CHAPTER

7 VALIDATION OF BATRISU VASANU IN RAT MODEL

One of the practices selected for the animal studies was herbal product namely, Batrisu vasanu. A commonly practiced galactagogue cum nutraceutical polyherbal mixture in Gujarat.

7.1 SAMPLE CHARACTERISTIC FOR COLLECTED PRODUCTS OF BATRISU VASANU

While surveying the marketed Batrisu vasanu products, total 16 products were collected and are labelled henceforth in the study as BV01 to BV16.

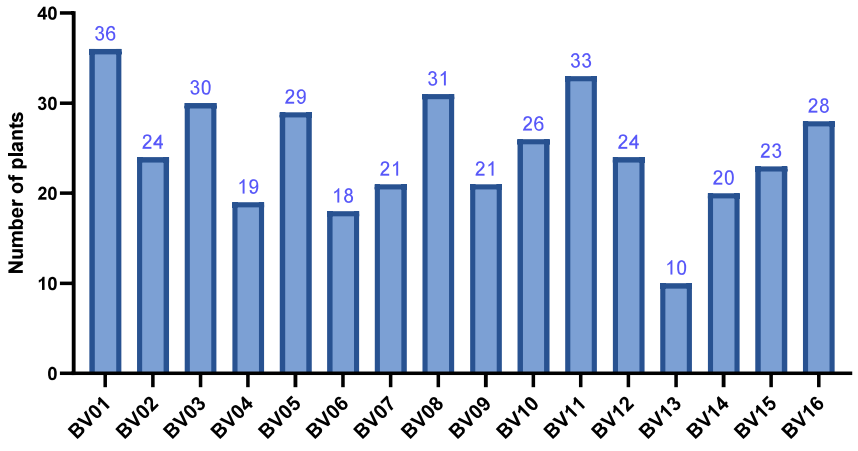


FIGURE 6. BAR GRAPH SHOWING NUMBER OF INGREDIENT PLANTS IN EACH BATRISU VASANU (BV) SAMPLES. DIGITS ON EACH BAR REPRESENTS THE NUMBER OF PLANTS PRESENT IN SAMPLE.

Names of the herbal ingredients were found written either in local language (Gujarati), English or botanical name. the number of herbs per sample in BV01 was highest and BV13 was the lowest as shown in Figure 6. There was a minimum of 10 herbs to a maximum of 36 herbs with range of 26 herbs per sample. Among the samples collected, the average number of herbs added as ingredients was 24.5±6.33.

After botanical validation of local and traditional names of the herbs, a total of 69 medicinally important herbs were listed from these products. It is important to understand the sample wise distribution of each of these 69 herbs. The information for all 69 herbs in BV samples is coded as present (1) or absent (0) in the Table 15.

7.2 BOTANICAL VARIABILITY IN COLLECTED PRODUCTS OF BATRISU VASANU

To determine the botanical ingredient variability in the samples collected, statistical analysis was performed. For collected samples, a statistical test was performed to find the average number of herbs shared (N_h) among. It was found that all products shared an average of 12.80 ± 4.62 medicinal herbs in common.

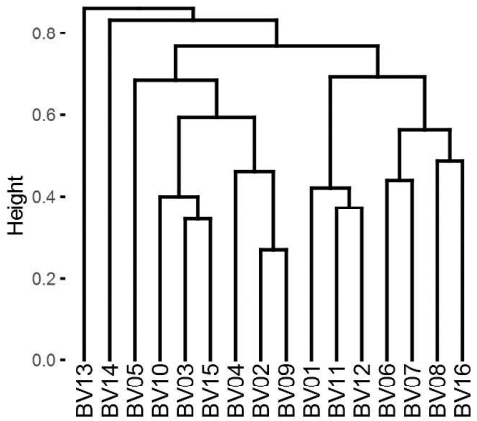


FIGURE 7. CLUSTER DENDROGRAM USING JACCARD COEFFICIENT DEPICTING THE SIMILARITY OF EACH BATRISU VASANU (BV) SAMPLES.

Then to test the similarity in ingredients between each pair of samples, a hierarchical cluster dendrogram was prepared as shown in Figure 7. It shows two large clusters with a couple of sub-clusters in each of them. There are 5 closely similar pairs in terms of herbs they share.

Jaccard coefficient of 0.79 between BV02 and BV09 shows maximum similarity, and 0.13 between BV08 and BV13 shows minimum similarity. Further, the average Jaccard coefficient for all sample pairs was found to be 0.35 (± 0.12), indicating poor similarity for ingredients among samples.

7.3 BOTANICAL DETAILS OF THE PLANTS FOUND IN BATRISU VASANU

The plants reported in the Table 16 are described with their scientific name, common name, API (Ayurvedic Pharmacopeia of India) name, Vernacular name (Gujarati language), Part used along with Relative frequency of citations (RFC^b).

As reported here in Table 16, botanically a sum of 64 species of plants belonging to 58 important Genera were found used in Batrisu vasanu. These medicinal plants belong to 38 diverse families, of which major families were Fabaceae (13.04%), Zingiberaceae (10.14%) and Piperaceae (8.69%). Of these medicinal plants, a total of 16 different parts like flower (Fl.), fruit (Fr.), heart wood (Ht. wd.), leaf (Lf.), root (Rt.), Rhizome (Rz.), seed (sd.), stem bark (St. bk.), stem (St.), tuberous root (Tub. Rt.), kernel (Kl.), floral bud (Fl. Bd.), aril, resin, gall, and gum were reported in the study.

The major plant part used in products was the seed (26.09%) followed by fruit (18.84%) and root (15.94%). The relative frequency of the herbs ranged from 0.06 being the lowest to 1.00 being the highest. Analysis further showed that there are 14.49% (n=10) herbs with ≥0.75 RFC, 13.04% (n=9) herbs with ≥0.5 RFC, 34.78% (n=24) herbs with ≥0.25 RFC and 37.68% (n=26) herbs with ≥0.00 RFC. Medicinal herbs namely Gokshura, Asvagandha, Pippali, Satavari, Maricha, and Shunthi were the most used in Batrisu vasanu product.

TABLE 15: LIST OF BATRISU VASANU SAMPLES WITH INFORMATION OF ADDED INGREDIENT PLANTS. BV01 TO BV16 ARE COLLECTED SAMPLES. 1 REPRESENTS PRESENCE AND 0 REPRESENTS ABSENCE OF THE ADDED HERB.

