Efficacy of Tulsi and Turmeric as antioxidants in combating heat stress in broilers

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Need to produce safe food

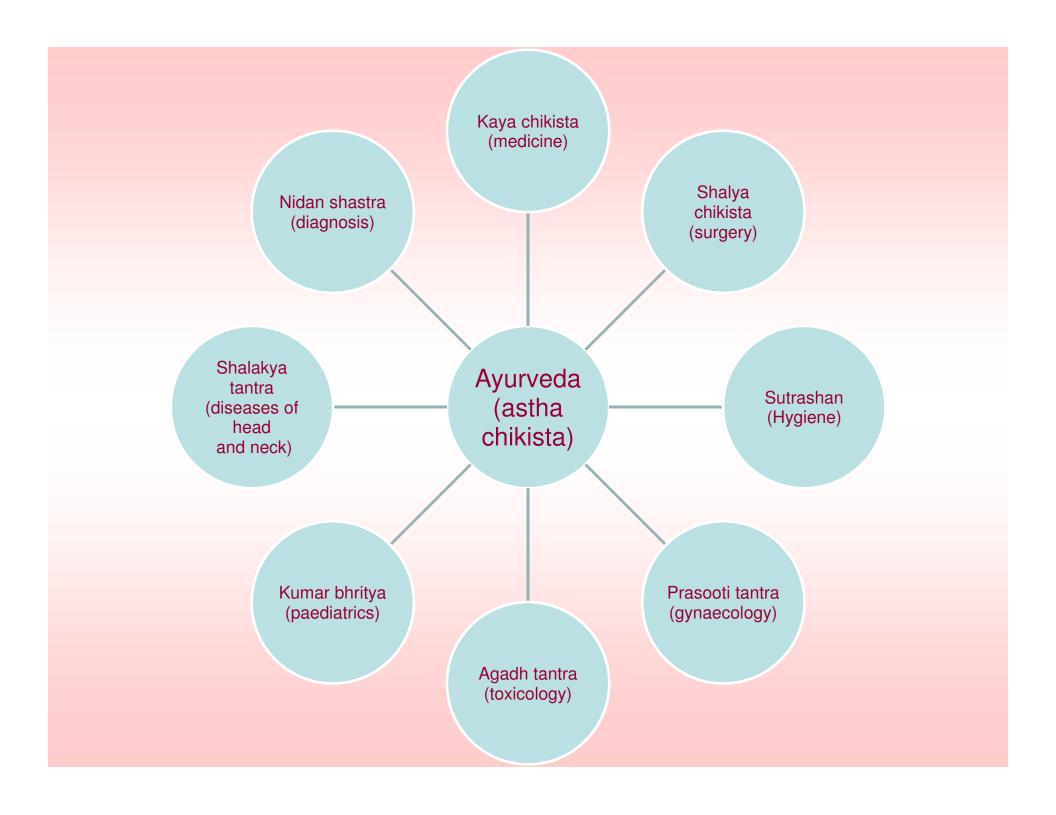
- Concern for food safety from the food born infections.
- Consumer demand for food free from chemical and antibiotic residues
- International standards are to be met to keep up the export of raw & processed foods
- To face the competition from the new entrants having claims for safety food
- Food bill 2002 by food processing industries ministry is emphasizing various standards for production and processing the safe food

phytobiotics

- These are plant derived products added to the feed in order to improve performance of livestock or for medicinal purpose.
- These may include
- Herbs (product from flowering, nonwoody and non persistent plants.
- Botanicals (entire or processed parts of a plant i.e. leaves, roots, bark)
- Essential oils (hydro distilled extracts of volatile plant compounds)
- Oleoresins (extracts based on nonaqeous solvents)

Rigveda, the oldest document of human knowledge written between 4500 and 1600 B.C mention the use of medicinal plants in the treatment of man and animals

Ayurveda dates back to 5000to 10000 years includes natural medication



Advantages

- Absence of side effects
- Absence of residual effects
- Non hazardous
- Eco-friendly
- Minimum problem of drug resistance

Limitations

- not easily quantifiable and standardized due to their complex composition
- The location, soil type, weather conditions, altitude, season during which the plant is grown, harvesting procedure & storage conditions may affect the composition of plants.
- although majority of herbals are stable, there are various constituents which are photo labile, thermo labile thus less stable

