were coded, tabulated and subjected to statistical analysis. Statistical analysis is performed using SPSS version 12. Quantitative and qualitative variables are described by proportions and percentages. Descriptive statistics are used to analyze

the response to individual items and the respondents' characteristics. A correlation test was used to test differences between the different groups.

3. RESULTS

3.1. Socio-demographic factors

Among 103 of case group participants, 49 (47.57%) were married and 49 (47.57%) were single while 5 (4.86%) were divorced. 50 (48.5%) of individuals age were less than30 years and 43 (41.7%) were between 30-39 years while 10 (9.8%) were more than 39 years. Regarding their literacy levels, 77 (74.7%) had university degrees, 21 (20.3%) were the secondary school, 2 (1.9%) had the primary school and 3 (2.9%) were illiterate.

Among 103 of control group participants, 40 (38.8%) were married and 56 (54.3%) were single while 7 (6.9%) were divorced. 57 (55.3%) of individuals age were less than30 years and 38 (36.9%) were between 30-39 years while 8 (7.8%) were more than 39 years. Regarding their literacy levels, 63 (61.1%) had university degrees, 24 (23.3%) were a secondary school, 8 (7.7%) had the primary school and 8 (7.7%) were illiterate. There were no significant differences in these variables between two groups.

3.2. Impact of the intervention program

As shown in Table 1, there was an improvement in all intervention items regards knowledge of breast cancer risk factors, between control and case groups of participant females. The highest percent change to the correct answer between the control and the case groups were reported for the items of "childlessness after the age of 35, genetic factors and previous benign tumors" (27.2%, 24.3%, and 22.3%) respectively. On the other hand, the lowest percent change to the correct answer (7.7%) was reported for an item of "breast cancer is the most common tumors between women". Concerning level of the knowledge about the breast cancer's signs, the percent change of correct answers ranged from 5.8% for the item of "difference in the shape and position of the nipples", to 27.2% for the item of "change the shape of the breast" (Table 2).

Table 3 showed that the females' knowledge and attitude about breast self-examination factors clarifies a highly improvement in all answers of case group participants, concerning: use the palm of the hand, place of another hand, appropriate time and frequency of breast self-examination, (46.9, 38.8, 38.8 and 33) respectively.

Table 1. Comparison of the control and case group intervention knowledge about risk factors for breast cancer among females' participating.

Risk factors for breast cancer	Correct answer No. (%)		No. (%) of change
	Control group n= 103	Case group n= 103	
Breast cancer is the most common tumors among women	84(81.6)	92(89.3)	8 (7.7)
Previous benign tumors increase breast cancer risk	27(26.2)	50 (48.5)	23(22.3)
Early menarche and late menopause increase the probability of incidence	4(3.9)	21 (20.4)	17(16.5)
Increase the incidence of breast cancer with age	26(25.2)	41(39.8)	15(14.6)
Genetic factors affect the incidence of breast cancer	26(25.2)	51(49.5)	25(24.3)
Users of oral contraceptives from factors breast cancer incidence	19(18.4)	38(36.9)	19(18.5)
Obesity has strong relationship with breast cancer occurrence	26(25.2)	37(35.9)	11(10.7)
Feeding a key role in the prevention of breast cancer	42(40.8)	54(52.4)	12(11.6)
Breastfeeding causes of breast cancer prevention	76(73.8)	87(84.5)	11(10.7)
Childlessness or having children after the age of 35 factors that may increase the incidence of breast cancer	16(15.5)	44(42.7)	28(27.2)

Table 2. The knowledge's level about symptoms and signs of breast cancer incidence.

Breast cancer's signs	Answers	Control group	Case group	No. (%) of change
Exit means of nipple without pressure	Correct	74(71.8)	89(86.4)	15(14.6)
	False	7(6.8)	6(5.8)	
	Don't know	22(21.4)	8(7.6)	
Change the shape of the breast may be a sign of breast cancer	Correct	47 (45.6)	75 (72.8)	28 (27.2)
	False	21 (20)	22 (21.4)	
	Don't know	35 (34)	6 (5.8)	
The presence of mass under the armpit evidence of the breast tumors	Correct	67 (65)	85 (82.5)	18 (17.5)
	False	5 (5)	4 (4)	
	Don't know	31 (30)	14 (13.6)	
Difference in the shape and position of the nipples refers to the existence of the breast tumors	Correct	70 (68)	76 (73.8)	6 (5.8)
	False	14 (13.6)	20 (19.4)	
	Don't know	19 (18.4)	7 (6.8)	
The presence of pain in the breast during the scan	Correct	15 (14.6)	23 (22)	8 (7.4)
	False	56 (54.4)	71 (69)	
	Don't know	32 (31)	9 (7.8)	
Change the color of skin of the breast and the increased thickness is evidence of cancerous tumors of the breast	Correct	77 (74.8)	96 (93)	19 (18.2)
	False	4 (3.9)	4 (3.9)	
	Don't know	22 (21.4)	3 (2.9)	

Table 3. The knowledge and attitude level about breast self-examination.

Breast self-examination factors	Answers	Control group	Case group	No. (%) of change
Appropriate time for performing breast self-examination	Correct	8(7.8)	48(46.6)	40 (38.8)
	False	13(12.7)	24(23.3)	
	Don't know	82(79.6)	31(30)	
Frequency of breast self-examination practice	Correct	36 (35)	70 (68)	34 (33)
	False	59 (57)	31 30)	
	Don't know	8 (7.8)	2 (1.9)	
Appropriate age for start a breast self-examination	Correct	26 (25.2)	50 (48.5)	24 (23.3)
	False	23 (22.4)	27 (26.3)	
	Don't know	54 (52.4)	26 (25.2)	
Use the palm of hand when breast examination	Correct	25 (24)	73 (70.9)	48 (46.9)
	False	3 (2.9)	13 (12.6)	
	Don't know	75 (72.8)	17 (16.5)	
Place of another hand during breast self-examination	Correct	12 (11.7)	52 (50.2)	40 (38.8)
	False	18 (17.5)	30 (29)	
	Don't know	73 (70.9)	21 (20.4)	
Breast self-examination is the most important means of early detection of breast cancer	Correct	72(70)	97 (94.2)	25 (24.2)
	False	2 (1.9)	1 (1)	
	Don't know	28 (27.2)	5 (4.9)	
Early diagnosis improves treatment outcomes	Correct	92 (89)	96(93)	4 (3.9)
	False	2 (3.9)	2 (1.9)	
	Don't know	7 (6.8)	4 (3.9)	
The discovery and treatment of breast cancer early often lead to full recovery	Correct	81(78.6)	89(86.4)	8 (7.8)
	False	7 (6.8)	4 (3.9)	
	Don't know	14 (13.6)	10 (9.7)	

99 (96.1%) of the control group participants, has heard about breast cancer and 102 (99%) of the case group participants, heard about breast cancer. Only, 21 (20.4%) of the control group participants, know about breast self-examination procedure, while 102 (99%) of the case group participants, know about breast self-examination procedure. In addition, 83.5% did not practice breast self-examination in the control group compared to 66% practicing it in the case group after the intervention. Furthermore, the main reason for not practicing breast self-examination in the control group was not knowing how to perform it (58.1%), while in the case group the main reason given for not practicing breast self-examination was forgetting (48.6%).

Almost of the case group and the control group participants together agreed that there is no adequate awareness by the authorities concerned about breast self-examination. And almost of the case group and the control group participants, together agreed that awareness about the importance of self-examination may help in early detection of breast cancer. Also, almost of the case group and the control group participants, together encourage awareness campaigns about breast self-examination.

Radio and TV media were the highest percentages (41.7%), as a source of knowledge about breast cancer and breast self-examination in the control group participants. While health education programs, represents the highest percentages (46.6%), as a source of knowledge for both breast cancer and breast self-examination in the case group participants. (Table 4).

Table 4. The practice and other factors of breast cancer and breast self-examination.

Factors	**Answers**	**Control group No. (%)**	**Case group No. (%)**	**No. (%) of change**
Heard of breast cancer	Yes	99 (96.1)	102(99)	3 (2.9)
	No	4(3.9)	1(1)	
Know about breast self-examination procedure	Yes	21 (20.4)	102 (99)	81 (78.6)
	No	82 (79.6)	1 (1)	
Practice breast self-examination	Yes	17 (16.5)	68 (66)	51 (49.5)
	No	86 (83.5)	35 (34)	
Causes of didn't practice breast self-examination	I do not know how	50 (58.1)	2 (5.7)	
	Fear of discovery of something unnatural	16 (18.6)	9 (25.7)	
	Pain	7 (8.1)	7 (20)	
	Forgetting	13 (15.1)	17 (48.6)	
	Total	86 (100)	35 (100)	
Is there adequate awareness by the authorities concerned about breast self-examination	Yes	16 (15.1)	16 (15.5)	0 (0)
	No	87 (84.5)	87 (84.5)	
Is awareness of the importance of self-examination may help in early detection of breast cancer	Yes	102 (99)	102 (99)	0 (0)
	No	1 (1)	1 (1)	
Do you encourage awareness campaigns about breast self-examination	Yes	99 (96.1)	101(98.1)	3 (2.9)
	No	4 (3.9)	2 (1.9)	
Source of breast cancer knowledge	Radio and TV	43 (41.7)	21 (20.4)	
	Internet	3 (2.9)	0 (0)	
	Education program	2 (1.9)	48 (46.6)	
	Family and friends	30 (29.1)	10 (9.7)	
	Journals and posters	21 (20.4)	23 (22.3)	

Table 5. The correlation between knowledge and behavior of individuals control and case groups participants.