Sr. No.	Scientific Name	Batrisu vasanu samples															
		BV01	BV02	BV03	BV04	BV05	BV06	BV07	BV08	BV09	BV10	BV11	BV12	BV13	BV14	BV15	BV16
1	*Abutilon indicum* (L.) Sw.	1	1	1	1	1	0	0	0	1	1	1	0	1	0	1	0
2	*Acacia nilotica* L.	0	0	0	0	0	1	1	1	0	0	1	1	0	1	0	1
3	*Acorus calamus* L.	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
4	*Alpinia galanga* Willd.	1	0	1	0	0	1	0	1	0	1	1	1	0	0	0	0
5	*Amomum subulatum* Roxb.	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
6	*Anacyclus pyrethrum* DC.	1	0	1	0	0	0	0	0	0	1	1	1	0	1	1	0
7	*Anethum sowa* Roxb. ex Flem.	1	0	0	0	0	0	1	1	0	0	1	1	0	0	0	1
8	*Asparagus adscendens* Roxb.	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1
9	*Asparagus racemosus* Willd.	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
10	*Asteracantha longifolia* Nees.	1	1	1	1	1	0	0	0	1	0	0	0	0	0	1	0
11	*Bambusa bambos* Druce.	0	1	1	1	0	0	0	1	1	1	0	0	0	0	1	0

12	*Buchnania lanzan* Spreng.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
13	*Butea monosperma* (Lam) Kuntze	0	1	0	0	1	0	0	0	1	1	0	0	0	0	1	0
14	*Careya arborea* Roxb.	1	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0
15	*Cassia absus* L.	1	0	0	0	1	0	0	0	0	0	1	0	1	0	0	1
16	*Cinnamomum tamala* (Buch. Ham.) Nees & Eberm.	1	0	0	0	0	0	1	0	1	0	1	1	0	0	0	1
17	*Cinnamomum zeylanicum* Blume.	1	1	1	1	1	0	0	0	0	0	1	1	0	0	0	1
18	*Corchorus depressus* L.	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1
19	*Coriandrum sativum* L.	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1
20	*Curculigo orchioides* Gaertn.	1	0	1	0	0	1	1	1	0	0	1	1	0	0	0	1
21	*Curcuma anguistifolia* Roxb.	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
22	*Curcuma longa* L.	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1
23	*Cydonia oblonga* Mill.	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
24	*Dactylorhiza hatagirea* (D. Doon) Soo	0	1	1	0	1	0	0	0	0	1	0	0	0	0	1	0
25	*Eletteria cardamomum* (L.) Maton	1	1	1	0	0	0	0	1	1	1	1	1	0	1	1	0

26	*Embelia ribes* Burm. F.	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
27	*Foeniculum vulgare* Mill	1	0	0	0	1	1	1	1	0	0	1	0	0	0	0	1
28	*Glycyrrhiza glabra* L.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
29	*Hedychium spicatum* Ham. ex Smith	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
30	*Illicium verum* Hook. F.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	*Indigofera glandulosa* Wendl.	1	0	0	0	1	0	1	0	0	0	0	0	1	0	0	1
32	*Ipomoea hederacea* (L.) Jacq.	1	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0
33	*Lepidium sativum* L.	0	0	0	0	1	0	1	1	0	0	1	0	0	0	0	0
34	*Mesua ferrea* L.	1	1	1	1	0	0	1	0	1	1	1	1	0	0	0	0
35	*Mucuna pruriens* Baker.	0	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1
36	*Mucuna pruriens* Baker.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
37	*Myristica fragrans* Houtt.	1	1	1	0	0	1	1	1	1	1	0	0	0	1	1	0
38	*Myristica fragrans* Houtt.	1	0	1	0	0	1	1	0	0	1	0	0	1	1	1	0
39	*Nelumbo nucifera* Gaertn.	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
40	*Papaver somniferum* L.	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	1
41	*Piper chaba* Hunter non-Blume.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

42	*Piper longum* L.	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1
43	*Piper longum* L.	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1
44	*Piper nigrum* L.	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1
45	*Piper nigrum* L.	1	1	0	1	1	0	1	1	1	0	1	1	1	0	1	1
46	*Piper retrofractum* Vahl.	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
47	*Pistachia vera* L.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
48	*Plantago ovata* Forssk.	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	1
49	*Plumbago zeylanica* L.	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
50	*Polygonatum verticillatum* (L.) All.	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0
51	*Prunus amygdalus* Batsch	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
52	*Pterocarpus marsupium* Roxb.	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0
53	*Pueraria tuberosa* DC.	1	0	1	0	0	0	0	1	0	1	1	1	0	0	0	0
54	*Quercus infectoria* Olivo	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
55	*Salmalia malabarica* (DC) Schott & Endl.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
56	*Sida cordifolia* L.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
57	*Smilax china* L.	1	0	1	0	1	0	0	0	0	0	1	1	0	0	1	1
58	*Sphaeranthus indicus* L.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1

59	*Symplocos racemosa* Roxb.	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
60	*Syzygium aromaticum* (L.) Merr. And L.M. Perry	0	1	0	0	1	0	0	1	0	1	1	0	0	1	0	1
61	*Trachyspermum ammi* (L.) Sprague ex Turril	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
62	*Tribulus terrestris* L.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
63	*Trigonella foenum-graecum* L.	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
64	*Vitex negundo* L.	0	0	1	0	1	0	0	0	0	1	0	0	0	0	1	0
65	*Vitis vinifera* L.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
66	*Withania somnifera* Dunal.	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
67	*Zanthoxylum armatum* DC.	1	1	1	1	1	0	0	0	1	1	0	0	0	1	1	0
68	*Zanthoxylum armatum* DC.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
69	*Zingiber officinale* Roxb.	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1

TABLE 16: DETAILS OF THE PLANTS REPORTED IN BATRISU VASANU SAMPLES ALONG WITH THEIR RFC[b] VALUES IN ALPHABETICAL ORDER.