Tulsi

- Though whole plant has medicinal value, mostly leaves, sometimes seeds are used.
- Leaves contain 0.7% volatile oil comprising of 71% eugenol,20% methyl eugenol and carvacol, caryophylline and ursolic acid.
- Nair et al (1982) also isolated apigenin, luteolin, orientin, molludisin and phenolic groups such as crislineol crismartin, isothymonin, rosemarinic acid and traces of Zn, Mn & Na
- Seeds of tulsi posses the fatty oil (17.82%) consisting of 6.9% palmitic acid,2.1% stark acid,15.7% linolenic acid,66% linoleic acid 7 9% oleic acid

Pharmacological profile

- Antimicrobial and antimycotic
- Hepato protective activity
- Immunomodulatory activity -
- Hypoglycemic& hypolipedemic
- Anti ulcerogenic & anti carcinogenic
- Anti inflammatory, analgesic, antipyretic and anti diarrheal activity
- Radio protective activity
- Wound healing
- Snakebite poisoning

Turmeric (Curcuma longa)

Biological activities

- Consists of essential oils (2.4- 4%), fatty oils (3%)
- Active ingredients includes curcumin (diferulolyl methane), curcuminoids, fats, minerals, fiber, vitamins, proteins, CHO's (Bakhru.,1997)

- Anti inflammatory & anti arthritic (Chandra & gupta.,1972)
- Antioxidant (Toda et al.,1985)
- Antimicrobial (lutomski et al.,1974)
- Anti leishmanial (Gomez et al.,2002)
- Hepatoprotective (Kiso et al.,1982)
- Anticancer (Kuttan et al.,1985),
- Vasodilator(Sasaki etal.,2003)
- Hypolipedemic (Dixit et al.,1988)
- Hypoglycemic (Arun and nalini.,2002)
- Choleritic (Deters et al.,1999)
- Immunomodulatory (Antony et al.,1999)
- Neuroprotective (Rajakrishnan et al.,1999)
- Anti depressant (Yu etal.,2002)

Experimental design

- G 1: HS+ Basal diet without inclusion of any antioxidant
- G 2: HS+ vitamin E (200 mg/kg)
- G 3: HS+ vitamin E (200mg/kg)+ selenium (0.15 ppm)
- G 4: HS+ Tulsi (0.25% level)
- G 5: HS+ Tulsi (0.5% level)
- G 6: HS + Turmeric (0.2% level)
- G 7; Hs+ Turmeric (0.4% level)
- G 8: HS+ Tulsi (0.25%) +Turmeric (0.2%)
- G 9: Hs+ Tulsi (0.5%) +Turmeric (0.4%)
- Heat stress free group(Control)

Materials & Methods

- 1. Glutathione peroxidase activity was assessed as per the method of Rotruck et al(1973)
- 2. Catalase activity was determined by the method of Caliborne (1985)
- 3. Superoxide dismutase activity in the plasma was measured by the method of Marklund and Marklund (1974)
- 4. The glutathione level in plasma was measured by the method of Moron et al.(1979)

Table 1. Glutathione peroxidase activity in plasma of heat stressed broilers supplemented with various antioxidants

GSH-Px (μg /min/mg protein)				
Treatment	4 th wk	6 th wk		
Stress free control	4.05±0.032°	4.85±0.021a		
Heat stress + BD	2.47±0.021 ^m	2.63±0.252 ¹		
BD+ Vitamin E	3.26±0.021g	3.95±0.033 ^d		
BD+ Vitamin E + Se	3.74±0.024 ^e	4.25±0.021 ^b		
BD+ Turmeric(0.2%)	2.93±0.041 ^{jk}	3.21±0.021gh		
BD+ Turmeric(0.4%)	3.13±0.031 ^h	3.26±0.033g		
BD+Tulsi(0.25%)	3.030±0.053 ⁱ	3.24±0.022g		
BD+Tulsi(0.5%)	3.17±0.023gh	3.41±0.021 ^f		
BD+Tulsi(0.25%)+Turmeric (0.2%)	2. 87±0.032 ^k	2.96±0.042 ^{ij}		
BD+Tulsi(0.5%)+Turmeric(0.4%)	2.64±0.031 ¹	2.71±0.023 ¹		
Means with different superscripts for attributes differ significantly at P≤0.01				