	Case group	**Knowledge**	**Behavior**	**Control group**	**Knowledge**	**Behavior**
Case group						
Pearson Correlation	.(a)	.(a)	.(a)	.(a)	.(a)	.(a)
Sig. (2-tailed)	.	.	.	.	.	.
N	103	103	102	103	103	39
Knowledge						
Pearson correlation	.(a)	1	.(a)	.(a)	.052	.(a)
Sig. (2-tailed)	.	.	.	.	.605	.
N	103	103	102	103	103	39
Behavior						
Pearson correlation	.(a)	.(a)	1	.(a)	.025	.428(**)
Sig. (2-tailed)	.	.	.	.	.806	.007
N	102	102	102	102	102	39
Control group						
Pearson correlation	.(a)	.(a)	.(a)	.(a)	.(a)	.(a)
Sig. (2-tailed)	.	.	.	.	.	.
N	103	103	102	103	103	39
Knowledge						
Pearson correlation	.(a)	.052	.025	.(a)	1	.373(*)
Sig. (2-tailed)	.	.605	.806	.	.	.019
N	103	103	102	103	103	39
Behavior						
Pearson correlation	.(a)	.(a)	.428(**)	.(a)	.373(*)	1
Sig. (2-tailed)	.	.	.007	.	.019	.
N	39	39	39	39	39	39

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

a Cannot be computed because at least one of the variables is constant.

The correlation tests revealed a significant difference between knowledge and behavior scores in the control group participants (P= 0.019). And it was a significant difference in the behavior scores of the control and case group participants (P= 0.007) as shown in Table 5.

4. DISCUSSION

The analysis of the present study revealed that breast cancer and breast self-examination awareness program provided during the study had a great impact on the responses of the case group. Also, results of our study demonstrated that education can improve individuals' level of knowledge, attitude, and behavior about breast cancer and breast self-examination. This is similar to findings of previous studies conducted in different countries. A study conducted in Egypt reported that there was a highly significant improvement in all the knowledge items of the intervention group from pre to post-test [11]. Other study conducted in Egypt reported that there was a significant improvement in the studied women regarding general knowledge of breast cancer and breast self-examination through-out the educational program in all items and in total score [14]. A study conducted in the Kingdom of Saudi Arabia reported that there was a significant improvement in studied women's regarding general knowledge of breast cancer and breast self-examination after implementation of the intervention program [9]. Other study conducted in the Kingdom of Saudi Arabia reported that the total mean knowledge scores of the participants increased significantly after the educational program [15]. A study conducted in Turkey reported that the difference between their knowledge before and after the training sessions was exceptionally significant [13]. A study conducted in Iran reported that the mean knowledge and behavior score related to

breast cancer and breast self-examination were increased after education [2].

As regards to knowledge of risk factors for breast cancer, this study results showed that the recognition of childlessness after the age of 35, genetic factor and previous benign tumors as risk factors increased significantly among of case group participants. The recognition of oral contraceptives users, early menarche, late menopause, and women's age, as risk factors also increased in the present study among the case group participants. This is agreed with findings previous study conducted in Egypt reported that the highest percent change to correct answer between pre and post test was reported for women's age and early age of menarche [11].

In relation to the average of the percentage change in knowledge of breast cancer signs between the control group and case group was low something (15.1%), that because of the control group had high knowledge about most of breast cancer signs' items. This agreed with findings of other studies. A study conducted in Egypt reported that there was 27.1% increase in the percentage of correct answer regarding knowledge of the breast cancer's signs [11]. Other study conducted in the Kingdom of Saudi Arabia reported that there was an improvement in the knowledge and knowing the dangerous signs and symptoms between studied groups [9]. Another study conducted in the Kingdom of Saudi Arabia reported that after the intervention program, there were statistically significant improvements in knowledge in relation to breast cancer signs and symptoms [15].

Regarding to knowledge and attitude of breast self-examination, this study results showed that, the education program showed an impact on the remarkable increase in the use the palm of hand when breast examination from 24% to 70.9%, place of other hand during breast self-examination from 11.7% to 50.2%, appropriate time for perform breast self-examination from 7.8% to 46.6% and frequency of breast self-examination practice from 35% to 68%. This agreed with findings of other study conducted in Egypt and reported that the women's knowledge about breast self-examination clarifies also a highly significant improvement in all answers concerning procedure, frequency and appropriate time from pre to post-test [11]. Study conducted in Iran reported that the average point of positive attitude about breast self-examination has increased after intervention [16].

Results of this study showed that the knowledge and practice ratios of breast self-examination were increased from 20.4% to 99% and from 16.5% to 66% respectively. This agreed with findings of other studies. A study conducted in Egypt reported that 75% practiced breast self-examination in post-test compared to 70% who did not practice it in pre-test [11]. Other study conducted in Egypt reported that before the program all students don't practice breast self-examination, after the program a significant was observed in the students' practice in relation to methods and techniques and total practice score [14]. A study conducted in the Kingdom of Saudi Arabia reported that 83.3% of women successfully performed breast self-examination after implementation of the program compared to 25% pre-program [9]. A study conducted in Western Turkey reported that the difference was statistically important when compared the breast self-examination practices of the women who participated in the study before and after the training program [1].

This study results also showed that the main reason for not practicing breast self-examination among control group participants was that they did not know how to perform it (58.1%), while the forgetting was the main reason for not practicing breast self-examination among case group participants (48.6%). This agrees with findings of other studies. A study conducted in Egypt reported that the main reason for nonpracticing breast self-examination prior to the program was that they did not know how to perform it [14]. Other study conducted in the Kingdom of Saudi Arabia reported that the reason for not performing breast self-examination regularly was forgetfulness [15].

As regards the main source of females' knowledge of breast cancer and breast self-examination, it changed from the media (Radio and TV) in the control group respondents to the intervention program in the case group respondents. This agreed with findings of other studies. A study conducted in Egypt reported that media was the highest percentage in the pre-test as a source of knowledge about breast cancer and breast self-examination while the intervention program rated

high as a source of knowledge of the post-test individuals [11]. Other study conducted in the Kingdom of Saudi Arabia reported that TV and doctor throw intervention program were high as a source of information about breast cancer [9].

Based on results of this study, the correlation tests revealed that the control group individuals' knowledge and behavior are significantly related to each other (P = 0.019). Also, the correlation tests revealed that there is a statistical relationship between behavior level between the control and case group individuals (P = 0.007). This agreed with findings of other studies. A study conducted in Egypt reported that women's education and knowledge score were significantly associated with the practice of breast self-examination [11]. Other study conducted in Egypt reported that women's knowledge score was significantly associated with the practice of breast self-examination [14]. A study conducted in Iran reported that a significant statistical correlation was seen between knowledge level and attitude (p = 0.001) [5]. Another study conducted in Iran reported that the correlation test revealed that after education there was a significant relationship between knowledge and behavior (P = 0.005) [2].

5. CONCLUSIONS

This study was conducted in order to evaluate the effects of interventions for increasing breast cancer and breast self-examination awareness. The study results indicate that females who received intervention program had the higher level of knowledge, attitude, and behavior regarding breast cancer and breast self-examination. As mentioned, education can significantly improve individuals' levels of knowledge, attitude, and behavior about breast cancer and breast self-examination.

Recommendations: According to aforementioned points, we suggest to considering comprehensive educational programs to improve females' knowledge about breast cancer and advantages of breast self-examination as an effective method for early recognition of the disease.

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TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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Research Article

Rarity study of endemic mammals of India

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ABSTRACT

India is one of the most biodiverse region of the world, representing four biodiversity hotspots. It represents 7.90% of the diversity (427 species, 48 families and 14 orders). The study reveals there are 43 mammals endemic to India transboundary. From which 15 are restricted to western Ghat, 17 from peninsular India, 2 from northeast India, 4 are in Himalaya and 9 are in islands so far known. According to the IUCN Red List, of the 43 endemic mammals 28 are considered to be threatened. These threatened are further divided into 7 as Critically Endangered (CR), 13 as Endangered (EN), and 8 as Vulnerable (VU). The study provided information on distribution of endemic species of India and rarity status of each endemic mammal based on its geographical range, habitat specificity and population size, known on the basis of information provided by the IUCN. The study also reveals the categorization of endemic species according to the threat level on them.

Keywords: Endemic; Threatened; Mammals; Rarity; Biogeography; IUCN.

1. INTRODUCTION

India is one of the world's most bio-diverse region. The country's political boundary encompass a wide range of ecozones: desert, high mountains, highlands, tropical and temperate forests, swamplands, plains, grasslands, areas surrounding rivers, as well as island. It is presented as parts of 4 global biodiversity hotspots; the Western Ghats, the Himalayas, the Indo-Burma region and the Sundaland region [1]. India is located at the confluence of Oriental, Palaearctic and Ethiopian biogeographical realms [2] and Indian mammals are the admixture of the three realms [3]. There are 668 Protected Areas including 102 National Parks, 515 Wildlife Sanctuaries, 47 Conservation Reserves and 4 Community Reserves covering a total of 161221.57 km^2 of geographical area which is approximately 4.90% of the country. In addition there are 47 Tiger Reserves, 18 Biosphere Reserves, 25 Elephant Reserves, 5 Natural World Heritage sites and 25 Ramsar Wetland sites in India [4].

India has very rich biological resource, and its most important attribute is the mammalian diversity [5]. About 5416 species of mammals belonging to 154 families and 29 orders recorded from the World [6], out of which 427 species recorded from India [3]. Due to growing population there is high pressure on the mammalian fauna and about half of the fauna have reduced their distributional habitat [3]. The country has already lost the four mammals species *i.e. Acinonyx jubatus*, *Bos javanicus*, *Dicerorhinus sumatrensis* and *Rhinoceros sondaicus* [3].

Biogeography is the study of the distribution of species (biology), organisms, and ecosystems in geographic space and through geological time [7]. Out of the ten recognizable bio-geographic zones

[7] of India, the Himalayas are less studied although it is more fragile and more diverse in flora and fauna [8]. The area coverage by the biogeographic zones of the country: Trans-Himalaya (5.62% of India), Himalaya (6.41%), Desert (6.56%), Semi-Arid (16.60%), Western Ghats (4.03%), Deccan Peninsula (41.99%), Gangetic Plain (10.79%), Coasts (2.52%), North-East (5.21%) and Islands (0.25%) [7].

