



Screening and evaluation of *Sonchus asper* n-hexane extract against Phenylhydrazine induced anemia

Sanghmitra S. Kamble¹, Suresh G. Killedar², Ravindra J. Jarag³, Hemalata S. Dol⁴

¹Student, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra

^{2,3}Head of Department, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra

⁴Head of Department, Late Adv. Dadasaheb Chavan Memorial Institute of Pharmacy, Malwadi, Maharashtra

ABSTRACT

Proposed work was conducted to screen and evaluate the activity of an n-Hexane extract of *Sonchus asper* against anaemia by analyzing its effects on red blood cells (RBC), Hemoglobin (Hgb) and hematocrit (HCT) level of blood in Sprague Dawley rats, which were induced anaemia using phenylhydrazine. Collection and authentication of *Sonchus asper* were done followed by extraction with Soxhlet extractor using n-Hexane as a solvent. The phytochemical investigation was carried out using a standard protocol. Anemia was induced in Sprague Dawley rats by a single Phenylhydrazine (i.p.) administration at a dose of 40mg/kg. *Sonchus asper* n-Hexane extract was administered to test group and standard marketed formulation, Folic acid Tablets (Fol*-5) was given to the standard group of animals. Evaluation of blood profile was carried out on the 14th day followed by a comparison between test, control and standard groups of animals for their red blood cells, Hemoglobin, hematocrit level of blood. The data obtained from this work suggests that *Sonchus asper* n-Hexane extract shows a significant increase ($p < 0.05$) in red blood cells, haemoglobin and hematocrit level of blood in Sprague Dawley rats. The *Sonchus asper* n-Hexane extract shows the most promising activity as compared to standard formulation. This study concludes that *Sonchus asper* n-Hexane extract shows antianemic activity hence it acts as a potent therapeutic agent against anaemia as it shows hepatoprotective potential.

Keywords— *Sonchus asper*, Antianaemic, Hematoprotective potential, Phenylhydrazine

1. INTRODUCTION

Nowadays herbal medicines are preferred by most of the people. The reasons for that are quite simple, as herbal medicines have dynamic medicinal values with fewer side effects and low cost so, it adopts a healthier method for healing various ailments which contributed to the fame of these medicines. Many disorders can be cured by using herbs and herbal products. *Sonchus asper* belonging to family-Asteraceae, Kingdom-Plantae, Order-Asterales, Tribe- Cichorieae, Genus-*Sonchus*, Species-*asper*, is an edible plant which is extremely nutritious. It is found in many countries like India, Pakistan, and South Asian countries. In North America, it is found as a common weed in open fields and roadsides. Analysis of the nutritional values of *Sonchus asper* was shown the presence of phenolic compounds, vitamin C, flavonoids, ascorbic acid and carotenoids. [1, 2] which possess an anticancer, antioxidant, anti-inflammatory properties while sesquiterpene lactones glycosides have anti-inflammatory and antioxidant activities. [2, 3, 4] It has the potential to act against various disorders. Blood disorders are those which affect single or multi-component of blood and prevent blood from doing its normal function. They can be acute or chronic in which anemia is the most common blood disorder. Many people are at risk of anemia, the reasons may be non-nutritional diet, chronic diseases, intestinal disorders, infections and other conditions. It is common in nearly 2 billion people throughout the world. Unless urgent preventive steps are taken, it will become a major health crisis. [5] These blood disorders can be controlled with herbs and herbal products. Hence herbal remedies are useful against blood disorders.