Fig: 1 Plasma glutathione peroxidase activity-GSH-PX (µg/min/mg protein) in different group of broiler chicks

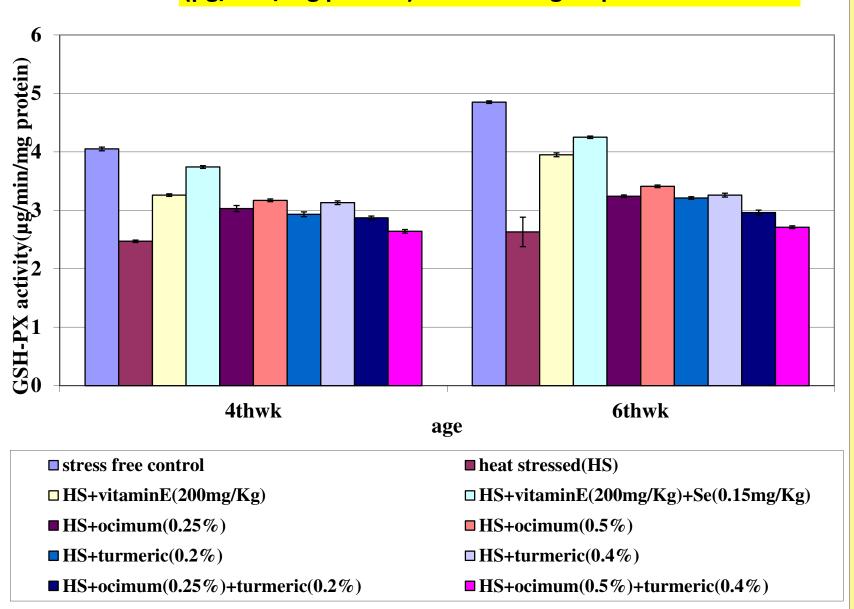


Table 2. catalase activity in plasma of heat stressed broilers supplemented with various antioxidants

Catalase (µm/min/mg protein)				
Treatment	4 th wk	6 th wk		
Stress free control	20.02±0.081g	37.48±0.021 ^a		
Heat stress + BD	16.48±0.033 ¹	26.11±0.052 ^f		
BD+ Vitamin E	19.02±0.042 ⁱ	28.56±0.023 ^d		
BD+ Vitamin E + Se	20.72±0.034 ^g	36.62±0.042a		
BD+ Turmeric(0.2%)	18.67±0.024 ⁱ	29.19±0.023 ^d		
BD+ Turmeric(0.4%)	18.68±0.012 ⁱ	30.25±0.033°		
BD+Tulsi(0.25%)	19.64±0.023 ^h	31.42±0.022°		
BD+Tulsi(0.5%)	19.81±0.021 ^h	33.37±0.024 ^b		
BD+Tulsi(0.25%)+Turmeric (0.2%)	18.32±0.013 ^j	28.01±0.051e		
BD+Tulsi(0.5%)+Turmeric(0.4%)	17.38±0.071 ^k	27.86±0.023e		

Means with different superscripts for attributes differ significantly at P≤0.01

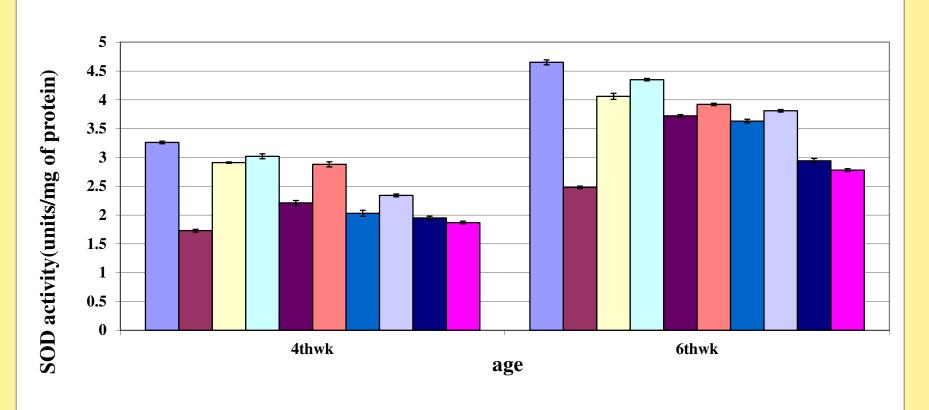
Fig: 2 Plasma catalase activity (µm/min/mg protein) in different groups of broiler chicks **40** 35 catalase activity(µm/min/mg protein) **30 25 20 15** 10 5 4thwk 6thwk age ■ stress free control ■ heat stressed(HS) □ HS+vitaminE(200mg/Kg) \square HS+vitaminE(200mg/Kg)+Se(0.15mg/Kg) \blacksquare HS+ocimum(0.25%) \blacksquare HS+ocimum(0.5%) \blacksquare HS+turmeric(0.2%) \square HS+turmeric(0.4%) \blacksquare HS+ocimum(0.25%)+turmeric(0.2%) \blacksquare HS+ocimum(0.5%)+turmeric(0.4%)