Among a set of ecologically similar species, those that are rare will have a greater extinction risk. Small populations are more likely to be impacted by demographic and environmental events, like failure to find a mate, diseases, floods, and fires. The limited budget for biodiversity conservation has restricted the work for the threatened group of mammals. Its important to bring into focus how much does biodiversity matters? The study is conducted with the aim to known the threatened status of the endemic mammals from the IUCN Red List [9], distribution and species categorisation into the seven forms of rarity model [10, 11] on the basis of the description given by different authors.

2. MATERIAL AND METHODS

Of the 427 species of mammals found in India, 43 are considered to be endemic to the Indian political boundary [3]. The status of these endemic one in the IUCN (International Union for Conservation of Nature) Red List of Threatened Species [16]: Categories (CR: Critically Endangered, EN: Endangered, VU: Vulnerable, NT: Near Threatened, LC: least Concern, DD: Data Deficient); the Indian Wildlife (Protection) Act, 1972 [19] (Schedule I, II, III, IV and V) and lastly in the CITES [20]: Convention on International Trade in Endangered Species of Wild Fauna and Flora (Appendix I, II and III) are checked and tabulated with cross-checking from the ZSI, Checklist of Mammals of India, 2015. The distribution of the endemic mammals in the concern biogeography zones were presented in the manner as present '1' and absent as '0' (Table-III).
The IUCN Red List of Threatened Species [16] (also known as the IUCN Red List or Red Data List), founded in 1964, is the world's most comprehensive inventory of the global conservation status of biological species. Individual species accounts including IUCN Red List threat category, range map, ecology information, and other data for every endemic mammal species was gathered (Table-II), which was used for ranking of species in the rarity model [17, 18]. For every mammal species, the following data were collected from the IUCN Red List:

- Species classification
- Geographic range (including a distribution map, extent of occurrence, area occupied)
- Red List category and criteria
- Population information
- Habitat preferences
- Major threats
- Conservation measures

In this context, the term "Threatened" refers to those species classified under the IUCN Red List categories of Vulnerable, Endangered, or Critically Endangered. In habitat type, the term used "artificial terrestrial habitat" refers to plantation areas, cultivated fields.

2.1. Seven forms of rarity model

For more emphasis on the conservation of the endemic mammals further the seven forms of rarity model [17, 18] was studied. The model focuses on: (1) local population density (2) the area of the species range, and (3) the number of different kinds of habitats that species occupy. If species are dichotomized for each of these variables, an eight-celled model is created that reflects different types of rarity and commonness.

For geographic range the extent of occurrence (more than 50,000 sq km as wide range and below it as narrow), for habitat specificity the suitable land cover (more diverse forest type are taken into broad and less diverse as restricted) are taken into consideration. At last for population size the mammals having less than thousands individuals with decreasing trend are considered as everywhere small and more than thousands with stable or no change in trend as somewhere large.

The species for which the population size is not given, body size is taken for grouping. Body size is considered as larger body mass less the population and vice-versa. Due to significant associations of body size with population density and species range area, they incorporated body mass into analyses [17].

Geographic range					
Population Size		**Large**		**Small**	
	Somewhere large	Common	Locally abundant over a large range in a specific habitat type	Locally abundant in several habitats, but restricted geographically	Locally abundant in a specific habitat, but restricted geographically
	Everywhere small	Constantly sparse over a large range and in several habitats	Constantly sparse in a specific habitat, but over a large range	Constantly sparse and geographically restricted in several habitats	Constantly sparse and geographically restricted in a specific habitat
		Broad	**Restricted**	**Broad**	**Restricted**
		Habitat specificity			

3. RESULTS

3.1. Endemic status of India

Out of the 5487 mammals species 427 are found in India [3]. From this 43 mammals are endemic which is of about 10.07% of the mammals found in India (Table 1). According to the IUCN categorisation into threatened categorises, out of the 43 endemic mammals 28 are considered under the threatened list which needs prior care and management ways to prevent them from being extinct. The table is further presented in the pie graph (Fig. 1) with the key colours as taken in the IUCN threatened list.

3.2. Distribution of endemic mammals according to the bio-geographic zones

The bar diagram (Fig. 2) helps to know the number of endemic mammals in the zones. It indicates the bio-geographic zone rich species diversity, threatened ones. The threatened endemic mammals are found in the Western Ghats i.e. 10 species, followed by 9 species found in the Islands, and 8 in Deccan peninsula. The endemic mammals found in the Islands are all in the threatened criteria. The mammals found in the islands are mostly of “Erinaceidae” family.

3.3. Threatened family of endemic mammals

From the bar diagram (Fig. 3) it is found that most of the endemic mammals belong to family “Muridae” (old world rats and mice family), 14 species (18.67%) are endemic out of the 75 species belonging to this family found in India.

Table 1. Endemic mammals threatened categorization.

Endemic threatened mammals of India		
Criteria	**Mammals found**	**% mammals found in the categories**
CR	7	16.28
EN	13	30.23
VU	8	18.60
NT	1	02.32
LC	11	25.58
DD	3	06.98
Total	43	

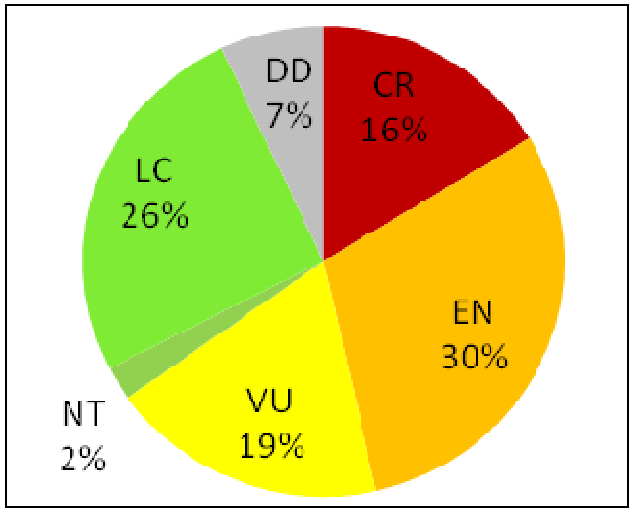

Figure 1. IUCN status of the endemic mammals of India.

3.4. Categorisation into different habitats

For getting more specific idea on the spread of the endemic mammals in different vegetations or degraded areas further information about their natural habitats are studied from the data given in the IUCN red list represented in the bar daigram (Fig. 4). The endemic animals are divided into two categories: non threatened and threatened, in the bar diagram. Most of the threatened mammals are found to be present in forest areas and with the decrease in forest their habitat is depleting. This results in the migration of these mammals from forest areas to artificial terrestrial habitats like plantation, garden, sacred grooves, etc., which rather increase man-animal conflict, hunting or poaching, competition etc.

3.5. Rarity study of mammals

Further these endemic mammals are categorised into 8 groups according to the seven forms of rarity model [10, 11] was studied. According to the grouping of the mammals the grading was done in which "H" cell needs to be given priority and "A" cell the least.

The endemic species varied considerably among categories of rarity, but about 14% of the species fell into the most common category (cell A) and about 63% of the endemic mammals come under the most rare category (cell H, Fig. 5). From this 4 mammals: Mitred horseshoe bat (*Rhinolophus mitratus*), Sombre bat (*Eptesicus tatei*), Peter's tube nosed bat (*Harpiola grisea*) and Royle's Mountain vole (*Alticola roylei*) are still not listed under the threatened categories even though they come in the rare category according to this model.

3.6. Causes of extinction

The bar (Fig. 6) show major threats listed in the IUCN red list for the endemic mammals. The threatened mammals have the major threat of habitat loss and fragmentation, hunting or human disturbance. Exponential rise of human population and competition for better survival among the human race is the key point for these threats to be rising at an alarming rate. Majority of endemic threatened mammals are affected due to loss of their habitat loss followed by hunting, human disturbance and natural disaster.

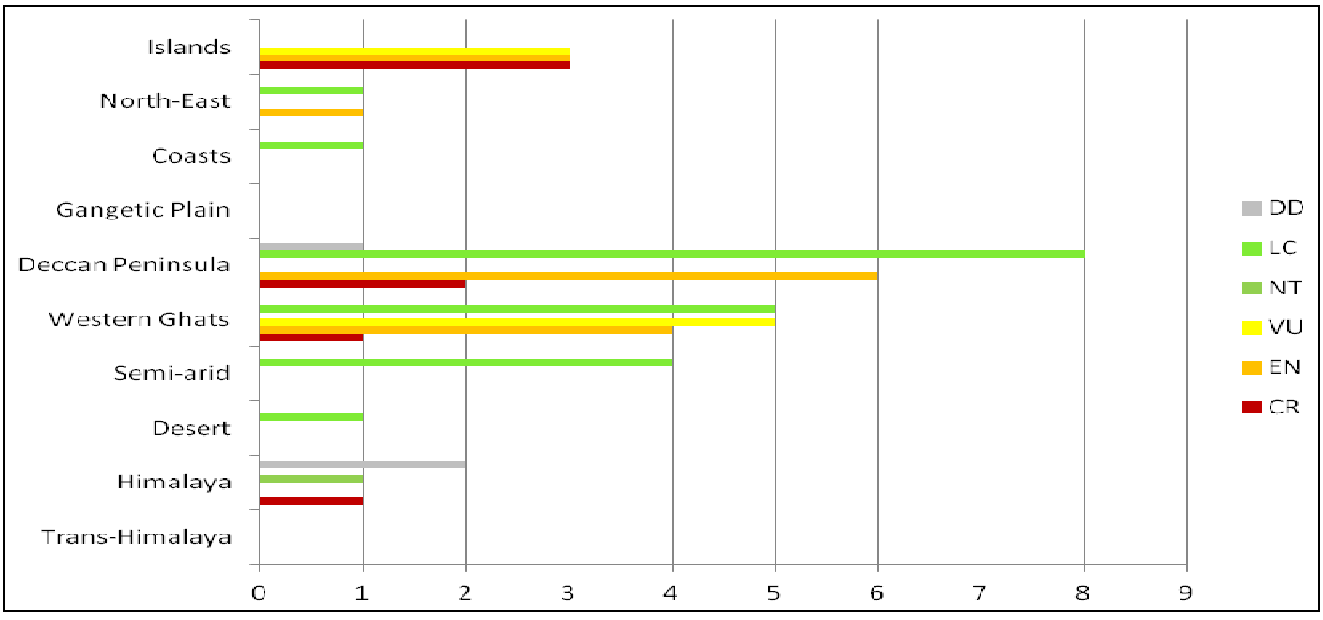

Figure 2. Bar diagram showing endemic and threatened mammals of India (IUCN criteria) according to bio-geographic zones.