Free radical generation is a major cause of many blood disorders. Due to free radicals, oxidative stress of cells is increasing which alter the permeability of cell membrane and also cause the formation of pores which ultimately invites the blood-related disorders. [6] In previous research, the antioxidant activity of *Sonchus asper* has been proved using different in-vitro models hence we could assume that the antioxidant activity of *Sonchus asper* can be used against free radicals in blood disorders. [2, 3] Antioxidant potential of *Sonchus asper* may be used against hematotoxicity but its scientific validation is necessary hence proposed work helps to screen and evaluate the activity of *Sonchus asper* n-Hexane extract (SAHE) against blood disorder i.e., anemia. As *Sonchus asper* is grown like a weed, it is thrown away by cultivators. If its proposed medicinal values will prove, then it can be utilized by many pharmaceutical and herbal industries for formulation development which ultimately helps farmers to promote its cultivation and job creations at local and national level hence by this research, from the waste we will get a product with dynamic medicinal properties. This may contribute to various national health promotion programmes.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

n-Hexane, Sodium hydroxide, Copper sulphate, Millon's reagent, Ether, Ethanol, Sodium sulphate, Sulphuric acid, Potassium bisulphate, Pyridine, Sodium nitroprusside, Hydrochloric acid, Bromine water, Antimony trichloride were purchased from Loba Chemie Pvt. Ltd., Colaba, Mumbai -400005 Maharashtra, India.

Sudan red-III reagent, Benzene, Chloroform, Lead acetate, Nitric acid was purchased from MOLYCHEM: 78/80, Babu Genu Road, Souri Building, 1st Floor, Mumbai 400 002, India.

2.2 Collection and authentication of *Sonchus asper* Plant

The *Sonchus asper* plants were collected from R. K. Nagar region of Kolhapur, Maharashtra during their flowering in August 2017. Then plant was identified and authenticated by taxonomist Dr S. R. Yadav and herbarium was deposited in Botany department of Shivaji University, Kolhapur (Maharashtra).

The plants were dried in shadow for 10 days and milled into powder with an electric grinder and were sieved through mesh no. 80#. Then it was stored in sealed containers.

2.3 Extraction

Extraction was done by Soxhlet extractor using n- Hexane as a solvent. It is a continuous process of extraction using hot menstrum. The powder to solvent ratio was selected as 1:20 (175 g of powder for 3.5 L solvent). Here one cycle took 2 min 20sec. For the complete process of extraction, total 72 cycles were followed which took 2 hr 48 min. Percentage yield of extract was calculated after the collection and drying of extract.

2.4 Physical characterization

Physical evaluation of extract was carried out. Here organoleptic characters were studied which involves, colour, odour, taste, pH and its percentage yield.

2.5 Chemical characterization

2.5.1 Qualitative estimation

Phytochemical screening of *Sonchus asper* n-Hexane extract was carried out for alkaloid, tannin, phenolic compounds, protein, fats and oil, flavonoids, glycosides and vitamins as per the standard protocol.^[7]

2.5.2 Quantitative estimation

Estimation of iron: Acid digestion/Atomic absorption spectroscopy: 1gm of *Sonchus asper* dry extract was digested with 10ml of an equal proportion of strong perchloric acid, nitric acid and sulphuric acid for 10min. or till fumes were ceased. The mixture was filtered through Whatman filter paper no. 42 and volume were diluted to 10ml with double glass distilled water. The sample was analyzed for mineral content after sufficient dilutions by atomic absorption spectroscopy using Analytikjena, NOVAA 350. The averages of triplicate results for mineral content were reported.

2.6 Spectral characterization

UV absorption spectroscopy: UV spectra of *Sonchus asper* n-Hexane extract

The ultraviolet absorption spectrum was obtained from UV/VIS spectrometer (Jasco V-630). About 1µg/ ml solution of *Sonchus asper* n-Hexane extract was prepared in respective solvent. The sample was scanned from 200- 400 nm wavelength. Then the λ max was determined where maximum absorbance was found.

2.7 Pharmacological screening

2.7.1 Selection of animals: Sprague Dawley female rats were obtained from GLOBAL bioresearch solution PVT LTD., Gat no. 531, Nhavi, Tal: Bhor, Dist: Pune, CPCSEA Reg. No. 1899/PO/BT/S/16/CPCSEA. Rats were kept in the animal house of Bharati Vidyapeeth College of Pharmacy, Kolhapur CPCSEA Reg. No. 988/c/PO/06/CPCSEA. They were placed in polypropylene cages which were bedded with paddy husk. The temperature of the animal house was maintained at 24 ± 2° C and relative humidity was 50±8 %. The light-dark cycle of 12 hrs was followed. Rats had free access to fed (standard pellet) and purified water *ad libitum*. The whole animal study protocol was reviewed and approved by the Institutional Animal Ethics Committee (approval no. BVCPK/CPCSEA/IAEC/01/03/2017-2018).

