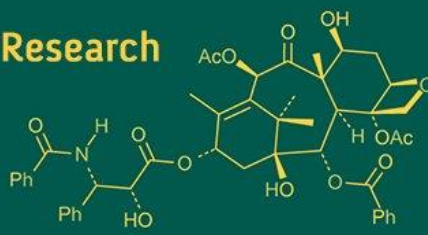


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A study on the efficiency of *Saraca asoca* leaves on serum biochemical, antioxidant and hormonal profile in postpartum anestrus buffaloes

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Abstract

The present study was evaluated on the biochemical parameters, antioxidant and hormonal profile of postpartum anestrus buffaloes treated with *Saraca asoca* leaves powder. A total of 16 postpartum anestrus buffaloes were selected and divided into two groups @ 8 buffaloes in each group. Group I (Control group) were treated with ovsynch protocol (GnRH-PGF2 α -GnRH on 0-7-9th day respectively) and Group II were fed with air dried and powdered *Saraca asoca* leaves @ 130gm orally daily for 24 days. Blood samples were collected at an interval of 0, 8, 16 and 24th days. Serum was isolated and analyzed for biochemical parameters like glucose, protein, albumin, globulin, A/G ratio, total cholesterol, and triglycerides, antioxidants viz lipid peroxidase, SOD and catalase, hormonal profile-progesterone and estrogen. The serum parameters like glucose, protein, albumin and globulin which were low showed increased levels in group II indicated that *Saraca asoca* leaves have regulated to their normal values. Triglycerides concentration has decreased while A/G ratio was non-significant. In the antioxidants profile, LPO decreased, SOD and catalase increased, in hormonal profile, progesterone and estrogen increased in group II indicated that *Saraca asoca* leaves have showed positive effect.

Keywords: Postpartum anestrus buffaloes, *Saraca asoca* leaves powder, biochemical, antioxidant, hormonal profile

Introduction

Productive and reproductive performance in buffaloes is greatly affected by postpartum anestrus condition (Kalyan *et al.*, 2021) [6]. Major cause of anestrus in buffaloes is feeding defects, unbalanced diets leading to deficiency of energy, total protein, cholesterol, triglycerides and some other metabolites. Oxidative stress is caused by an imbalance between reactive oxygen metabolites and antioxidants (Ullah *et al.*, 2019) [15] and various oxidative status markers (Saqib *et al.*, 2022) [11]. Antioxidants play a vital role in reproductive processes by modulating follicular dynamics and other critical activities. The balance between ROS and antioxidants may have an important role in reproductive processes such as follicular development (Heba *et al.*, 2014) [3]. Antioxidant enzymes like SOD acts as defense against reactive oxygen species. Post-partum buffaloes experience hormonal changes, specifically in oestradiol (E2) and progesterone (P4) levels, which affect the quality of oocytes and influence reproductive stages (Husamaldeen A *et al.*, 2023) [14]

Materials and methods:

A total of Sixteen postpartum anestrus buffaloes (45-90 days) were identified through rectal palpation twice at an interval of 10 days. Blood samples were also collected twice 10days before and 0th day for serum hormonal levels (17 β -estradiol and progesterone) estimation. On 0th day, one time blood sample was collected for biochemical, antioxidant profile. Anestrus in buffaloes was confirmed by Ultrascan machine with B-mode scanner (Aloka). The selected anestrus buffaloes were divided into two groups @ 8 buffaloes in each group. Group I (Control group) were treated with ovsynch protocol (GnRH-PGF2 α -GnRH on 0-7-9th day respectively), Group II were fed with 130 gm of *Saraca asoca* leaves powder orally for 24 days. Blood samples were collected for further analysis of various parameters.

For serum analysis, a volume of 10 ml blood sample was collected from the jugular vein of buffalo into serum vacutainer at 0, 4, 8, 12, 16, 20 and 24th day of the treatment. The blood was allowed to clot and serum was separated from blood clot by microcentrifuge at 3000 rpm for 10 min and serum samples were stored at -20 °C for further analysis using microtiter plate reader.