Scientific name	Family	API name	Common name	Local Name	Part used	RFC[b]
Abutilon indicum (L.) Sw.	Malvaceae	Atibalaa	Country mallow	Balbij, Baladana	Sd.	0.63
Acacia nilotica L.	Mimosaceae	Babbuula	Babul	Bawal gunder	Gum	0.44
Acorus calamus L.	Araceae	Vacha	The sweet flag	Vacha, Vaj, Ghodvach	Rz.	0.19
Alpinia galanga Willd.	Zingiberaceae	Kulanjana	Greater galangal	Panjad, Kulinjan	Rz.	0.44
Amomum subulatum Roxb.	Zingiberaceae	Sthulaila	Greater or Nepal cardamom	Elacho, Moti Elchi	Sd.	0.13
Anacyclus pyrethrum DC.	Asteraceae	Akarakarabha	Pellitory	Akkalkaro, Akkalgaro	Rt.	0.44
Anethum sowa Roxb. ex Flem.	Apiaceae	Satahva	Indian dil fruit	Suva	Fr.	0.38
Asparagus adscendens Roxb.	Asparagaceae	Musali	White musli	Safed mushali	Rt.	0.75
Asparagus racemosus Willd.	Liliaceae	Satavari	Asparagus	Shatavari	Rt.	0.88
Asteracantha longifolia Nees.	Acanthaceae	Kokilaksha	Long leaved barleria	Ekharo	Sd.	0.44
Bambusa bambos Druce.	Poaceae	Tugaksiri	Bamboo manna	Vaskapoor, Vanslochan	Resin	0.44
Buchnania lanzan Spreng.	Anacardiaceae	Priyala	Cuddapah almond	Charoli	kl.	0.06

Butea monosperma (Lam) Kuntze	Fabaceae	Palasa	Butea gum	Kamarkas	Gum	0.31
Careya arborea Roxb.	Lecythidaceae	Kumbhika	Kumbi	Vapumbha, Kumbhi	Fl.	0.25
Cassia absus L.	Fabaceae	Chakshushyaa	-	Chimed	Sd.	0.31
Cinnamomum tamala (Buch. Ham.) Nees & Eberm.	Lauraceae	Tvakapatra	Indian cinnamon	Tamal patra, Tejpatra	Lf.	0.38
Cinnamomum zeylanicum Blume.	Lauraceae	Tvak	Cinnamon bark	Taj, Dalchini	St. bk.	0.50
Corchorus depressus L.	Malvaceae	Chanchuka	Bahu phali	Bahuphali	Sd.	0.25
Coriandrum sativum L.	Umbelliferae	Dhanyaka	Coriander fruit	Dhana	Fr.	0.25
Curculigo orchioides Gaertn.	Amaryllidaceae	Talamuli	Golden eye grass	Kali musli, kalirnusali	Rz.	0.50
Curcuma anguistifolia Roxb.	Zingiberaceae	Tavkshir	East Indian arrowroot	Tavkir, Tavkheer	Rt.	0.06
Curcuma longa L.	Zingiberaceae	Haridra	Turmeric	Haldar	Rz.	0.25
Cydonia oblonga Mill.	Rosaceae	Amritaphala	Quince fruit	Bihidana, Bedaana	Sd.	0.13
Dactylorhiza hatagirea (D. Doon) Soo	Orchidaceae	Hattajari	Marsh orchids	Salampanja, Punjabi salam	Rt.	0.31

Eletteria cardamomum (L.) Maton	Zingiberaceae	Sukshmaila	Cardamom	Elaichi	Fr.	0.63
Embelia ribes Burm. F.	Myrsinaceae	Vidanga	Embelia	Vavding, Vayavadang	Fr.	0.25
Foeniculum vulgare Mill	Umbelliferae	Mishreya	Fannel fruit	Variyali	Fr.	0.44
Glycyrrhiza glabra L.	Fabaceae	Yashtimadhu	Licorice	Jethimadh, Mulethi	Rt.	0.06
Hedychium spicatum Ham. ex Smith	Zingiberaceae	Shati	Spiked ginger lily	Kapurkachri, Kapurkachali	Rz.	0.06
Illicium verum Hook. F.	Magnoliaceae	Takkola	Star anise of china	Badiyaan	Fr.	0.06
Indigofera glandulosa Wendl.	Fabaceae	-	-	Vakeriyo	Sd.	0.31
Ipomoea hederacea (L.) Jacq.	Convolvulaceae	Krishna bij	ivy-leaved morning glory	Mughalai	Sd.	0.25
Lepidium sativum L.	Cruciferae	Chandrasura	Common cress	Asaliyo, Aseriya	Sd.	0.25
Mesua ferrea L.	Guttifereae	Nagakesara	Cobras saffron	Nagkesar	Fl. bd.	0.56
Mucuna pruriens Baker.	Fabaceae	Atmagupta	Cowhage	Safed kaucha	Sd.	0.81
Mucuna pruriens Baker.	Fabaceae	Atmagupta	Cowhage	Kala kaucha	Sd.	0.06
Myristica fragrans Houtt.	Myristicaceae	Jatiphala	Nutmeg	Jaiphala, Jayfar	Sd.	0.63
Myristica fragrans Houtt.	Myristicaceae	Jatipatri	Mace	Javintri	Aril	0.50

Nelumbo nucifera Gaertn.	Nymphaeaceae	Kamala	Sacred lotus	kamal gatta, kamal kakdi	Sd.	0.13
Papaver somniferum L.	Papavaraceae	Khaskhasa	Poppy seeds	Khaskhas	Sd.	0.56
Piper chaba Hunter non-Blume.	Piperaceae	Gajapippali	Java long pepper	Gajapipar	Fr.	0.06
Piper longum L.	Piperaceae	Pippali	Long pepper	lindipeepar, Pipali	Fr.	0.88
Piper longum L.	Piperaceae	Pippalimula	Piper root	Pipali mool, Ganthoda	Rt.	0.81
Piper nigrum L.	Piperaceae	Maricha	Black pepper	Kala mari	Sd.	0.88
Piper nigrum L.	Piperaceae	Maricha	Black pepper	Safed mari	Sd.	0.75
Piper retrofractum Vahl.	Piperaceae	Chavya	Cubeb	Chavaka, Chavka	St.	0.13
Pistachia vera L.	Anacardiaceae	Mukuulaka	Pistachio	Pista	Kl.	0.06
Plantago ovata Forssk.	Plantaginaceae	Snigdhajeerak	Ispaghula seed	Isabgol dana, Othamijiru	Sd.	0.25
Plumbago zeylanica L.	Plumbaginaceae	Chitraka	Lead war	Chitrak, Chitrakmula	Rt.	0.13
Polygonatum verticillatum (L.) All.	Liliaceae	Meda	Solomon's seal	Salamdana, Salam misri	Rt.	0.19
Prunus amygdalus Batsch	Rosaceae	Vaataama	Almond	Badamgir	Kl.	0.06
Pterocarpus marsupium Roxb.	Leguminoseae	Asana	Indian kino tree	Asan, Biyo	Ht. wd.	0.19