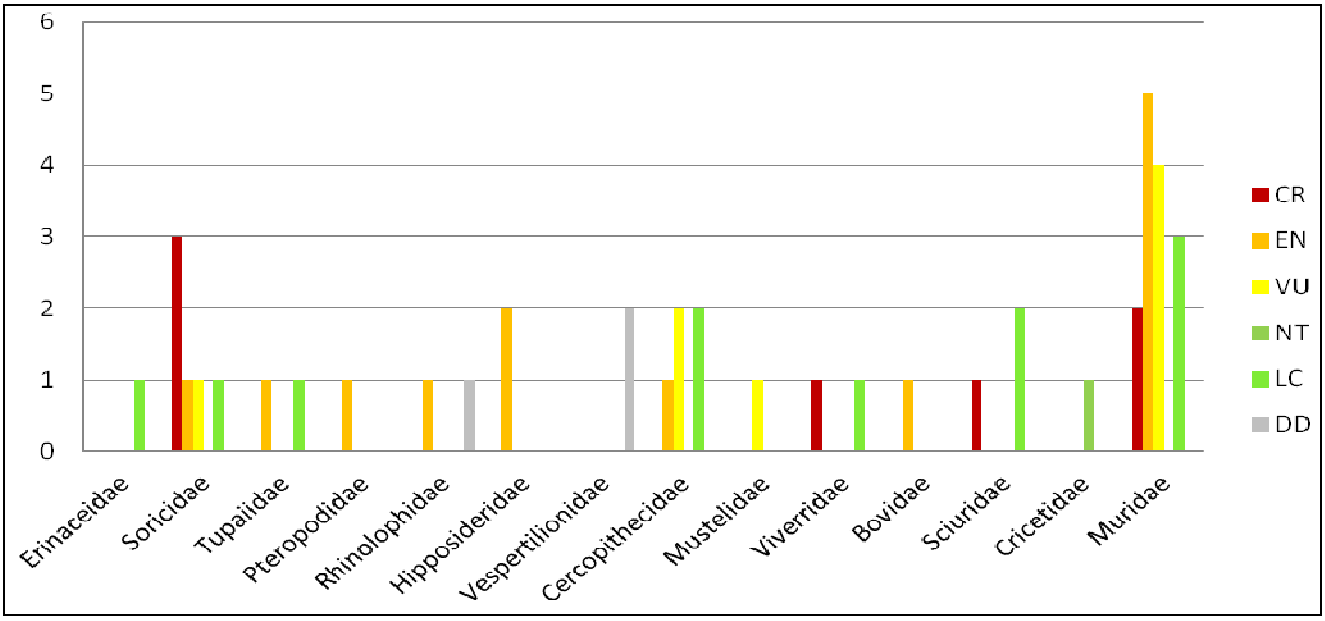

Figure 3. Categorisation of endemic mammals on threatened criteria, according to the families.

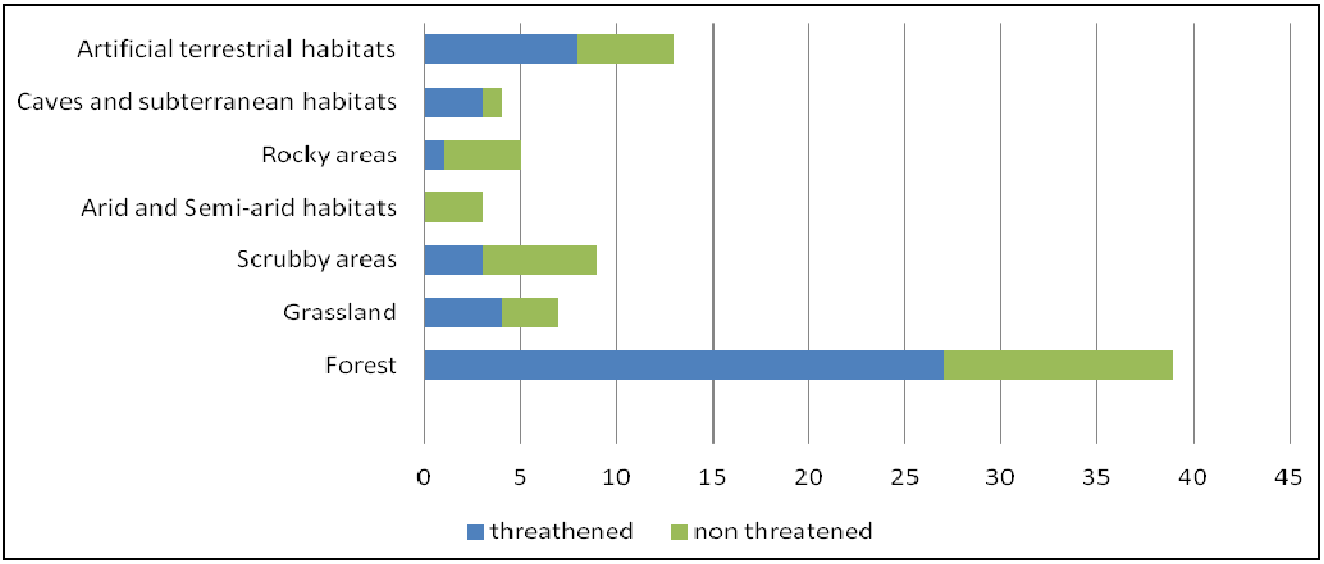

Figure 4. Endemic mammals in different habitat types.

Local population size	**Geographical distribution**			
	Wide		Narrow	
	Broad	Restricted	Broad	Restricted
Somewhere large	6 mammals (A)	2 mammals (C)	2 mammals (E)	2 mammals (G)
Everywhere small	(B)	(D)	4 mammals (F)	27 mammals (H)
	Habitat specificity			

Figure 5. Mammals species in cell of the rarity model.

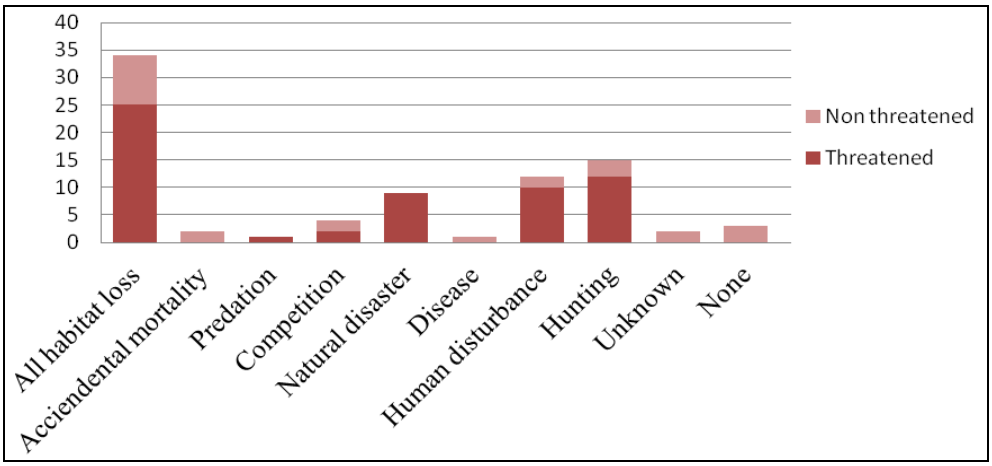

Figure 6. Endemic species under various threat categories.

4. DISCUSSION

In India, the classification of mammals was dealt by many authors such as fauna of British India - Mammalia [14, 15], checklist of Indian and Palaeartic mammals [16], book of Indian animals [17], Indo-malayan region mammals [18], checklist of mammals of India [19], South Asian mammals [20], mammals of South Asia [21], Indian mammals [22], Critically Endangered Animal Species of India [23] and IUCN threatened list [9]. After the detail study of each mammals distribution status described by authors the endemic list was gathered. Of the so far known endemic mammals of India (43 species) "Muridae" family has highest number (14) of endemic mammals, followed by the family "Soricidae" (6) and the family "Cercopithecidae" (5). While other families: "Erinaceidae", "Mustelidae", Bovidae" and "Cricetidae" has only single endemic mammals. From the endemic list its seen that most are in the threatened category (28), which is alarming point. As the species are restricted to the geographical boundary, its population needs to be conserved or else it will go extinct. The presence of large faunal elements in a landscape is not only decisive to assure the completeness of the respective biocenosis; it also has an emergent effect on the ecosystem itself [24]. Being in the IUCN threatened list [9] they are not neither listed in the Wildlife (Protection) Act, 1972 [12] nor in the CITES [13] and also no protection measures are carried for these mammals.