2.7.2 Induction of anemia: Sprague Dawley rats were divided into group I, II, III, IV each with six animals. Initially, 1 ml of blood was withdrawn from retro-orbital plexus for primary monitoring of blood parameters of each group.

Group II, III, IV of rats were induced by single phenylhydrazine administration at a dose of 40 mg/kg by intraperitoneal injection.^[8, 9]

After 2 days blood was withdrawn from retro-orbital plexus of rats and evaluated for various blood parameters. The rats with anemia i.e., erythrocyte conc. and HgB level lowered than normal values were used for the experiment. (Reference ranges for blood parameters of sprague Dawley rat are, HgB: 11.5-16.1g/dl, RBC: 6.76-9.75×10⁶/µl, HCT: 36-45%.)

2.7.3 Anti-anaemic study: After induction of anemia, all groups were supplemented as follows:

Group I: Normal control rats were fed with standard pellets and purified water *ad libitum*.

Group II: Anemic control rats were fed with standard pellets and purified water *ad libitum*.

Group III: Rats have received STD folic acid tablet, Fol[®]-5 (1 mg/kg), feed (standard pellets) and purified water *ad libitum*.

Group IV: Rats were received *Sonchus asper* n-Hexane extract (350 mg/kg), standard pellets and purified water *ad libitum*.

Blood samples were drawn on 14th day of treatment from retro-orbital plexus of rats under anaesthesia using glass capillary tube. Then blood Erythrocyte level, HgB and HCT count were estimated by using automated blood cell counter, ERMA, Japan.

2.8 Statistical analysis

All Results from the study were expressed as the mean \pm standard error of the mean (SEM) and statistical analysis was carried out using GraphPad Prism 7 software. Differences among the groups were investigated using one-way analysis of variance (ANOVA). $p < 0.05$ was considered as statistically significant.

3. RESULT AND DISCUSSION

3.1 Physical characterization

It was carried out by examination of organoleptic characters which indicates, n-Hexane extract of *Sonchus asper* has an intense green colour, aromatic odour, bitter taste and alkaline pH i.e., 10. After extraction, the percentage yield of extract was calculated and this was found to be 3.66%

3.2 Chemical characterization

3.2.1 Qualitative Estimation: It was carried out by the phytochemical investigation. Table 1 indicates the qualitative determination of phytoconstituents of *Sonchus asper* n-Hexane extract which show the presence of Tannin and phenolic compounds, Fats and oil, Flavonoids, Vit A and D.

Table 1: Results of phytochemical screening of *Sonchus asper* n-Hexane extract

Test	Observation
Alkaloid determination	
Dragendroff's test	-
Hager's test	-
Wagner's test	-
Mayer's test	-
Tannin, Phenolic compounds	
Bromine water test	++
Lead acetate test	++
Dil. HNO ₃ test	++
Protein determination	
Millon's test	-
Biuret test	-
Fats and oil	
Microscopic determination	+++
Physical observation (Permanent staining of filter paper with oil)	+++
Solubility test	+++
Saponification test	+++
Flavonoids	
Shinoda test	+++
Sulphuric acid test	++
Glycosides	
Saponine glycoside: Foam test, Hemolytic test	-
Cardiac glycoside: Baljet test	-
Legal's test	-
Vitamins: A, D	+++
Vitamins: C	-

+++ = evidently present; ++ = moderately present; + = present; - = absent

3.2.2 Quantitative estimation

Iron Estimation: It was carried out by Atomic absorption spectroscopy (Analytikjena, NOVAA 350) using acid digestion method which was shown the presence of 1.644 ± 0.002 mg of Iron per 100 mg of *Sonchus asper* n-Hexane extract. Values obtained from result are expressed in terms of mean \pm SEM.