Pueraria tuberosa DC.	Fabaceae	Vidarikanda	Indian kudju	Vidarikand, Bhonykoru	Tub. rt.	0.38
Quercus infectoria Olivo	Fagaceae	Mayyaku	Oak-gall	Mayafal, Maujoophal	Gall	0.19
Salmalia malabarica (DC) Schott & Endl.	Bombacaceae	Mocarasa	Silk cotton tree	Semul musli, Shaalmali	Rt.	0.06
Sida cordifolia L.	Malvaceae	Bala	Country mallow	Bala	Rt.	0.06
Smilax china L.	Liliaceae	Madhusnuhi	China root	Chopcheenee	Tub. rt.	0.44
Sphaeranthus indicus L.	Asteraceae	Mahamundi	East indian thistle	Bodiokalara, Mundi	Lf.	0.13
Symplocos racemosa Roxb.	Symplocaceae	Lodhra	Symplocos bark	Lodhar, Lodhra	St. bk.	0.13
Syzygium aromaticum (L.) Merr. And L.M. Perry	Myrtaceae	Lavanga	Clove	Laving	Fl. bd.	0.44
Trachyspermum ammi (L.) Sprague ex Turril	Umbelliferae	Yavani	Bishop's weed	Ajwain, Ajmo	Fr.	0.19
Tribulus terrestris L.	Zygophyllaceae	Gokshura	Caltrops fruit	Gokharu	Fr.	1.00
Trigonella foenum-graecum L.	Fabaceae	Methi	Fenugreek	Methi	Sd.	0.19
Vitex negundo L.	Verbenaceae	Renuka	Five-leaved chaste tree	Nirgundi, Nagodbiya, Harenu, Renuka	fr.	0.25

Vitis vinifera L.	Vitaceae	Draksha	Raisin	Draksh	Fr.	0.06
Withania somnifera Dunal.	Solanaceae	Asvagandha	Winter cherry	Ashwagandha, Aasandh	Rt.	0.94
Zanthoxylum armatum DC.	Rutaceae	Tumburu	Winged prickly ash	Tejbal	Fr.	0.56
Zanthoxylum armatum DC.	Rutaceae	Tejohva	Winged prickly ash	Tejovati	St. bk.	0.06
Zingiber officinale Roxb.	Zingiberaceae	Shunthi	Ginger	Sunth	Rz.	0.88

[API (Ayurvedic pharmacopeia of India), flower (Fl.), fruit (Fr.), heart wood (Ht. wd.), leaf (Lf.), root (Rt.), Rhizome (Rz.), seed (sd.), stem bark (St. bk.), stem (St.), tuberous root (Tub. Rt.), kernel (Kl.), floral bud (Fl. Bd.)]

7.4 BIOCHEMICAL TESTS OF COLLECTED BATRISU VASANU PRODUCTS

As the diversity of the products in terms of herbal ingredients was large, biochemical characterization of the samples was conducted. For this purpose, total phenols, flavonoids and flavonols were assessed in samples.

As shown in Figure 8, a stacked bar graph was prepared to understand each sample better. While performing the One-way ANOVA for multiple comparisons using Tukey's post-hoc test, many of the sample pairs were found significantly different ($p \leq 0.001$). For total phenols test (mg GAE/g of extract), BV01 (77.39±7.41), BV02 (79.83±8.08), BV11 (96.06±1.74), BV12 (117.02±0.86), BV13 (78.12±9.5), and BV16 (79.9±13.89) were found to be significantly high than rest of the samples. Further, the biochemical test for total flavonoids were also performed and is represented as mg RE/ g of extract. Samples BV07 (99.49±9.56), BV08 (78.75±16.01), BV10 (58.21±6.54), and BV16 (64.35±11.59) were found to have high level of total flavonoids. Apart from flavonoid content, total flavonols was also analyzed and presented as mg RE / g of extract. It was found that BV07 (117.12±21.08), BV08 (81.39±29.77), BV13 (79.63±21.06) and BV16 (69.00±24.95) were having high total flavonol content. According to higher phenol content, representing better antioxidant property (Rice-Evans et al., 1997; Santas et al., 2008), the samples were marked in Figure 8 with # mark. These key samples were namely BV01, BV02, BV11 and BV12 Batrisu vasanu products.

Apart from Phenol, flavonoid and flavonol, it is important to validate the antioxidant property for the samples. For the purpose, three antioxidant tests were done namely DPPH radical scavenging assay IC_{50} value (μg/ml), total antioxidant capacity (mg AAE/ g), and FRAP value (μM Fe (II) / g). The results were tested using one-way ANOVA for multiple comparisons (Tukey's post-hoc test). A very significant difference was observed for all sample pairs ($p \leq 0.001$).

FIGURE 8. STACKED BAR DIAGRAM SHOWING TOTAL PHENOLS (mg GAE/ g), TOTAL FLAVONOIDS (mg RE/ g) AND TOTAL FLAVONOL (mg RE/ g) FOR ALL BATRISU VASANU (BV) SAMPLES. # REPRESENTS KEY SIGNIFICANT SAMPLES.