Table 3 . superoxide dismutase (SOD) activity in plasma of heat stressed broilers supplemented with various antioxidants

SOD (units/mg protein)				
Treatment	4 th wk	6 th wk		
Stress free control	3.26±0.022 ^f	4.65±0.042a		
Heat stress + BD	1.73±0.024°	2.48±0.023 ^j		
BD+ Vitamin E	2.91±0.011 ^h	4.06±0.054 ^c		
BD+ Vitamin E + Se	3.02±0.043 ^g	4.35±0.021 ^b		
BD+ Turmeric(0.2%)	2.03±0.052 ^m	3.63±0.032e		
BD+ Turmeric(0.4%)	2.34±0.025 ^k	3.81±0.022 ^d		
BD+Tulsi(0.25%)	2.21±0.043 ¹	3.72±0.023 ^e		
BD+Tulsi(0.5%)	2.88±0.044 ^h	3.92±0.021 ^d		
BD+Tulsi(0.25%)+Turmeric (0.2%)	1.95±0.031 ^{mn}	2.94±0.044gh		
BD+Tulsi(0.5%)+Turmeric(0.4%)	1.87±0.024 ^h	2.78±0.025 ⁱ		

Means with different superscripts for attributes differ significantly at P≤0.01

Fig: 3 Plasma superoxide dismutase-SOD (units/mg protein) activity in different group of broiler chicks



- stress free control
- □ HS+vitaminE(200mg/Kg)
- \blacksquare HS+ocimum(0.25%)
- \blacksquare HS+turmeric(0.2%)
- \blacksquare HS+ocimum(0.25%)+turmeric(0.2%)

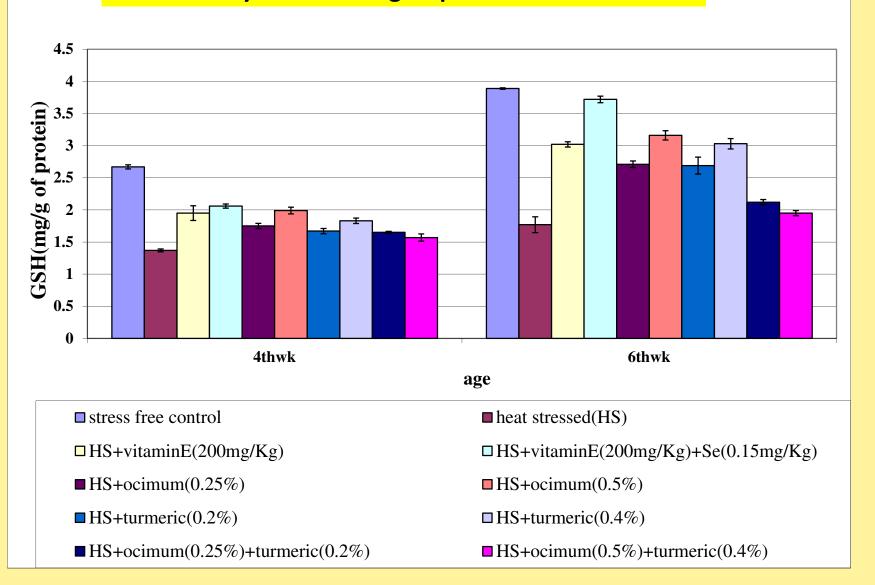
- heat stressed(HS)
- \square HS+vitaminE(200mg/Kg)+Se(0.15mg/Kg)
- \blacksquare HS+ocimum(0.5%)
- \square HS+turmeric(0.4%)
- \blacksquare HS+ocimum(0.5%)+turmeric(0.4%)