3.3 Spectral characterization

UV spectra of SAHE

The ultraviolet absorption spectra of *Sonchus asper* n-Hexane extract was obtained from UV/VIS spectrometer (Jasco V-630). About 1 μ g/ml solution of *Sonchus asper* n-Hexane extract was prepared in respective solvent. The sample was scanned from 200-400 nm wavelength. From the UV spectroscopy, λ max of the n-Hexane extract was found to be 353 nm. (Figure 1)

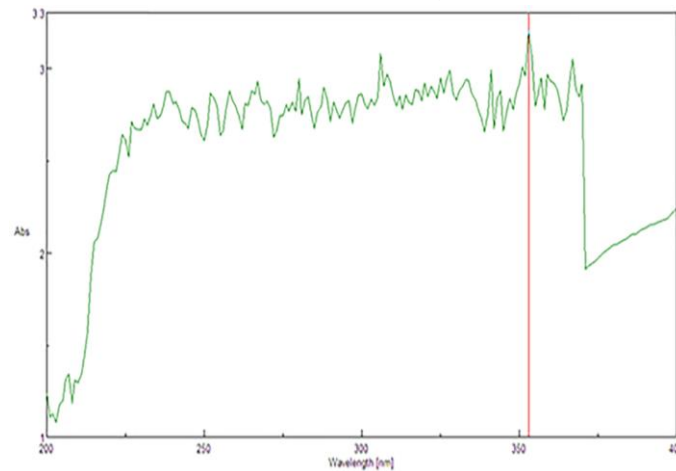


Fig. 1: UV Spectra of *Sonchus asper* n-Hexane extract

3.4 Induction of anemia

After induction of anemia using Phenylhydrazine to experimental rats, blood collection was carried out after two days of Phenylhydrazine administration. Data obtained from haematological evaluation using automated blood cell counter, ERMA was revealed that there was reduction in haemoglobin from 16.00 ± 0.034 (g/dl) to 5.00 ± 0.23 (g/dl) with Percentage reduction of 31.25%, Red blood cells count was decreased from 7.25 ± 0.26 ($10^6/\mu\text{l}$) to 2.02 ± 0.023 ($10^6/\mu\text{l}$) with percentage reduction of 27.86%, and Hematocrit level was decreased from $42.5 \pm 0.22\%$ to $26.29 \pm 1.31\%$ with percentage reduction of 61.85%, when normal control was compared with anaemic control. (Table no 2)

3.5 Evaluation of *Sonchus asper* n-Hexane extract against anemia

Evaluation of effects of *Sonchus asper* n-Hexane extract and STD folic acid tablet, Fol*-5 on haematological parameters of Sprague Dawley rats was carried out by the animal study (*in-vivo*). Data (Table 2) revealed that after administration of *Sonchus asper* n-Hexane extract (350mg/kg) daily for 2 weeks to group-IV was significantly increased the Hemoglobin level from 5.00 ± 0.23 (g/dl) to 13.00 ± 0.23 (g/dl), RBC level was significantly increased from 2.02 ± 0.023 ($10^6/\mu\text{l}$) to 7.00 ± 0.048 ($10^6/\mu\text{l}$) and Hematocrit level was significantly increased from $26.29 \pm 1.31\%$ to $36.06 \pm 0.93\%$. The increase is statistically significant ($p < 0.05$). After administration of standard Folic acid tablet, Fol*-5 (1mg/kg) daily for 2 weeks to group-III was significantly increased the Hemoglobin level from 5.00 ± 0.23 (g/dl) to 9.60 ± 0.22 (g/dl), RBC level was significantly increased from 2.02 ± 0.023 ($10^6/\mu\text{l}$) to 5.60 ± 0.20 ($10^6/\mu\text{l}$) and Hematocrit level was significantly increased from $26.29 \pm 1.31\%$ to $30.00 \pm 1.57\%$. The increase is statistically significant ($p < 0.05$). Table 2 indicates the haematological findings of HgB concentration, RBC count and percentage hematocrit after treatment with SAHE and STD (Figure 2), (Figure 3).