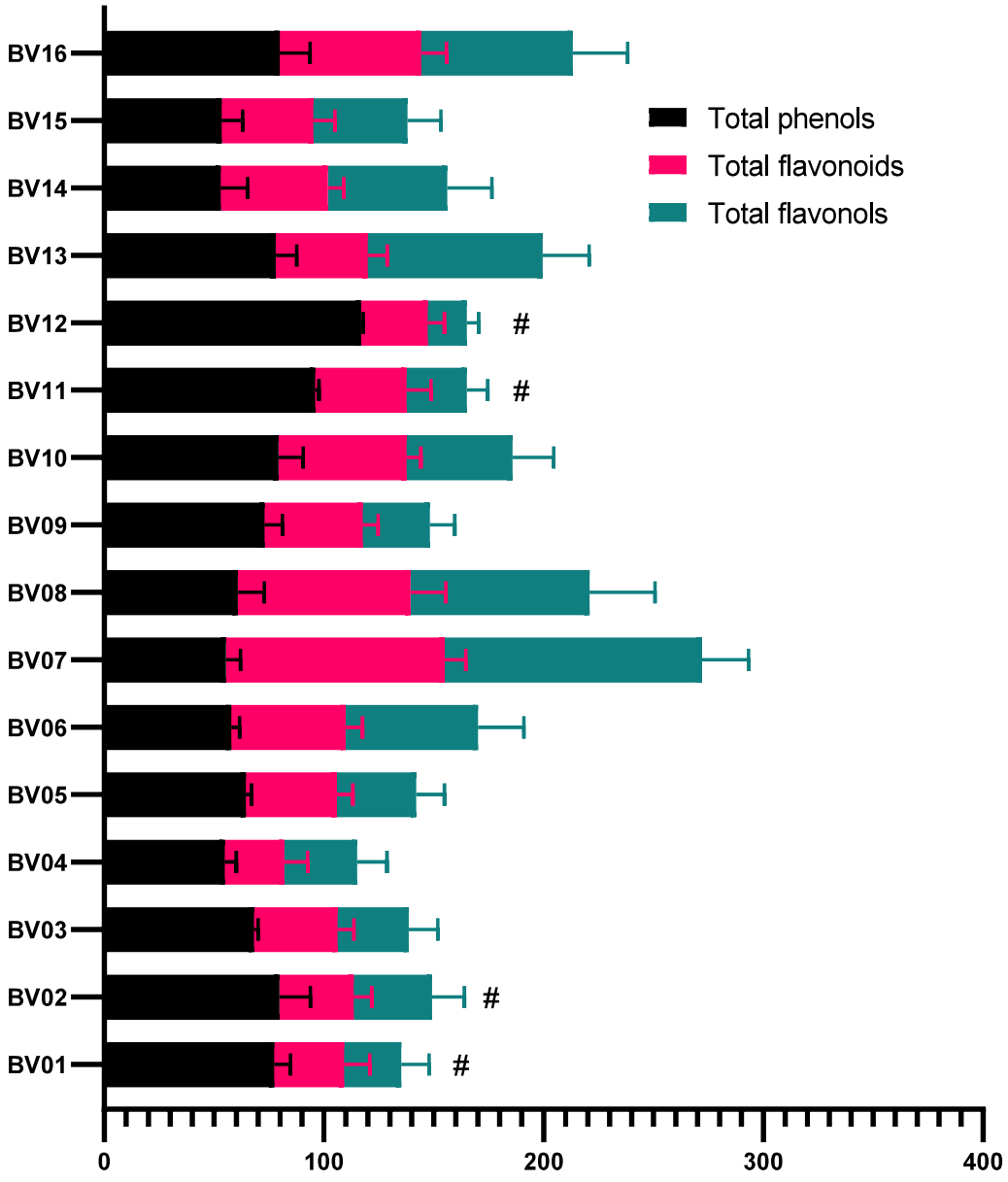


Figure 9 shows the stacked bar diagram for the DPPH radical scavenging activity IC_{50} value (μg/ml), total antioxidant capacity (mg AAE/ g), and FRAP value (μM Fe (II) / g) for all Batrisu vasanu (BV) samples. It was found that BV01 (3.65±0.07), BV03 (4.13±0.22), BV11 (3.44±0.4) and BV12 (2.48±0.18) had significantly low IC_{50} value tested as μg/ml. The lowest IC_{50} value indicates the potent antioxidant capacity of these samples.

Further, BV01 (2.14±0.13), BV02 (2.0±0.19), BV11 (2.19±0.01), BV12 (2.39±0.11) and BV13 (2.14±0.13) had significantly high total antioxidant capacity as mg AAE/g of extract tested. The data about the FRAP (Ferric ion reducing antioxidant potential) represents the capacity of the extract to convert Fe (III) to Fe (II). In FRAP assay, BV01 (443.11±4.37), BV02 (427.1±7.93), BV09 (404.33±0.33), BV10 (414.0±5.76), BV12 (617.46±9.7) and BV13 (424.6±6.11) were found to have highest antioxidant capacity expressed as μM Fe (II) / g of extract.

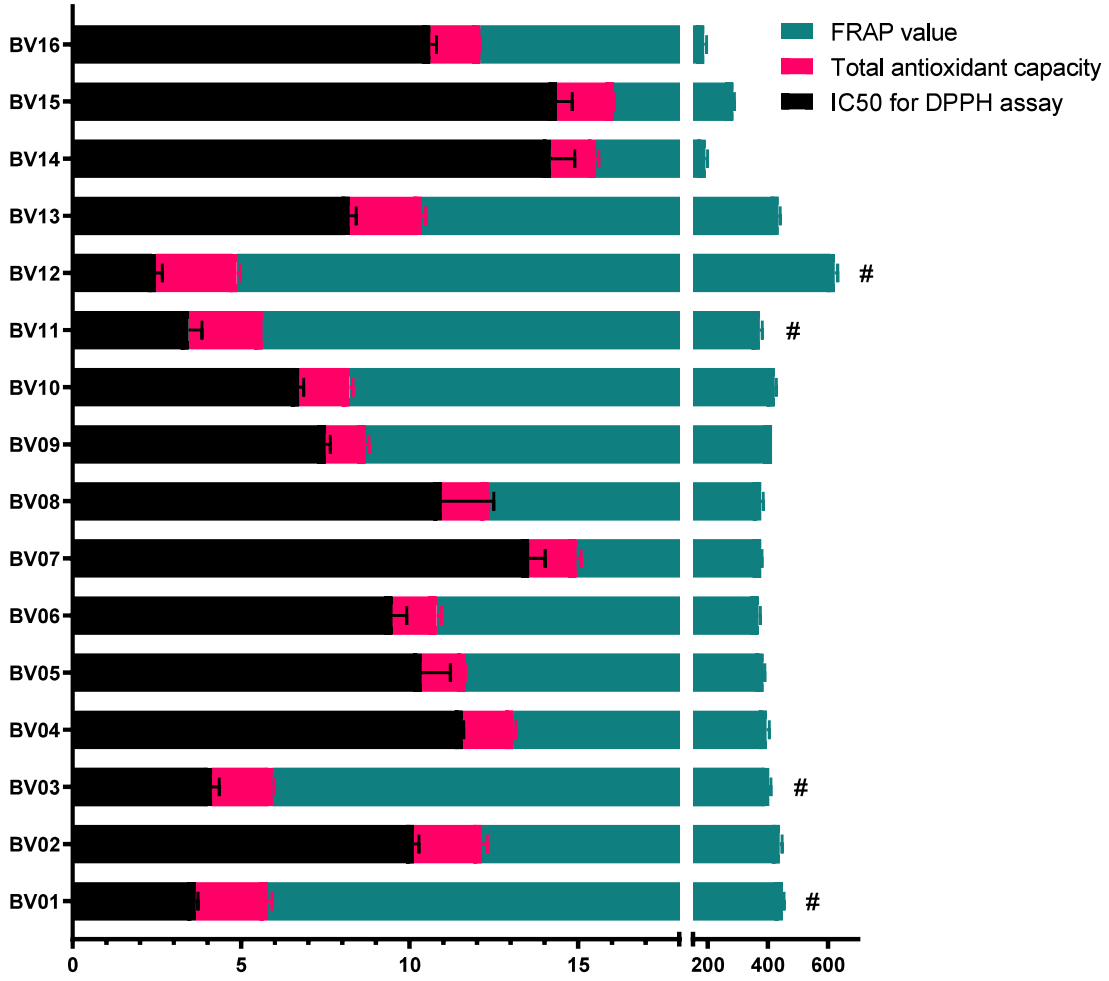
As presented in the graph (Figure 9) with #, from the three-antioxidant assay, key samples identified and shortlisted were BV01, BV03, BV11 and BV12 to show highest antioxidant capacity.