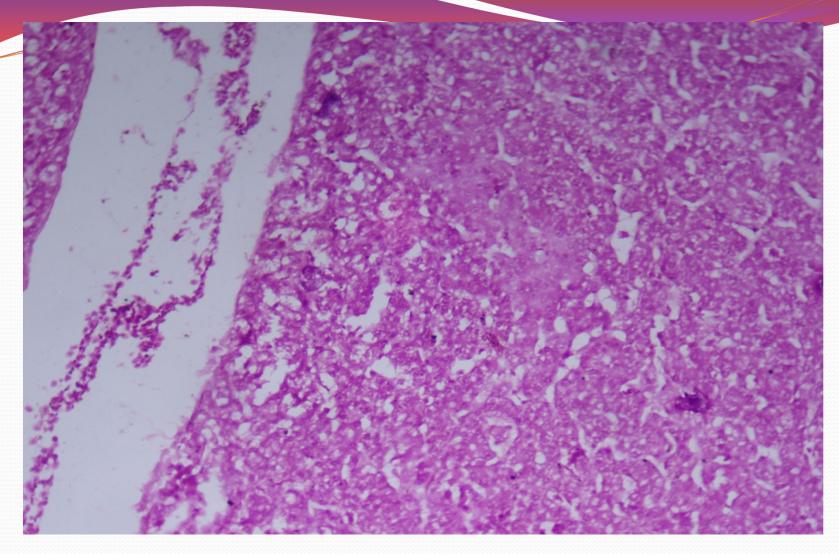
Table 4 Non enzymatic (reduced glutathione- GSH) activity (mg GSH / g of protein) in plasma of heat stressed broilers supplemented with various antioxidants

Reduced glutathione (mg GSH / g of protein)				
Treatment	4 th wk	6 th wk		
Stress free control	2.67±0.032°	3.89±0.011 ^a		
Heat stress + BD	1.37±0.023 ⁱ	1.77±0.123 ^{fgh}		
BD + Vitamin E	1.95±0.114 ^{def}	3.02±0.041 ^g		
BD+ Vitamin E+ Se	2.06±0.032 ^d	3.72±0.052 ^a		
BD+ Turmeric (0.2%)	1.67±0.042 ^{gh}	2.69±0.132 ^c		
BD+ Turmeric (0.4%)	1.83±0.043 ^{ef}	3.03±0.082 ^b		
BD+Tulsi(0.25%)	1.75±0.041 ^{fgh}	2.71±0.051e		
BD+Tulsi(0.5%)	1.99±0.053 ^{def}	3.16±0.072 ^b		
BD+Tulsi(0.25%)+Turmeric (0.2%)	1.65±0.017 ^{gh}	2.12±0.041 ^d		
BD+Tulsi(0.5%)+Turmeric(0.4%)	1.57±0.055 ^h	1.95±0.041 ^{def}		

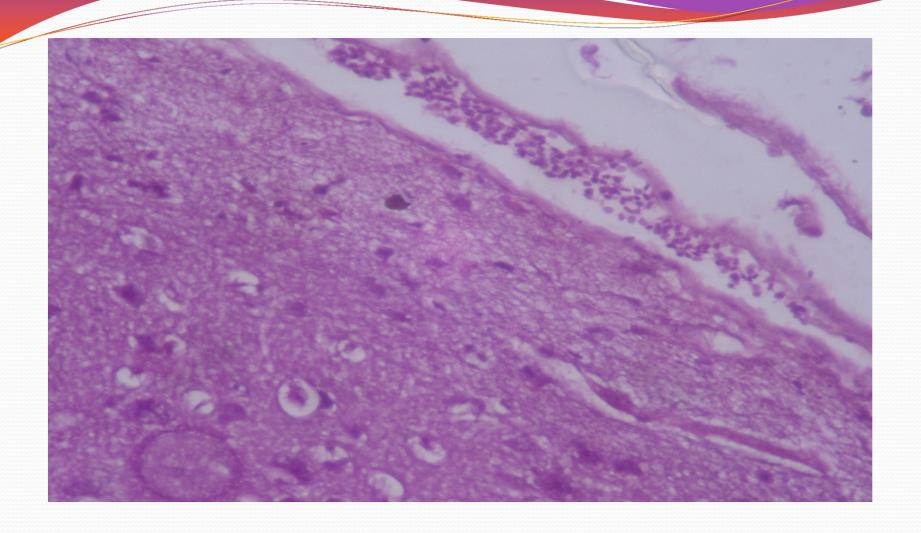
Means with different superscripts in a column differ significantly at P≤0.01