Table 2: Hematological findings of haemoglobin concentration, red blood cells count and percentage hematocrit level

Parameters	Group-I (Normal control)	Group-II (Anaemic control)	Group-III STD (Folic acid)	Group-IV Test (SAHE)
HgB (g/dl)	16.00 ± 0.034	5.00 ± 0.23	9.60 ± 0.22	13.00 ± 0.23
RBC($10^6/\mu\text{l}$)	7.25 ± 0.26	2.02 ± 0.023	5.60 ± 0.20	7.00 ± 0.048
HCT (%)	42.5 ± 0.22	26.29 ± 1.31	30.00 ± 1.57	36.06 ± 0.93

All Values are expressed in terms of mean \pm SEM n= (6)., the increase is significant ($p < 0.05$) when Group-III and Group-IV were compared with Group II.

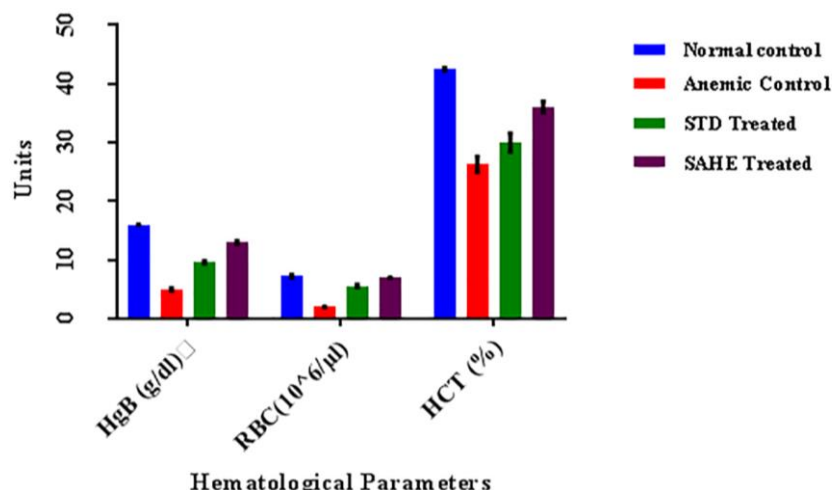


Fig. 2: Effect of SAHE on HgB, RBC, HCT level of blood

(SAHE= *Sonchus asper* n-Hexane extract; HgB=Hemoglobin; RBC=Red blood cells; HCT=Hematocrit)

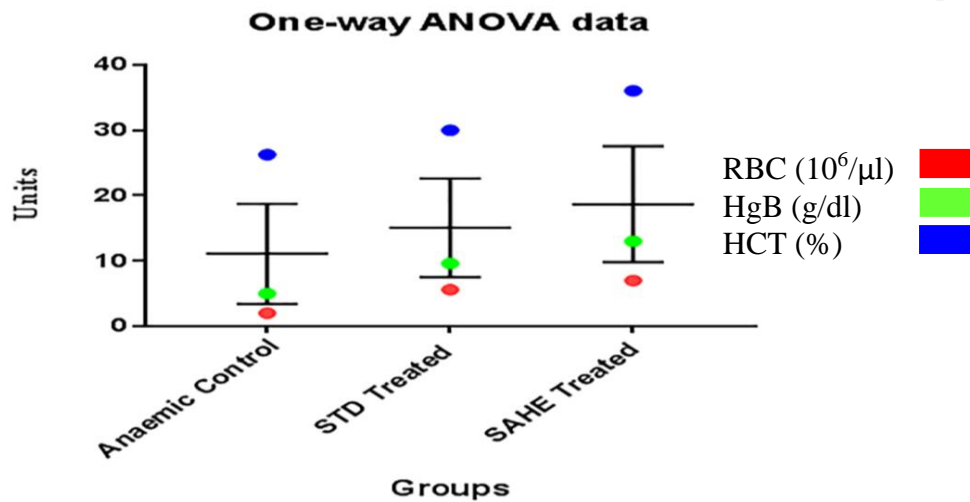


Fig. 3: Comparison between groups

(STD treated=Standard treated; SAHE treated=*Sonchus asper* n-Hexane extract treated; HgB=Hemoglobin; RBC=Red blood cells; HCT=Hematocrit)

3.6 Comparison between STD and test

Treatment using standard Folic acid tablet (Fol*-5) and Test-*Sonchus asper* n-Hexane extract (SAHE) was compared on the basis of their respective percentage recovery.