In conclusion, total phenol, flavonoid and flavonol tests presented BV01, BV02, BV11 and BV12 while antioxidant assay presented BV01, BV03, BV11 and BV12. So, it can be deduced from the data that samples BV01, BV11 and BV12 products were potent antioxidant polyherbal mixtures.

7.5 BODY WEIGHT AND FOOD EFFICIENCY RATIO IN BATRISU VASANU FED RATS

To analyze the nutraceutical effect of Batrisu vasanu, nulliparous female Wistar Rats were divided into three groups as Control, Treated 1 and Treated 2 as briefed in the methodology section. Rats in a group of four animals were dosed for three weeks and their body parameters were measured as discussed here.

FIGURE 9. STACKED BAR DIAGRAM DEPICTING DPPH RADICAL SCAVENGING ACTIVITY IC_{50} VALUE (µg/ml), TOTAL ANTIOXIDANT CAPACITY (mg AAE/ g), AND FRAP VALUE (µM Fe (II) / g) FOR ALL BATRISU VASANU (BV) SAMPLES. # REPRESENTS THE KEY SIGNIFICANT ANTIOXIDANT SAMPLES.



As presented in Table 17, Initial body weight of control group was 209.75±11.35 g) after three weeks became 229.5(15.5 g with 19.75(4.14 g) weight gain. Similarly, there was non-significant weight gain in treated 1 animal group of 15.75±4.21 g. However, for treated 2 group, there was a significant ($p \leq 0.001$) reduction in weight gain after three weeks of treatment. It was 6.0±1.5 g) reduction compared to both control and treated 1 animal groups. The same can be observed in Figure 10 (b). The trend of change in body weight from initial weight (g) to final weight (g) can be observed in Figure 10 (a). It can be noted that control group animals showed steady increase in weight over the study period, while treated 1 and 2 had maintained a flat increase.

TABLE 17: BODY WEIGHT, FOOD INTAKE AND FOOD EFFICIENCY RATIO OF RATS FED WITH CHOW DIET (CONTROL), CHOW DIET WITH BATRISU VASANU AS TREATED 1 AND TREATED 2.

Parameters	***Control***	***Treated 1***	***Treated 2***
Initial weight (g)	209.75 ± 11.35	198.5 ± 15.42	213 ± 3.24
Final weight (g)	229.5 ± 15.50	214.25 ± 19.62	207 ± 4.74
Weight gain (g)	19.75 ± 4.14	15.75 ± 4.21	- 6.0 ± 1.50
Total food intake (g)	874 ± 5.6	830 ± 7.2	847 ± 8.4
Total fecal content (g)	145.1 ± 4.2	137.1 ± 5.6	182 ± 4.2
Food efficiency ratio (%)	2.26 ± 0.5	1.89 ± 0.3	-0.71 ± 0.01

As shown in Table 17, total food intake was observed to be 874±5.6 g, 830±7.2 g and 847±8.4 g for control, treated 1 and treated 2, respectively. Additionally, total fecal content and food efficiency ratio is also displayed in the table.

Figure 10 (c) presents the Food efficiency ratio, calculated as percentage of body weight gain for total food consumed during study period. FER was significantly negative for treated 2 compared to control and treated 1 ($p \leq 0.001$). The said results can be equated to decreased body weight over treatment period but steady food consumption. Total fecal material (g) was also found to be significantly ($p \leq 0.001$) increased in treated 2 compared to control and treated 1 group as shown in Figure 10 (d).

For each group, food and water intake were monitored daily as described in the methods section. The food consumption trend by each group is presented in Figure 11 (a). It can be observed that food consumption (g) was found to have non-significant changes throughout the study period. Food consumption was same in all groups of animals. Similarly, the water intake (ml) was equal among control, treated 1 and treated 2 groups as presented in Figure 11 (b).

FIGURE 10. PARAMETERS OF BODY WEIGHT, FOOD EFFICIENCY AND FECAL CONTENT IN RATS: (A) BODY WEIGHT CHANGES IN CONTROL, TREATED 1 AND TREATED 2 GROUPS OVER TREATMENT PERIOD; (B) WEIGHT GAIN IN GRAMS FOR ALL GROUPS AS FRACTION OF INITIAL WEIGHT DIVIDED BY FINAL WEIGHT; (C) FOOD EFFICIENCY RATIO (FER) CALCULATED AS WEIGHT GAINED BY THE TOTAL FOOD CONSUMED OVER THE TREATMENT PERIOD; (D) AVERAGE FECAL WEIGHT (G) PRODUCED PER GROUPS FOR THE TREATMENT PERIOD. DATA ARE REPRESENTED AS MEAN±SEM AND $p \leq 0.05$ WAS CONSIDERED SIGNIFICANT.