Fig: 4 Plasma reduced glutathione-GSH(mg/g protein) activity in different group of broiler chicks

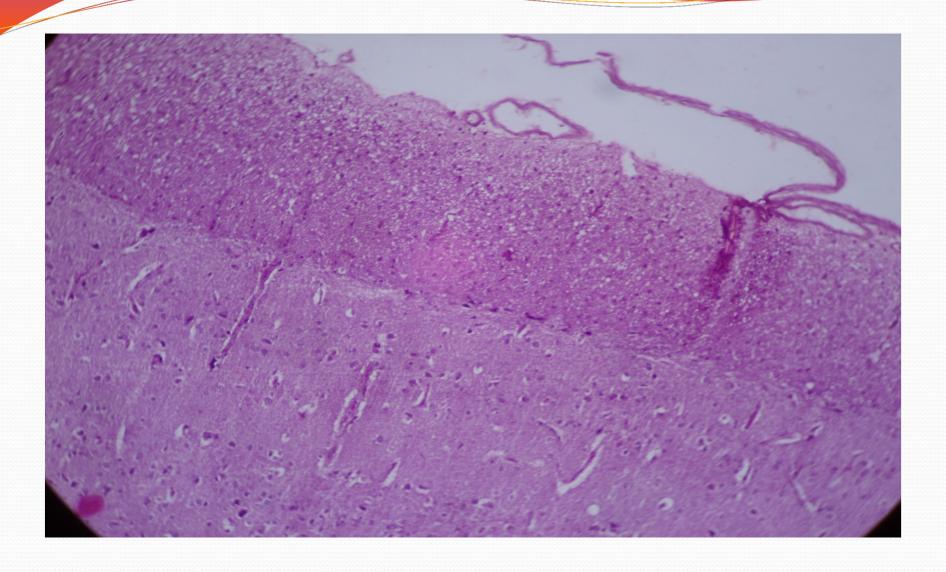




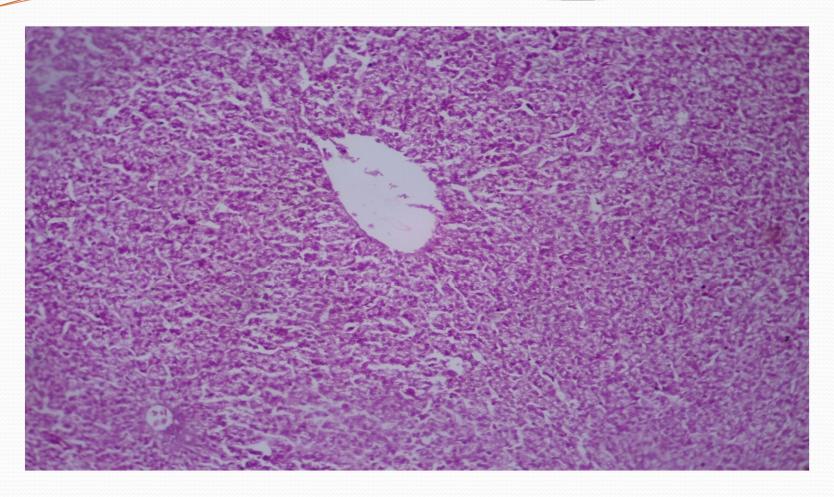
Photomicrograph of liver showing central venous congestion, franc necrosis and degenerative fatty changes HE X 20 (Heat stress group)