From biogeographic zones such as North-east and Western Himalaya, there may be further unrecorded mammals as these areas are inaccessible. Also these regions share common habitat and their transboundary in nature, so taking out the political boundary of endemicity might yield less endemic in Indian landscape. The North-east having a large diversity, only 2 species are known to be endemic as most of the species are also observed in Bhutan, Myanmar, Bangladesh. In the Western Ghats, the species are restricted to the region as it has the Great plain on one side and the Arabian Sea on other. The Islands having 9 threatened mammals which is a limited geographical range due to habitat fragmentation and edge effect. The population is decreasing as its migration is inhibited by the Indian Ocean.

Further when the mammals are categorised on family basis "Muridae" has highest threatened mammals (11). It's generally a concept to have a large population size as its smaller body mass. The smaller mammals are difficult to survey and not conserved in any ex-situ conservation. Due to their smaller body size and less ecological importance, they are not thought to be in the endangered category by researchers. The rarity model is the best way to know the threatened species which purely based on the area of occurrence, habitat type and population size. In most of the protected areas large body sized mammals are given more priority for conservation as they are being the "keystone species" or "umbrella species" in the ecosystem. All the protected areas and hotspots are also affected due to human interference. Natural disaster like Tsunami affected the island species by destruction of habitat and habitat fragmentation. The human

interference and conversion of the natural habitats into urban, agricultural lands, mining or industrialisation must be limited. The species in the top most position of the threatened list i.e. critically endangered needed to be kept in captivity in their natural habitat with proper availability of food and suitable environment for their breeding cycle to enhance their number of progenies.

5. CONCLUSIONS

As a group, mammalian species exhibit a strong bimodal pattern of many relatively common and rare species. The exceptionally high proportion of mammalian species listed as threatened by the IUCN still there are species that exhibit characteristics of all forms of rarity. The islands are needed to be given special attention as its being the dwelling place for most of the endemic mammals of India. As the terrestrial ecosystem is limited the mammals are restricted to the place, and any further disturbance cause a depletion of the populations.

According to the results obtained from categorising the mammals in the methods it is also being observed that the small mammals are rarer in both criteria and very few information is being available on these species. Though these are a little away from the verge of extinction, still no protection region is being established for them. Along smaller body size, if the mammals is further burrowing or nocturnal in nature there is a problem of citation and the limit of occurrence is not known.

AUTHOR'S CONTRIBUTION

JD: Data collection, compilation of data, collection of references and manuscript preparation. SPP: Collection of references, final editing and checking of manuscript. The final manuscript has been read and approved by both authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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Appendix I. Distribution and status of endemic mammals.

Order	Family	Common Name	Scientific Name	IUCN status	WPA, 1972	CITES	Geographical Range	Habitat specificity	Population size
Insectivora	Erinaceidae	Madras hedgehog	*Paraechinus nudiventris* (Horsefield, 1851)	LC			southern India, up to an elevation of 700 m	dry deciduous scrubby areas with acacia and rocky habitats	locally common (decreasing)
	Soricidae	White toothed Andaman Shrew	*Crocidura andamanensis* (Miller, 1902)	CR			South Andaman Island	tropical moist deciduous and evergreen forests	not known
		Jenkin's Shrew	*Crocidura jenkinsi* (Chakraborty, 1978)	CR			South Andaman Island, up to 100 m asl	tropical moist deciduous forest	not known
		Nicobar Shrew	*Crocidura nicobarica* (Miller, 1902)	CR			southern tip of Greater Nicobar Island, upto about 100m	tropical moist deciduous forest	decreasing
		Day's shrew	*Sunucus dayi* (Dodson, 1888)	EN			Western Ghats in southern India - Thrissur (Kerala), Eravikulam National Park (Kerala), Palni Hills (Tamil Nadu) and Upper Bhavani (Mukurthi National Park, Tamil Nadu); 1,500-2,500 m asl	montane grassland-shola	not known
		Andaman spiny shrew	*Crocidura hispida* (Thomas, 1913)	VU			Middle Andaman Island	tropical evergreen forests	unknown
		Assam mole shrew	*Anourosorex assamensis* (Anderson, 1875)	LC			Northeastern India, ranges in altitude from 1,500 to 3,100 m asl	both subtropical and tropical montane forest, stony escarpments, and in sewage lines	unknown
Scandentia	Tupaiidae	Nicobar treeshrew	*Tupaia nicobarica* (Zelebor, 1869)	EN		II	Great Nicobar and Little Nicobar, Sea level to 1,000 m	lower and mid-canopy of rainforest areas.	locally common (decreasing)
		Madras Treeshrew	*Anathana ellioti* (Waterhouse, 1850)	LC		II	eastern, central, southwestern and southern parts of peninsular India	scrub jungle, dry and moist deciduous forests and in montane sholas (wet evergreen forests)	locally common (decreasing)
Chiroptera	Pteropodidae	Salim Ali's fruit bat	*Latidens salimalii* (Thonglongya, 1972)	EN	I		Periyar Tiger Reserve in Kerala and Kalakkad-Mundunthurai Tiger Reserve, Kardana Coffee Estate, Megamalai, High Wavy Mountains in Tamil Nadu(extent of occurance 1,100 sq km)	montane evergreen forest and coffee/cardamom plantations	50-400 bats (decreasing)

Order	Family	Common Name	Scientific Name	IUCN status	WPA, 1972	CITES	Geographical Range	Habitat specificity	Population size
Chiroptera	Rhinolophidae	Andaman horseshoe bat	*Rhinolophus cognatus* (K. Andersen, 1906)	EN			three Andaman Islands (North Andaman, South Andaman and Narcondom Island), extent of occurance >5,000sq km and area of occupancy 11-500sq km	rainforests and mangrove forests	decreasing
		Mitred horseshoe bat	*Rhinolophus mitratus* (Blyth, 1844)	DD			Chaibassa in Jharkhand , at an elevation of 300 m	still not confirmed	unknown
	Hipposideridae	Khajuria's leaf nosed bat	*Hipposideros durgadasi* (Khajuria, 1970)	EN			Jabalpur district of Madhya Pradesh, extent of occurance >5,000sq km and area of occupancy >500 sq km, recorded from sea level to 200 m asl.	dry tropical forest	uncommon (decreasing)
		Leafletted leaf-nosed bat	*Hipposideros hypophyllus* (Kock & Bhat, 1994)	EN			Hanumanahalli and Theralli, in Kolar District, Karnataka; 500 to 570 m asl; extent of occurrence 101-5,000 km² and the area of occupancy >500 km²	granite caves in subterranean	unknown
	Vespertilionidae	Sombre bat	*Eptesicus tatei* (Ellerman & Morrison-Scott, 1951*)*	DD			Darjeeling in West Bengal	montane forests	unknown
		Peter's tube nosed bat	*Harpiola grisea* (Peters, 1872)	DD			Sairep in Mizoram and Jairipanee in Mussourie, Uttarakhand; an elevation of 1,692 m asl	thick montane forests	unknown
Primates	Cercopithecidae	Lion tailed macaque	*Macaca silenus* (Linnaeus, 1758)	EN	I	I	Western Ghats hill ranges	upper canopy of primary tropical evergreen rainforests, in monsoon forest in hilly country and in disturbed forest	less than 4,000 individuals (decreasing)
		Nilgiri black langur	*Trachypitheus johnii* (J. Fischer, 1829)	VU	I	II	Western Ghats in southwestern India	evergreen, semi-evergreen, moist deciduous forests, mountain evergreen forests and in riparian forests in lower altitudes (300 to 2,000m)	less than 20,000 individuals (decreasing)

Order	Family	Common Name	Scientific Name	IUCN status	WPA, 1972	CITES	Geographical Range	Habitat specificity	Population size
Primates	Cercopithecidae	Black-footed gray langur	*Semnopithecus hypoleucos* (Blyth, 1841)	VU		I	south-western India (Goa, Karnataka and Kerala), centred on the Western Ghats, 35,000 sq km	tropical rainforest, moist deciduous forest, sacred groves, gardens, and riparian forest, from 100 to 1,200 m in elevation	unknown
		Bonnet macaque	*Macaca radiata* (É. Geoffroy, 1812)	LC	II	II	southern tip of India up to the southern banks of Tapti River in the north, and to the Krishna River in the northeast, found up to 2,600 m.	all forest types from scrub to evergreen and deciduous forest, plantations, agricultural lands, and urban areas, also in disturbed habitats	21.5% decline in 20 years (decreasing)
		Southern plains gray langur	*Semnopithecus dussumieri* (I. Geoffroy, 1843)	LC		I	the Godavari east to Diguvamatta and south through the foothills of the Nilgiri and other hill systems to Shernelly	100-1,700 m in tropical rainforest, dry deciduous forest, sacred groves, moist deciduous forest, gardens, riparian forest, and open scrub	several hundred thousands (stable)
Carnivora	Mustelidae	Nilgiri marten	*Martes gwatkinsii* (Horsfield, 1851)	VU	II	III	Western Ghats (a wide range of elevations from 300 to 2,600 m asl)	evergreen forests and montane forest-grassland mosaics, moist deciduous forest, plantations area	thousands (stable)
	Viverridae	Malabar large spotted civet	*Viverra civettina* (Blyth, 1862)	CR	I	III	coastal district of the Western Ghats, >600 m	lowland forests, lowland swamp and riparian forests	unknown
		Brown palm civet	*Paradoxurus jerdoni* (Blanford, 1885)	LC	II	III	the Western Ghats, 500-2000 m elevation	evergreen forest and occasionally in coffee plantations, common in altitudes above 1,000 m	fairly common
Artiodactyla	Bovidae	Nilgiri tahr	*Nilgiritragus hylocrius* (Ogilby, 1838)	EN	I		limited to approximately 5% of the Western Ghats, confined to altitudes of 1,200 to 2,600 m	high elevations on cliffs, grass-covered hills, and open terrain	1,800-2,000 individuals (decreasing)
Rodentia	Sciuridae	Namdapha flying squirrel	*Biswamoyopt-erus biswasi* (Saha, 1981)	CR			Namdapha National Park (at altitudes of 100-350 m asl)	dry deciduous montane forests	rare species (decreasing)
		Western ghats squirrel	*Funambulus tristriatus* (Waterhouse, 1837)	LC			Western Ghats, at 700 to 2,100 m asl and widely distributed in the mountain ranges	tropical evergreen forest, moist deciduous forests, plantations and pasturelands; occupy tea, cardamom and coffee estates	locally common (decreasing)