Results obtained indicates that the treatment using standard shown percentage recovery of 41.81%, 68.45% and 22.88% for haemoglobin, red blood cells, and hematocrit level respectively. While treatment using *Sonchus asper* n-Hexane extract(SAHE) shown percentage recovery of 72.72%, 95.21% and 60.27% for haemoglobin, red blood cells, and hematocrit level respectively. (Table 3) Both were shown recovery from anemia but *Sonchus asper* n-Hexane extract was shown greater recovery than standard Folic acid Tablets (Fol*-5) formulation. (Figure 4)

Table 3: Percentage recovery

Parameters	Recovery of STD (%)	Recovery of SAHE (%)
HgB (g/dl)	41.81	72.72
RBC (10 ⁶ /μl)	68.45	95.21
HCT (%)	22.88	60.27

STD=Standard; SAHE= *Sonchus asper* n-Hexane extract

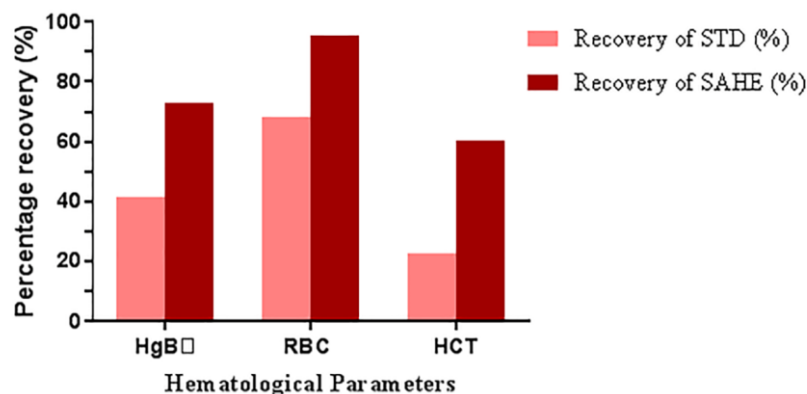


Fig. 4: Percentage recovery

(STD=Standard; SAHE=*Sonchus asper* n-Hexane extract)

4. DISCUSSION

This study was designed to investigate the effects of *Sonchus asper* n-Hexane extract on haematological parameters of Phenylhydrazine induced-anemic Sprague Dawley rats. Previous literature has been reported that *Sonchus asper* used for wound healing, fever, diuretic, sedative, antiseptic, heart diseases, scabies, kidney inflammation, asthma, constipation etc., but its action on blood disorder has not been reported so, present work was undertaken to evaluate the effect of *Sonchus asper* on anemia. Anemia is a medical condition where the haemoglobin and red blood cell's count is decreases than the normal. It is caused due to a decrease in production of RBC or haemoglobin, destruction of RBC or an increase in its loss. It is most common blood disorder which is observed in nearly 2 billion people throughout the world. In India, 70% of pregnant women and children and 40% of males get affected with anemia which is a major health crisis. Free radical generation is one of the leading cause of many blood disorders. Due to free radicals, oxidative stress of cells is increasing which alter the cell membrane permeability and also cause the formation of pores which ultimately invites the blood-related disorders. As in case of hydrogen peroxide free radical, peroxidation of lipid membrane of cells occurs due to excess generation of hydrogen peroxide which alter the permeability of cell membrane, formation of pores and also leads to increase the exposure of phosphatidylserine to outer cell surface so, cell recognize and engulfed by macrophages with phosphatidyl serine-specific receptors which finally causes degradation of cells [6]. Likewise,