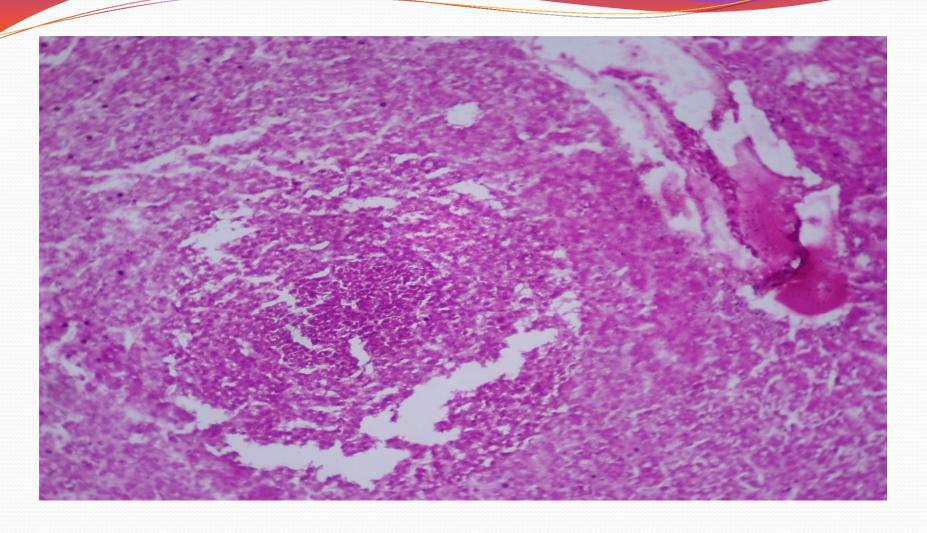
Photomicrograph of brain showing hemorrhages HE X 40 (Heat stressed group)



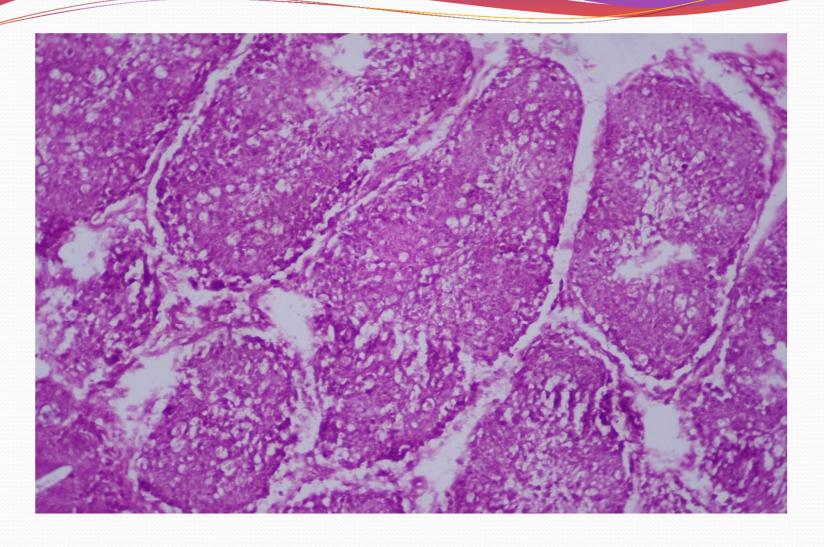
Photomicrograph of brain showing normal histology(Heat stress free group)



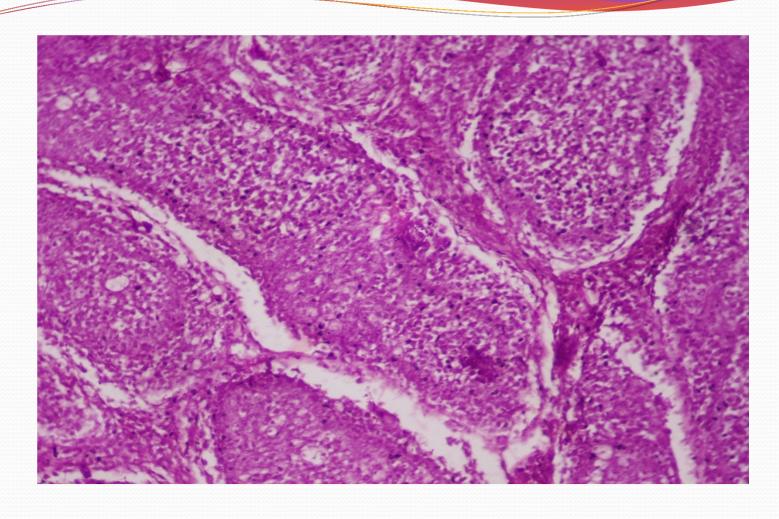
Photomicrograph of liver showing mild changes HE X 200 (group supplemented with turmeric 0.4%)