Serum glucose, total protein, albumin, total cholesterol and triglycerides were determined by using microtiter plate reader and globulin, A/G ratio were calculated by total protein and albumin. The serum parameters were estimated by-Serum glucose (GOD/POD method) (Trinder, 1969), total protein (Biuret method) (Gornall *et al.*, 1949 & Doumas, 1975), albumin (BCG method) (Doumas and Watson, 1971). Total cholesterol (CHOD/PAP method) (Trinder, 1969, Allain 1974 and Flegg, 1972) and triglycerides (GPO/PAP method) (Trinder, 1969, Bucolo & David, 1973 and Fossati & Prencipe, 1982). Hormonal profile included 17 β -Estradiol and Progesterone in the serum was analysed by ELISA Kit method using UV-VIS spectrophotometer. Antioxidant profile like lipid peroxidase (Stock and Dormandry, 1971), Catalase: (Sinha, 1972), and superoxide dismutase were estimated in serum sample.

Results and Discussion

Postpartum anestrus buffaloes were estimated for biochemical profile of serum glucose, total protein, albumin, globulin A/G ratio, cholesterol and triglycerides, antioxidant profile SOD, Catalase and LPO were estimated and hormonal profile for progesterone and 17 β -estradiol were estimated. The mean values of biochemical profile and antioxidant profile of postpartum anestrus buffaloes of experimental groups are given in the Table 1 and Table 2.

Table 1: Mean values of biochemical profile and antioxidant profile of postpartum anestrus buffaloes of experimental groups

Parameters	Group I	Group II
Biochemical profile		
Glucose (mg %)	56.47 $\pm$ 2.50	61.69 $\pm$ 1.71
Total Protein (g %)	5.46 $\pm$ 0.092	5.36 $\pm$ 0.117
Albumin (g %)	2.48 $\pm$ 0.037	2.60 $\pm$ 0.121
Globulin (g %)	2.98 $\pm$ 0.110	2.76 $\pm$ 0.148
A/G ratio	0.84 $\pm$ 0.044	0.97 $\pm$ 0.094
Total cholesterol (mg %)	91.36 $\pm$ 4.05	92.70 $\pm$ 6.82
estradiol Triglycerides (mg%)	46.51 $\pm$ 0.54	45.30 $\pm$ 0.36
Antioxidant profile		
Lipid peroxidase (U/ml)	4.43 $\pm$ 0.057	4.51 $\pm$ 0.009
Superoxide dismutase (U/ml)	1.88 $\pm$ 0.085	1.91 $\pm$ 0.054
Catalase (U/ml)	1.46 $\pm$ 0.42	1.48 $\pm$ 0.047

Table 2: Mean values of hormones in postpartum anestrus buffaloes of experimental groups

Parameter	Group I	Group II
Progesterone (ng/ml)		
-10 th day	0.430 $\pm$ 0.023	0.415 $\pm$ 0.028
0 th day	0.445 $\pm$ 0.029	0.405 $\pm$ 0.024
17β-estradiol (pg/ml)		
-10 th day	13.64 $\pm$ 0.45	13.01 $\pm$ 0.34
0 th day	15.27 $\pm$ 0.50	13.41 $\pm$ 0.34

The mean values pertaining to serum biochemical profile, antioxidant and hormonal profile after 24 days of treatment with Ovsynch protocol (group I) and *Saraca asoca* leaves powder (group II) were depicted in Table 3 and Table 4.

Table 3: Mean values of biochemical profile and antioxidant profile of postpartum anestrus buffaloes after 24 days treatment

Parameters	Group I	Group II
Biochemical profile		
Glucose (mg %)	49.83 $\pm$ 1.36	50.73 $\pm$ 1.75
Total Protein (g %)	6.29 $\pm$ 0.126	6.48 $\pm$ 0.156
Albumin (g %)	2.79 $\pm$ 0.043	3.11 $\pm$ 0.079
Globulin (g %)	3.49 $\pm$ 0.089	3.37 $\pm$ 0.103
A/G ratio	0.81 $\pm$ 0.015	0.94 $\pm$ 0.030
Total cholesterol (mg %)	101.94 $\pm$ 2.51	94.01 $\pm$ 2.78
Triglycerides (mg %)	38.12 $\pm$ 1.22	35.68 $\pm$ 1.60
Antioxidant profile		
Lipid peroxidase (U/ml)	4.15 $\pm$ 0.041	3.95 $\pm$ 0.063
Superoxide dismutase (U/ml)	2.28 $\pm$ 0.071	2.54 $\pm$ 0.098
Catalase (U/ml)	1.59 $\pm$ 0.024	2.15 $\pm$ 0.086