Order	Family	Common Name	Scientific Name	IUCN status	WPA, 1972	CITES	Geographical Range	Habitat specificity	Population size
	Sciuridae	Malabar gaint squirrel	*Ratufa indica* (Erxleben, 1777)	LC	II	II	southwestern, central and eastern peninsular India specifically in the Western Ghats, Satpuras and Eastern Ghats, at elevations of 180 to 2,300 m asl	tropical evergreen, semievergreen and moist deciduous forests	fairly to locally common (decreasing)
	Cricetidae	Royle's high mountain vole	*Alticola roylei* (Gray, 1842)	NT	V (vermin)		western Himalayas from Kulu Valley in Himachal Pradesh to Kumaon in Uttarakhand; from 2,500 to 4,300 m asl.	subtropical and temperate montane rocky areas (including cliffs and peaks), extending from the coniferous treeline to the snowline	little information (decreasing)
Rodentia	Muridae	Elvira rat	*Cremnomys elvira* (Ellerman, 1946)	CR	V (vermin)		Eastern Ghats of Tamil Nadu, about 600 m asl.	tropical dry deciduous scrub forest, seen in rocky areas.	no information (decreasing)
		Kondana soft-furred rat	*Millardia kondana* (Mishra & Dhanda, 1975)	CR	V (vermin)		small Singharh plateau (about one km²), near Pune in Maharashtra ; elevation of about 1,270 m asl.	tropical and subtropical dry deciduous forest and tropical scrub(bushy areas)	no information (decreasing)
		Manipur bush rat	*Hadromys humei* (Thomas, 1886)	EN	V (vermin)		northeastern India (900-1,300 m asl)	tropical evergreen, moist deciduous forests, also in secondary forests	no information (decreasing)
		Servant mouse	*Mus famulus* (Bonhote, 1898)	EN	V (vermin)		Western Ghats of Kerala and Tamil Nadu (1,540-2,400m asl)	tropical and sub tropical evergreen montane forest and shola grasslands	very rare (decreasing)
		Miller's nicobar Rat	*Rattus burrus* (Miller, 1902)	EN	V (vermin)		confined to the islands of Great Nicobar, Little Nicobar and Trinket	tropical evergreen and semi-evergreen forests	little information (decreasing)
		Nilgiri long tailed tree mouse	*Vandeleuria nilagirica* (Jerdon, 1867)	EN	V (vermin)		the Nilgiris and the Coorg Western Ghats (900-2,100m asl); 500-5,000sq km extent of occurance	evergreen montane forests and relatively undisturbed plantations	Very patchy in distribution (decreasing)
		Kerala rat	*Rattus ranjiniae* (Agrawal & Ghosal, 1969)	EN	V (vermin)		Alleppey, Thrissur and Tiruvananthapuram in Kerala (sea level up to above 1,000m asl)	arable land, waterlogged areas and inundated cultivated fields	no information (decreasing)
		Palm rat	*Rattus palmarum* (Zelebor, 1869)	VU	V (vermin)		Car Nicobar and Great Nicobar(50-150 m asl); <1,200sq km	tropical evergreen forests, mangrove areas and prefer crowns of palm trees .	no information (unknown)

Order	Family	Common Name	Scientific Name	IUCN status	WPA, 1972	CITES	Geographical Range	Habitat specificity	Population size
Rodentia	Muridae	Andaman rat	*Rattus stoicus* (Miller, 1902)	VU	V (vermin)		Henry Lawrence Island, South Andaman and Middle Andaman (sea level to 200 m); <5,000sq km	tropical evergreen forests	no information (stable)
		Malabar spiny tree mouse	*Platacanthomys lasiurus* (Blyth, 1859)	VU	V (vermin)		Western Ghats (600-2,000m asl); 2,000sq km	moist-deciduous, semi-evergreen, evergreen and shola forests and riparian forests	very few (decreasing)
		Sahyali's forest rat	*Rattus satarae* (Hinton, 1918)	VU	V (vermin)		northern Western Ghats (700-2,150m asl); <2,000sq km	pristine montane moist deciduous and evergreen forests	most common (decreasing)
		Cutch rat	*Cremnomys cutchicus* (Wroughton, 1912)	LC	V (vermin)		Andhra Pradesh, Bihar, Gujarat, Jharkhand, Karnataka and Rajasthan; >2000sq km and 150-1,500 m asl	tropical and subtropical dry deciduous and deserts, seen in thorn scrub sparse vegetation, plain grasslands, agriculture lands and rocky areas	little information (stable)
		Phillip's mouse	*Mus phillipsi* (Wroughton, 1912)	LC	V (vermin)		Andhra Pradesh, Madhya Pradesh, Maharashtra, Gujarat, Karnataka, Rajasthan and Tamil Nadu, 500 to 1,500 m asl; >2,001sq km	tropical and sub tropical thorn scrub forest, plain grassland with sparse vegetation, rocky, semi-arid, scrub,bush, dry forest patches	common (stable)
		Brown spiny mouse	*Mus platythrix* (Bennett, 1832)	LC	V (vermin)		Andhra Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra (including Pune), Rajasthan, Tamil Nadu and West Bengal; sea level up to 2,000 m asl; 2,000sq km	tropical and sub tropical dry deciduous, scrub forest	no information (stable)

Source: IUCN Red List of Threatened Species, 2015-4 and ZSI e publication, 2015

Abbreviations:

IWPA: Indian Wildlife (Protection) Act, 1972 (Schedule I, II, III, IV and V).

IUCN: International Union for Conservation of Nature.

IUCN Red list of Threatened Species: Categories (CR: Critically Endangered; EN: Endangered; VU: Vulnerable; NT: Near Threatened;

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Research Article

Immunopharmacological activity of flavonoids isolated from *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum*

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ABSTRACT

The objective of our present study is to investigate the immunosuppressive activity of variable doses of crude flavonoids (6.25-100 mg/ml; 50 µl) extracted from the leaves of *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum* on human whole blood using specific antigen (i.e. hepatitis B vaccine containing surface antigen, 20 µg/ml; 10 µl) is to estimate its blood counts, CD14 monocyte marker and observed its cytotoxicity. The results showed that the crude flavonoids extracted from these three medicinal plants showed maximum inhibition of blood counts, CD14 monocyte marker and cytotoxicity at higher doses. Overall, the data suggests that crude flavonoids extracted from these medicinal plants i.e. *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum* showed immunosuppressive activity against HBsAg.

Keywords: Flavonoid; *Mesua ferrea; Ficus benghalensis; Jasminum auriculatum*.

1. INTRODUCTION

Medicinal plants contained a wide range of bioactive compounds that are present in plant products (leaves, stem, root, seeds etc.) and used as alternative therapeutic tools for the prevention of many animal and human diseases [1, 2]. Lot of research work related to these medicinal plant products were taken and identified many valuable drugs (e.g. vincristine, vinblastine, taxol etc.) for various immunopharmacological activities e.g. anti-cancer, anti-inflammatory etc. [3-5]. As per literature, extensive research work is available for isolation and characterization of secondary metabolites especially flavonoids from medicinal plant products [6]. These flavonoids are found in higher concentration in legumes, fruits, seeds, vegetables etc. it is estimated that medicinal plant produces at least hundred thousand secondary metabolites during its growth and development [7, 8]. Out of these, more than 4000 flavonoids are being reported. In this regard, researchers focused only those flavonoids (phenolic compounds i.e. flavonones and chalcones) extracted from medicinal plant products and showed as anti-inflammatory, anti-allergic, anti-viral etc. [6-9]. Lot of flavonoids are still reported for various immunopharmacological activities e.g. antileukemic properties, vasodilators (treatment for heart diseases), anti-cancer and anti-aging properties etc. [7, 8].

Mesua ferrea (family *Guttiferae*), medicinal plant used for curing human as well as animal diseases. In India especially Assam use of this

medicinal plant as antiseptic, blood purifier and also showed its medicinal uses in different parts of the plant i.e. leaves and flowers are antidotes for snake bite and scorpion sting, roots are useful in gastritis and bronchitis etc. Moreover, this plant showed number of activities like anti-inflammatory, anti-microbial etc. [10-12].

Ficus benghalensis (family *Moraceae*), medicinal plant showed some medicinal properties e.g. leaves/fruits/bark are used as anti-cancer, anti-microbial properties and also used in the treatment of several diseases e.g. skin, vaginal disorders etc. [13-15].

Jasminum auriculatum (family *Oleaceae*) widely distributed in India, Sri Lanka and Nepal. Extensive research work on medicinal plant products of *Jasminum auriculatum* has already been done related to its immunopharmacological properties against various diseases e.g. roots for skin diseases (ringworm); flowers (aromatherapy, antiseptic, aphrodisiac etc.) and leaves (mouth ulcers) [16-18]. In recent years, further immunopharmacological studies have been carried out related to secondary metabolites especially flavonoid pertaining to explore its potential.

2. MATERIAL AND METHODS

2.1. Plant material

Leaves of medicinal plant were collected from Vidya Pratishthan's School of Biotechnology. For flavonoid extraction using fresh plant leaves samples and these samples were shade dried and prepared powdered form using mortar and pestle.