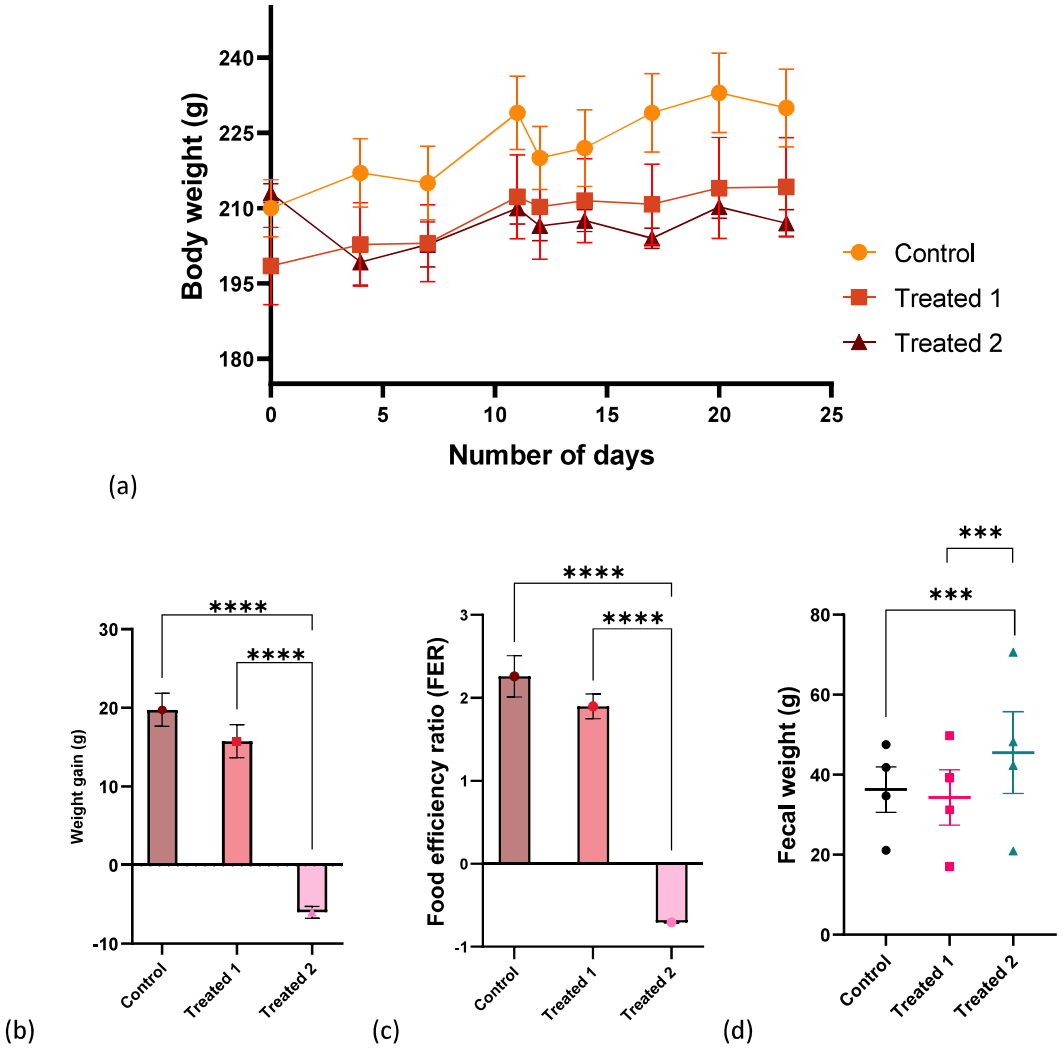
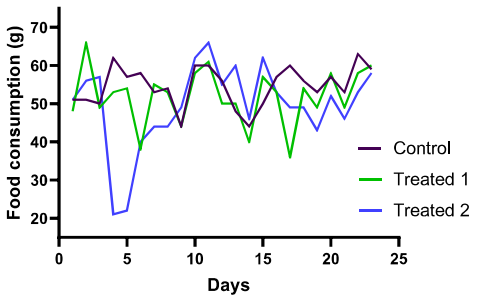
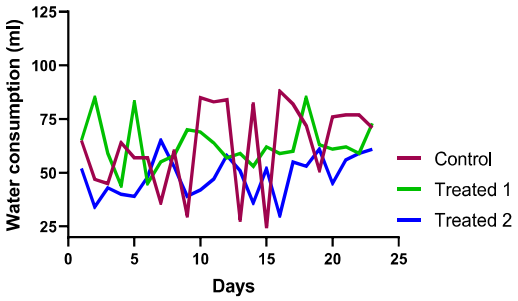


FIGURE 11. CONSUMPTION PATTERN OF FOOD AND WATER IN RATS AFTER DOSING WITH BATRISU VASANU. (A) FOOD PELLET CONSUMED IN GRAMS PER CAGE FOR TREATMENT PERIOD, (B) CONSUMPTION OF WATER IN ML PER CAGE WHILE TREATMENT PERIOD.



(a)



(b)

7.6 HEMATOLOGICAL PARAMETERS

After three weeks of treatment with Batrisu vasanu included diet, the blood was collected from all animals and were tested for hematological parameters. The parameters are listed as Mean ± SEM for each group in Table 18. Each group was statistically evaluated for the parameter using One-way ANOVA with Tukey's post-hoc test for multiple comparisons. Significance level is presented with alphabets in table if p-value was ≤0.05.

As shown in Table 18, Hemoglobin (g/dL) for each group was tested and found non-significant. Additionally, Red blood cells (10^6/μl) was also found to be non-significant for control, treated 1 and treated 2 groups. Mean corpuscular volume (μl), Mean corpuscular hemoglobin (g/dL) and Mean corpuscular hemoglobin concentrations (pg) were tested estimated and found non-significant among groups. Platelets count (10^3/ml) was also not changed after treatment.

However, White blood cells (per μl) was 2075±942.96 in control, 3975.0±428.9 in treated 1 and 3400.0±503.32 in treated 2. This was significantly ($p \leq 0.05$) elevated in treated 1 as well as in treated 2 compared to control. Further, lymphocytes (%) was significantly ($p \leq 0.001$) decreased in treated 1 and treated 2 compared to control (60.25±3.79). Other haematological parameters like Polymorphonuclear cells (%), Eosinophils (%), and Monocytes (%) were also compared among groups but was found non-significantly altered.

TABLE 18: EFFECT OF TREATMENTS ON HEMATOLOGICAL PARAMETERS OF RATS#.