Table 4: Mean values of hormone levels of postpartum anestrus buffaloes after 24 days treatment

Period (days)	Progesterone (ng/ml)		17 β -estradiol (pg/ml)	
	Group I (Synthetic hormone)	Group II (SAL)	Group I (Synthetic hormone)	Group II (SAL)
0 th day	0.445 $\pm$ 0.03	0.431 $\pm$ 0.02	15.27 $\pm$ 0.49	14.24 $\pm$ 0.48
4 th day	0.491 $\pm$ 0.03	0.489 $\pm$ 0.02	15.71 $\pm$ 0.48	14.63 $\pm$ 0.48
8 th day	0.544 $\pm$ 0.04	0.555 $\pm$ 0.03	19.79 $\pm$ 0.29	19.66 $\pm$ 0.52
12 th day	0.824 $\pm$ 0.18	0.608 $\pm$ 0.04	24.82 $\pm$ 5.48	26.64 $\pm$ 4.17
16 th day	1.466 $\pm$ 0.42	1.863 $\pm$ 0.38	15.7 $\pm$ 2.51	14.12 $\pm$ 2.77
20 th day	2.269 $\pm$ 0.66	2.746 $\pm$ 0.65	16.00 $\pm$ 1.79	15.51 $\pm$ 2.40
24 th day	2.659 $\pm$ 0.80	3.701 $\pm$ 0.94	17.35 $\pm$ 1.73	17.97 $\pm$ 2.26
Overall Mean $\pm$ S.E	1.141 $\pm$ 0.17	1.351 $\pm$ 0.20	17.29 $\pm$ 0.91	17.05 $\pm$ 0.88

The low values of total protein, albumin in postpartum anestrus buffaloes were probably associated with deficiency of certain amino acids required for the biosynthesis of gonadotropins and gonadal hormones and consequent reproductive hormonal disturbances led to inactive ovaries. The similar observations were recorded by Emad *et al.*, 2019, Kalasariya *et al.*, 2018^[2, 5].

The values of 17 β -estradiol and progesterone were lower than normal range (Susmitha *et al.*, 2024, Husamaldeen A. Alsalm *et al.*, 2023)^[14, 4] in anestrus buffaloes. After postpartum there will be negative energy balance, deficiency of minerals and clearance of ovarian steroids (17 β -estradiol and progesterone) led to anovulation. The above findings are in agreement with the results of Kumar *et al.*, 2014; Walsh *et al.*, 2007.^[7, 16]

The mean serum glucose, total protein, albumin, A/G ratio were significantly ($p < 0.01$) higher in group II compared to group I, while globulin, total cholesterol, triglycerides were significantly ($p < 0.01$) lower in group II compared to group I. SOD and catalase showed significantly higher values and LPO showed lower levels in group II compared to group I. On 24th day in group II progesterone levels were highest ($p < 0.01$) compared to group I.

The mean serum glucose levels were significantly ($p < 0.01$) lower in group II compared to group I indicating that glucose levels were reduced by *Saraca asoca* leaves on the treatment days but glucose has increased on the day of estrous and was regulated in Group II. β -sitosterol in *Saraca asoca* leaves on oral administration, regulates glucose uptake by increasing plasma insulin level, correspondingly decreases fasting hyperglycemia, increases glucose uptake by adipocytes and down-regulates GLUT4 gene expression (Sayeed *et al.*, 2016).^[12] Decreased cholesterol and triglycerides in group II was due to β -sitosterol will reduce LDL cholesterol from blood and prevent the synthesis of cholesterol in liver (Soodabeh *et al.*, 2014).^[13] Increase in concentration of antioxidant activity in group II compared to group I was due to β -sitosterol from *Saraca asoca* leaves which increase concentrations of the antioxidants lead to an increased ability to neutralize oxidative stress (Lakshminarayanan A, 2023).^[8] The antioxidant activity of *Saraca asoca* leaves was due to the ability to scavenge free radicals and inhibit lipid peroxidation (Prathapan *et al.*, 2012).^[9] The mean serum progesterone concentration tended to rise by treating with *Saraca asoca* indicated initiation of cyclicity and formation of corpus luteum (Rajkumar *et al.*, 2008).^[10] Beta-sitosterol from *Saraca asoca* leaves possess antioxidant, lipid lowering effect, antidiabetic property, hepatoprotective, effective on reproductive disorders. (Heba S and Jayaraman, 2020).

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