2.2. Phytochemical analysis

Preliminary studies of medicinal plant products especially leaves of *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum* were carried out pertaining to determined its secondary metabolites especially flavonoids in the aqueous leaves extract through various phytochemical analysis. After confirmation of these flavonoids in the aqueous leaves extract, our group isolated the crude flavonoids from leaves of these medicinal plants. For flavonoid extraction using fresh plant leaves (5 g) were macerated in liquid nitrogen to prepare fine leaves powder. Thereafter, reflux with 50 ml of 80% methanol along with leaves powder of three medicinal plants in different sets for 2h at 100 ^{o}C. Finally, collect the filtrate (after cooling down the solution using whatman filter paper) containing constituents of leaves powdered solution and then add ethyl acetate (10 ml) and distilled water (20 ml) in the ratio of 1:2. Incubate the solution for 4-5 h at room temperature and observed its two different layers i.e. upper layer containing ethyl acetate (evaporate it) and lower layer containing dried extracts (i.e. flavonoids) settled at the bottom [6]. For confirmation of its flavonoid content of three different medicinal plants using lead acetate test, small quantity of lead acetate solution is added and yellow precipitation appears. This yellow colour precipitation showed the presence of flavonoid.

2.3. Flow cytometric estimation on human blood samples pertaining to blood counts and CD14 monocyte surface marker

In order to determine the effect of crude flavonoid extracted from *Mesua ferrea, Ficus benghalensis* and *Jasminum auriculatum* using variable doses (6.25-100 mg; 50 µl) on human whole blood (samples received from Mangal Pathology lab, Baramati) for analysing its blood counts (lymphocytes, monocytes and granulocytes count) and estimated its CD14 monocyte surface marker using flow cytometer. For these studies, EDTA human whole blood (50 µl) was taken and then stained with or without CD14 FITC monocyte surface marker in presence of HBsAg (20 µg/ml; 10 µl) along with addition of variable concentration of crude flavonoid (6.25-100 mg; 50 µl) extracted from the leaves of *Mesua ferrea, Ficus benghalensis* and *Jasminum auriculatum.* Incubate the samples for 2-3 h at 37°C, 5% carbon dioxide incubator. After incubation, add FACS lysing solution (2-3 ml) and incubate it for 8-10 minutes. Centrifuging and washing the samples with buffer PBS (two times). Collect the pellet and dissolved in PBS. Afterwards, the samples were prepared and finally processed through FACS Calibur, flow cytometer (BD Biosciences) [19, 20].

2.4. Cytotoxicity assay

For these studies, lysed human whole blood was cultured for 24 h in presence of variable doses (6.25-100 mg; 50 µl) of crude flavonoids extracted from *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum* along with HBsAg (20 µg/ml; 10 µl) and proceeds for cytotoxicity assay. After 24 h incubation, add MTT solution (2.5 mg/ml; 10 µl) to each well of 96 well (flat bottom, tissue culture) plates. Incubate the plate for another 4 h in carbon dioxide incubator. Plates were centrifuged at 1800 rpm for 5 minutes and the supernatant was removed. Add DMSO (100 µl solution) to the formazon crystals (purple coloured, observed under the microscope) and dissolved it. Finally, absorbance was evaluated in an ELISA reader (Perkin Elmer) at 570 nm [19, 20].

2.5. Statistical analysis

To determine its statistical analysis using one-way ANOVA (Boniferroni multiple comparison) test for determine the comparison between the control and treated groups of crude flavonoids extracted from *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum.*

3. RESULTS

3.1. Total blood count and CD14 monocyte surface marker

The effect of variable doses of crude flavonoids of *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum* on total blood counts and CD14 monocyte surface marker of human whole blood using HBsAg. The results showed that there is dose dependent decline in total blood counts (Table 1) of human whole blood at higher doses as compared to control. Similarly, same response is reported in case of CD14 monocyte surface marker (Fig. 1). Over all, the data of total blood counts and CD14 monocyte surface marker at higher doses showed immunosuppressive activity.

3.2. Cytotoxicity assay

The effect of variable doses of crude flavonoids on lysed human whole blood pertaining to its cytotoxicity as shown in Fig. 2. Human lysed blood cells were cultured for 24 h in the presence of variable doses of crude flavonoid along with or without HBsAg. The results showed that there was a significant cytotoxicity at higher doses as compared to control.

4. DISCUSSION

Flavonoids may be categorized into various types i.e. aglycones (monomeric); bi or tri or oligo flavonoids or with different levels of O-methylation or with one or more saccharide units that includes various types of acyl substituents [7, 8]. Recently, extensive work on flavonoids has been done or in progress but these are normally present in crude form especially in case of medicinal plant products [7-9]. In other words, the amounts of flavonoids present in most medicinal plant products are relatively small even though the visual impression is quite striking.

Flavonoid isolated from these three medicinal plants and determined its immunobiological activity against HBsAg and showed marked decline in case of total blood counts and CD14 monocyte surface marker at higher concentrations. In this study we show that CD14 expression may be used to identify monocytes that are present in human whole blood samples. The capacity to enumerate monocytes in human whole blood as part of an existing lymphocyte immunophenotyping platform, is an important addition to the screening repertoire [19, 20]. In the present study our group focused on immunobiological activity of these flavonoids from these medicinal plants that are responsible to inhibit CD14 surface marker and total blood counts in a concentration-dependent manner. HBsAg was used as the standard and exhibited enhancement of total blood counts and CD14 monocyte surface marker when compared to the control.

In contrast, comparing the human blood profile using flow cytometry pertaining to blood counts (lymphocytes, monocytes and granulocytes) to assess immunosuppressive potential against HBsAg. From the results that are shown in Table 1; it can be concluded that human whole blood is well suited for the evaluation of its immunosuppressive activity of flavonoid extracted from three medicinal plants.

Table 1. Effect of variable doses of flavonoids on human blood counts using flow cytometry.

Plant material	Doses (mg/ml; 50 µl)	Lymphocytes	Monocytes	Granulocytes
Mesua ferrea	Control PBS	5.46 ± 0.78	3.86 ± 0.14	38.6 ± 2.18
	HBsAg, 20 µg/ml; 10 µl	11.94 ± 1.56	8.16 ± 0.52	52.14 ± 3.78
	6.25	9.18 ± 1.04	5.18 ± 0.92	46.8 ± 5.44
	12.5	8.76 ± 0.88	4.84 ± 0.78	39.4 ± 4.88
	25	7.48 ± 0.62	4.12 ± 0.34	36.2 ± 3.98
	50	6.14 ± 0.82	3.21 ± 0.18	32.2 ± 4.02
	100	4.24 ± 0.56	3.86 ± 0.14	28.12 ± 3.24
Ficus benghalensis	Control PBS	5.46 ± 0.78	3.86 ± 0.14	38.6 ± 2.18
	HBsAg, 20 µg/ml; 10 µl	11.94 ± 1.56	8.16 ± 0.52	52.14 ± 3.78
	6.25	12.24 ± 1.56	6.16 ± 0.24	38.2 ± 4.88
	12.5	10.14 ± 0.98	5.02 ± 0.58	32.18 ± 3.86
	25	7.42 ± 1.04	4.78 ± 0.82	29.6 ± 3.24
	50	6.24 ± 0.78	3.64 ± 0.76	24.6 ± 2.78
	100	5.18 ± 0.24	2.18 ± 0.18	20.18 ± 1.98
Jasminum auriculatum	Control PBS	5.46 ± 0.78	3.86 ± 0.14	38.6 ± 2.18
	HBsAg, 20 µg/ml; 10 µl	11.94 ± 1.56	8.16 ± 0.52	52.14 ± 3.78
	6.25	9.16 ± 1.46	6.88 ± 0.98	44.2 ± 5.78
	12.5	7.84 ± 1.12	6.16 ± 0.78	34.8 ± 4.84
	25	6.78 ± 0.76	5.94 ± 0.72	31.2 ± 3.16
	50	5.44 ± 0.86	4.54 ± 0.66	27.4 ± 2.88
	100	4.89 ± 0.28	3.12 ± 0.22	19.6 ± 1.88

Flow cytometric analysis of crude flavonoids (6.25-100 mg/ml; 50 µl) extracted from *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum* for determine its effect in human whole blood against HBsAg containing lymphocytes, monocytes and granulocytes count. Data acquisition of 10000 events and fraction or separation of cell populations representing different phenotypes analysed using cell quest software.

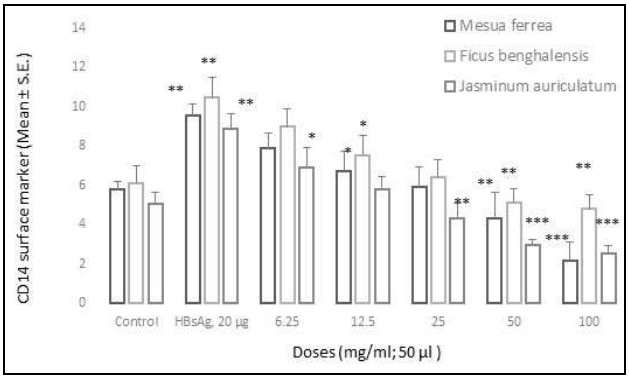

Figure 1. Effect of variable doses of flavonoid on CD14 monocyte surface marker. Lysed human whole blood were treated with variable doses of flavonoid along with HBsAg (described in materials and methods section) and then lysed and wash the cells with phosphate buffered saline and analysed through flow cytometer (FACS, Calibur) using forward and side scatter gating applied for data acquisition of 10000 events of cell populations representing different phenotypes analysed using cell quest software. *P* values: **P<0.05, **P<0.01, ***P<0.001* as compared to control.