phenylhydrazine induces hemolytic anemia by the formation of reactive oxygen species.^[9] Phenyl rings of phenylhydrazine have Para substitution which is accountable for its nucleophilic character so it generates free radicals during the oxidation process. When phenylhydrazine enters into bloodstream it causes oxidative alterations of various blood cell proteins which leads to hemolysis which initiates the events like premature ageing of erythrocytes which causes lack of hemoglobin and circulating erythrocytes. It ultimately leads to hemolytic injury of cells due to oxidative stress and as in previous study the antioxidant potential of *Sonchus asper* was proved^[2, 3] hence, it may contribute for its hematoprotective potential. *Sonchus asper* is a rich in phytoconstituents like antioxidants like tannin and phenolic compounds, Fats and oil, Flavonoids, vit A, vit D. It also has essential nutrients including protein, sugar and lifesaving elemental contents like calcium, potassium, phosphorus and zinc which is essential for maintaining normal health and as it is a rich source of iron (1.644± 0.002 mg of Iron per 100 mg) so it might contribute to its hematoprotective potential. But further investigation is obligatory for estimation and isolation of active compounds which shown potential against anemia. The results obtained from the study revealed that the hemoglobin, red blood cells and hematocrit level of Sprague Dawley rats were significantly increased (p<0.05) after treatment with *Sonchus asper* n-Hexane extract when group IV (test group treated with SAHE) was compared with group II (anaemic control). Group III also showed a significant increase (p<0.05) in haemoglobin, Red blood cells and hematocrit level after treatment with Folic acid Tablets, Fol*-5, when Group III (STD group treated with std marketed Folic acid Tablets, Fol*-5) was compared with group II (anaemic control). Percentage recovery was calculated for each hematological parameter. *Sonchus asper* n-Hexane extracts shown more percentage recovery as compared to standard. Hence it might have promising aspect against anemia. Many marketed synthetic hematinics causes severe side effects (e.g., stomach cramps, tightness in the chest, swelling of mouth are commonly observed side effects with folic acid supplements) therefore by using herbal medicines we can overcome it at some extent. Still, further studies are obligatory to derive its effects on humans.

5. CONCLUSION

The antianemic study was carried out by estimating hematoprotective effects of *Sonchus asper* n-Hexane extract on anemia induced Sprague Dawley rats by analyzing its haemoglobin, red blood cells and hematocrit level of blood. The data obtained from this work suggests that n-Hexane extract of *Sonchus asper* shows a significant increase (p<0.05) in HgB, RBC and HCT level of blood, therefore, it might be used as an antianemic. The n-Hexane extract of *Sonchus asper* shows the most promising activity than standard (Folic acid Tablets, Fol*-5), as it contains flavonoids, phenols and various fat-soluble vitamins i.e., vit A, vit D, that is used as an antioxidant against haemolytic anemia, which is here caused due to phenylhydrazine induced oxidative stress by generation of free radicals so antioxidant potential of *Sonchus asper* has been used against hematotoxicity and as it is a rich source of iron it also contributed in recovery of anemia. Hence from this study, we conclude that *Sonchus asper* n-Hexane extract acts as a potent therapeutic agent against anemia as it shows hematoprotective potential.

6. CONFLICT OF INTEREST

The Authors declare that there is no known conflict of interests associated with this work.

7. ACKNOWLEDGEMENT

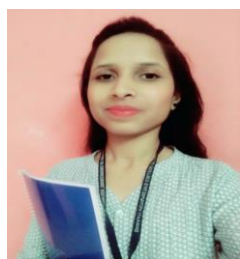
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BIOGRAPHY



Sanghmitra S. Kamble

Student, M. Pharmacy in Department of Quality Assurance
Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra



Dr. Suresh G. Killedar

Head of Department Pharmacognosy
Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra



Ravindra J. Jarag

Head of Department of Pharmacology
Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra



Hemalata S. Dol

Head of Department of Pharmaceutics
Late Adv. Dadasaheb Chavan Memorial Institute of Pharmacy, Malwadi, Maharashtra