*Parameters**	*Control*	*Treated 1*	*Treated 2*
Hb (g/dL)	12.50 ± 0.77	12.65 ± 0.21	12.85 ± 0.18
RBC (10^6/μl)	5.33 ± 0.69	5.67 ± 0.82	6.11 ± 0.41
MCV (fl)	63.60 ± 7.51	60.38 ± 3.34	65.53 ± 5.47
MCHC (g/dL)	34.68 ± 0.51	34.28 ± 0.76	33.93 ± 0.15
MCH (pg)	21.48 ± 2.92	18.90 ± 0.42	19.38 ± 0.65
Platelets (10^3/μl)	369.00 ± 30.47	386.50 ± 10.73	391.75 ± 35.78
WBC (per μl)	2075.00 ± 942.96a	3975.00 ± 428.90b	3400.00 ± 503.32b
PMN (%)	34.00 ± 3.08	54.50 ± 5.42	48.00 ± 8.72
Lymphocytes (%)	60.25 ± 3.79a	39.75 ± 5.72b	36.00 ± 5.03b
Eosinophils (%)	3.25 ± 0.48	3.25 ± 0.48	3.00 ± 0.58
Monocytes (%)	2.50 ± 0.65	2.50 ± 0.50	3.00 ± 0.58

#Values having different alphabets in the same row are significantly different (p-value≤0.05)

*Hb, Hemoglobin; RBC, Red blood cells; MCV, Mean corpuscular volume; MCHC, Mean corpuscular hemoglobin concentration; MCH, Mean corpuscular hemoglobin; WBC, White blood cells; PMN, Polymorphonuclear cells

7.7 SEROLOGICAL PARAMETERS

Rat serum was tested for various parameters including liver function and hormones as described in methodology. The results were tabulated as shown in Table 19. Each parameter was statistically evaluated using One-way ANOVA with Tukey's post-hoc test for multiple comparisons. Alphabets were used in table to represent statistical significance if p value was ≤0.05.

Total protein (g/dL), Albumin (g/dL) and globulin (mg/dL) concentrations were found non-significantly change as shown in Table 19. Further, Creatinine (mg/dL) and Bilirubin (mg/dL) in blood was also recorded as no change (non-significant). Liver function enzymes Aspartate aminotransferase (AST, U/L) and Alanine aminotransferase (ALT, U/L) were statistically non-significant.

While studying the lipid profile of the animals, parameters like total cholesterol (mg/dL), triglycerides (mg/dL), high density lipoprotein (HDL, mg/dL), low density lipoprotein (LDL, mg/dL) and very low-density lipoproteins (VLDL, mg/dL) were studied. Total cholesterol in treated 1 (80.75±6.02) and treated 2 (91.25±6.01) was significantly ($p \leq 0.05$) low compared with control (115.25±7.4). Similarly, triglycerides in treated 1 (191.75±28.77) and treated 2 (224.0±38.7) was also significantly lower than control group (421.0±124.8). In line of the altered lipid parameters, HDL (mg/dL) was found elevated in treatment groups (54.25±2.5 in treated 1, and 56.2±5.12 in treated 2) compared to control (41.23±7.04). However, LDL (mg/dL) and VLDL (mg/dL) were not significantly altered.

Additionally, serum parameters like Glucose (mg/dL), Uric acid (mg/dL) and urea (mg/dL) were reported without any significant change among control and treated groups.

Reproductive hormones like Follicle-stimulating hormone (FSH, μU/L), luteinizing hormone (LH, μU/L), Prolactin (ng/ml), Progesterone (ng/ml) and Estradiol (pg/ml) were also assayed. As presented in Table 19, all reproductive hormones of female rats under study were non-significant compared to control. Additionally. thyroid function tests for Triiodothyronine (T3, ng/ml) and Thyroxine (T4, μg/dL) was also estimated as unchanged among treatment groups.

Calcium (mg/dL) level in blood was also estimated for each study groups. However, there was no significant difference between control and treated 1 or treated 2.

TABLE 19: EFFECT OF TREATMENT ON SEROLOGICAL PARAMETERS OF RATS#.

*Parameters**	*Control*	*Treated 1*	*Treated 2*
Total protein (g/dL)	7.83 ± 0.34	7.15 ± 0.46	7.32 ± 0.76
Albumin (g/dL)	2.80 ± 0.25	2.58 ± 0.40	2.83 ± 0.23
Globulin (mg/dL)	5.03 ± 0.51	4.58 ± 0.84	4.73 ± 0.84
Creatinine (mg/dL)	0.85 ± 0.27	1.12 ± 0.06	1.33 ± 0.08
Bilirubin (mg/dL)	1.32 ± 0.24	0.88 ± 0.04	1.20 ± 0.23
ALT (U/L)	47.50 ± 16.09	56.25 ± 1.93	55.25 ± 2.95
AST (U/L)	39.00 ± 11.69	47.00 ± 1.29	44.25 ± 2.43
Total cholesterol (mg/dL)	115.25 ± 7.41a	80.75 ± 6.02b	91.25 ± 6.01b
Triglycerides (mg/dL)	421.00 ± 124.82a	191.75 ± 28.77b	224.00 ± 38.74b
Glucose (mg/dL)	70.00 ± 2.94	71.25 ± 6.66	61.50 ± 9.61
HDL (mg/dL)	41.23 ± 7.04a	54.25 ± 2.50b	56.25 ± 5.12b
LDL (mg/dL)	14.20 ± 0.35	8.63 ± 0.36	7.30 ± 0.68
VLDL (mg/dL)	84.20 ± 24.96	38.35 ± 5.75	44.80 ± 7.75
Uric acid (mg/dL)	9.55 ± 5.45	10.95 ± 0.51	11.28 ± 1.22
Urea (mg/dL)	24.00 ± 5.02	27.50 ± 3.18	29.50 ± 2.25
FSH (μU/L)	0.16 ± 0.02	0.17 ± 0.02	0.17 ± 0.01
LH (μU/L)	1.35 ± 0.18	1.53 ± 0.09	1.38 ± 0.10
Prolactin (ng/ml)	0.58 ± 0.01	0.60 ± 0.04	0.51 ± 0.04
Progesterone (ng/ml)	9.43 ± 1.48	16.84 ± 6.80	10.67 ± 4.28
Estradiol (pg/ml)	43.69 ± 20.95	31.37 ± 5.61	27.98 ± 5.11
T3 (ng/ml)	0.53 ± 0.06	0.49 ± 0.01	0.52 ± 0.01
T4 (μg/dL)	3.53 ± 0.51	3.18 ± 0.21	3.20 ± 0.83
Calcium (mg/dL)	9.17 ± 0.22	9.05 ± 0.35	9.36 ± 0.29

#Values having different alphabets in the same row are significantly different (p-value≤0.05)

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low-density lipoprotein; FSH, follicle-stimulating hormone; LH, Luteinizing hormone; T3, Triiodothyronine; T4, thyroxine.