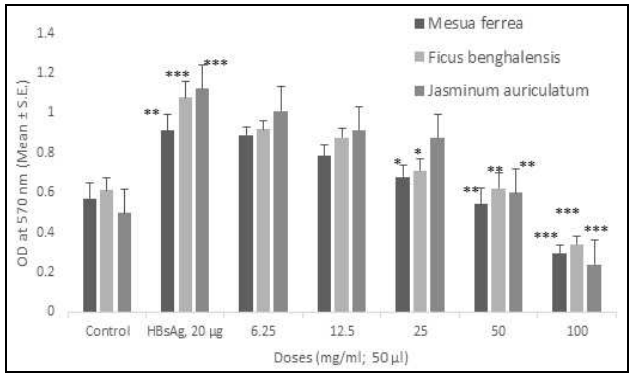

Figure 2. Cytotoxicity assay. Lysed human whole blood were cultured with HBsAg along with variable doses of flavonoid or HBsAg alone. After 24 h, proliferation was measured by MTT assay. The results are presented as Mean ± S.E. *P* values: **P < 0.05, **P < 0.01, ***P < 0.001* as compared to control.

In summary, flavonoids extracted from *Mesua ferrea, Ficus benghalensis* and *Jasminum auriculatum* were shown to suppress the release of mediators related to immunosuppression at higher doses in the form of CD14 monocyte surface marker and blood counts. The result obtained from the experiment it is concluded that flavonoids extracted from *Mesua ferrea, Ficus benghalensis* and *Jasminum auriculatum* having immunosuppressive activities. The results support the presence of biologically active components which may be worth further investigation and elucidation.

The effect of the crude flavonoid isolated from these medicinal plants at various concentrations on lysed human whole blood and was evaluated its cytotoxicity against HBsAg using MTT assay. The optical density of proliferation of lymphocytes at different concentrations were given in Fig. 2 and the results of this experiment showed that the flavonoid isolated from these medicinal plants inhibited HBsAg stimulations at higher concentrations, indicating its potential as an effective immunosuppressive compound.

5. CONCLUSIONS

These results, in assemblage with the potent inhibitory activity of crude flavonoid isolated from these medicinal plants in human whole blood against HBsAg. Further studies are necessary to isolate, characterize and elucidate the structures of these flavonoids that are present in crude form and should be responsible for eliminating several diseases.

AUTHOR'S CONTRIBUTION

This work was carried out in collaboration between two authors. AG performed the experimental work, collect the preliminary data and produced the initial draft. SRC managed the literature survey and approved the final manuscript. The final manuscript has been read and approved by both authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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Short Communication

Pilea microphylla (L.) Liebm. (Urticaceae): a naturalised taxon for the flora of Jammu and Kashmir State, India

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ABSTRACT

The present paper gives taxonomic accounts of *Pilea microphylla* (L.) Liebm., (Urticaceae), a species naturalised for Jammu and Kashmir State. Taxonomic description along with illustrations of floral parts and fruits and photo of plant and scanned copy of Herbarium sheet are provided to facilitate its identity in field.

Keywords: *Pilea microphylla*; J & K State; India.

Genus *Pilea* Lindl. is represented by over 700 species mostly confined to Tropical and subtropical region [1], 20 species has been reported from India [2] and only three species has been reported from Jammu and Kashmir including the current species under report. *Pilea microphylla* (L.) Liebm. represents a new record of a naturalised taxon for flora of Jammu and Kashmir State, India. Since the size of the plant is minute therefore, it overlooked by the plant explorers. Apart from *Pilea microphylla* (L.) Liebm., the other species of this genus that occur in this region area are *Pilea scripta* Wedd. and *P. umbrosa* Wedd. Of these species, *Pilea umbrosa* Wedd. is the most common occurring in Kashmir, reaching to the elevation of alpine zones. In Jammu region, the species extends to Batote, Kishtwar, Ramban, Chhatru, Bhaderwah and Udhampur. *Pilea microphylla* (L.) Liebm., was reported from other state of Indian republic such as Ranga Reddi District of Andhra Pradesh, India [3].

During field exploration in Jammu region, the author collected some specimens of a species inhabiting as a weed on shady and moist places of gardens and parks. The taxonomic study of the specimens indicated it to be a species of genus *Pilea*. On further investigation the specimen was confirmed as *Pilea microphylla* (L.) Liebm. with consultation of relevant literatue and comparison of the specimens.

Perusal of floristic literature such as [4-14] suggested that it has not been reported so far from Jammu and Kashmir State. However the species is known to be planted in the gardens for obtaining gun powder so this is also known as gun powder plant [15]. The present report is an occurrence of a naturalised taxon from Jammu and Kashmir. Therefore, the species is described as a new report of a naturalise taxon. The specimens are deposited in the Herbarium RRLH 23024 of CSIR-IIIM. Jammu under voucher number 20125. Taxonomic description and illustrations of floral parts (Fig. 1) and photo (Fig. 2) are given for its easy identification in the field.

During the floristic survey in Jammu and Kashmir between 2015 and 2016, the specimens of *Pilea microphylla* (L.) Liebm., were collected from shady and marshy places of Jammu and its immediate surroundings. These specimens were

pressed and mounted on the standard Herbarium sheet. The specimens ware deposited in RRRH 23024 of CSIR-IIIM (Fig. 3) for its future reference. The authenticity of the specimens was made by consultation of Kew Herbarium (K).

Pilea microphylla (L.) Liebm., Vidensk. Selsk. Skr. 5: 296. 1851; Manilal & Sivar., Fl. Calicut 279. 1982. *Parietaria microphylla* L., Syst. Nat. ed. 10, 1308. 1759. *Pilea muscosa* (L.) Lindl., Coll. Bot. t. 4. 1821.

Prostrate 2-12 cm tall creeping, succulent herb; stem slender, delicate, somewhat 4-angular, green to transparent. Leaves oblong to obovate, or elliptic, 2-5 x 1-3 mm, disticous, unequal pairs at nodes, margin entire, apex obtuse. Inflorescence in axillary cymes. Flowers minute, male flowers tepals 3-4, stamens 3-4, antitepalous, anthers bithecous; female flowers tepals 3, unequal, median one hooded partially enclosing the fruiting flowers. Achenes minute, about 1 mm long, slightly compressed; seeds white. Flowering and fruiting: June-November.

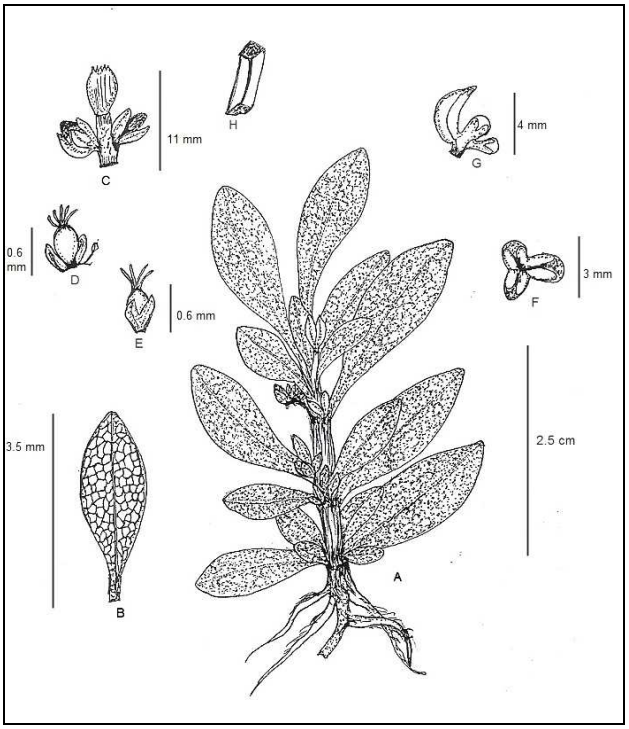

Figure 1. *Pilea microphylla* (L.) Liebm.; A - plant showing habit; B - leaf showing abaxial view; C - inflorescence; D - bisexual flower; E - pistillate flower; F - staminate flower; G - tepals of pistillate flower; H - part of stem.

Figure 2. Photograph of *Pilea microphylla* (L.) Liebm.

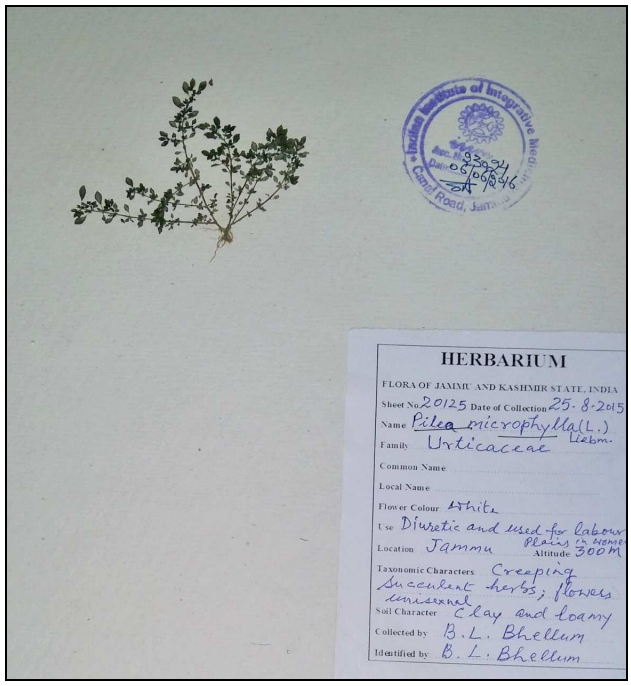

Figure 3. *Pilea microphylla* (L.) Liebm. Scanned Copy of Herbarium Sheet.

Ethno-botanical utility: Entire plant infusion is used as diuretic. The plant is used for labour pains in women. It is used for infertility and inflammation. Plant is used for urinary problems. It is also used for treatment of diarrhea through decoction of stem. The entire plant is used for antibacterial activity and for stomach and intestinal trouble. The leaves are applied to sores and bruises.

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AUTHOR'S CONTRIBUTION

Both authors have equally contribution in conducted studies and manuscript preparation. The final manuscript has been read and approved by both authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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