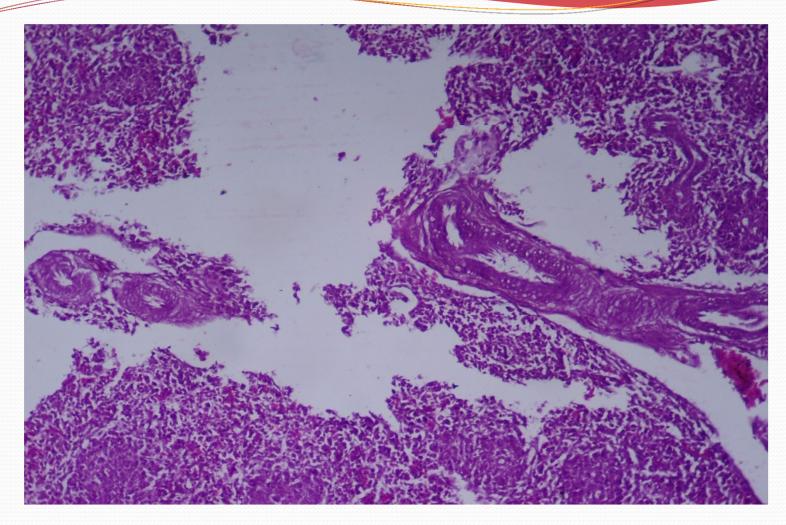
Photomicrograph of liver showing central venous congestion and granuloma HE X 20 (group supplemented with combination of Tulsi (0.5%) and Turmeric (0.4%))



Photomicrograph of bursa showing depletion of lymphoid follicles HE X 20 (Heat stressed group)

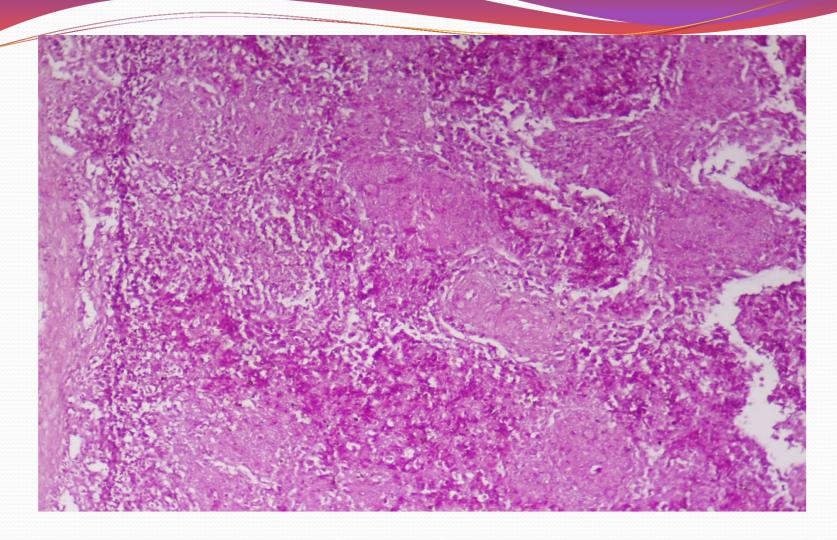


Photomicrograph of bursa revealing mild changes HE X 20 (group supplemented with Tulsi 0.5%)



Photomicrograph of spleen showing depletion of lymphocytes and thickening of trabecular system HE X 20 (Heat stressed group)

Photomicrograph of spleen showing mild changes HE X 20 (Group supplemented with Turmeric 0.4%)



Photomicrograph of spleen showing mild changes HE X 20 (Group supplemented with Turmeric 0.4%)

Conclusion

Tulsi at 0.5% and turmeric at 0.4% inclusion to broiler dites improved the antioxidant status. The combinations of herbals at either of the doses didn't have any added benefit than the independent inclusions.

