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Haemato-Clinical Changes in Pregnant and Non-Pregnant Rats, *Rattus rattus* Linnaeus, 1758 under Parasitic Stress

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SERUM chemistry of non-pregnant (Group I) and pregnant (Group II) female *Rattus rattus* (n=389) collected alive from wild conditions in rat traps was investigated in different weight groups (A: 50-100gm, B: 100-150gm and C: 150-200gm) under uninfected and infected (*Trypanosoma* and *Cysticercus*) conditions. Groups I and II were further subdivided into four subgroups each, a: Uninfected rats, b: *T. lewisi* infected rats, c: *C. fasciolaris*-infected rats, d: *T. lewisi* and *C. fasciolaris*-infected rats. A significant maximum fall of 38.01% in glucose was recorded in pregnant rats of Group IIb (weight category B). Changes in cholesterol were insignificant in most of the groups except Group IIc (weight category B) where the fall was maximum (25.32%). Total serum protein (TSP) changes were again insignificant in most of the groups except in weight categories B of Group IIb showing 18.54% rise and Group IIc showing 22.62% fall. Changes were significant ($P<0.05$) in hemoglobin in parasitized rats showing a maximum fall of 36.63% in Group IIb (weight category B). The results infer that glucose and hemoglobin are the most vulnerable biochemical constituents to parasitism and that older (weight category B and C) pregnant rats (Group II) and rats with dual infection (Group IId) exhibit greater changes in their biochemical constituents.

Keywords: Cholesterol, *Cysticercus fasciolaris*, Glucose, Haemoglobin, Protein, *Trypanosoma.*

Rats are most widely distributed rodents of the world and have served as excellent models for parasitological studies (Gill *et al.*, 2001). For several decades, trypanosomiasis has continued to contribute adversely to the economic and social well being of sub Saharan Africans. Because of the presence of trypanosomes in blood, these invading parasites produce numerous changes in the biochemical constituents of blood (Adenike and Stephen, 2010).

Absence of suitable animal research models has been one of the most challenging constraints in the understanding of disease. The current understanding of the pathology of *T. lewisi* and *C. fasciolaris* in the pregnant and non-pregnant rats rests largely on the pathogenesis of the parasites in laboratory animals.

Blood is the chief circulatory medium of animal, by virtue of its circulation through every organ, it participates in all major functional activities of the body (Guyton and Hall, 1996). Study of blood is important from parasitological point of view. It is reported that parasitic infection affects the blood picture and its composition (Chappel, 1980). Most of the studies have attempted to standardize normal reference values for biochemical parameters. Dynamics and features of blood chemistry in rats are clearly understood (Matsuzawa *et al.*, 1993, Boehm *et al.*, 2007), however during pregnancy, the reports are few (La Barde *et al.*, 1999, Honda *et al.*, 2008). Serum chemistry values are subjected to change during pregnancy and under parasitic stress. Clinical studies on rat's haematology are therefore not only demanding but would serve as useful indicators for evaluating rodent health with respect to disease as well as the blood-borne parasitic diseases.

In this context, the present investigation was undertaken to probe into the serum biochemistry using *Rattus rattus* as a model under parasitic stress of *T. lewisi* and *C. fasciolaris* under pregnant and non-pregnant conditions. It is envisaged that such studies will pave way for better understanding of the pathology of the parasites in higher mammals and man.

Material and Methods

Study populations

A total of 389 wild rats (*Rattus rattus)* collected from different localities of Rohilkhand region were maintained in the Animal House of the Department of Animal Science, M.J.P. Rohilkhand University, Bareilly, Uttar Pradesh, India (28.35ºN, 79.42ºE) under proper conditions of food and aeration. These rats were subjrcted to investigations pertaining to parasites and serum chemistry (glucose, cholesterol, serum protein and haemoglobin).

Parasite study

Blood was collected either from lateral tail vein, jugular vein, lateral saphenous vein, orbital plexus or by cardiac puncture using a 16 gauge, 5 cm. long hypodermic syringe. Up to 20 ml of blood `was withdrawn, sodium EDTA (2mg mL^{-l}) was used as the anticoagulant covered with a cover slip and examined microscopically to observe live parasites. Blood was also examined by microhaematocrit (7000 rpm for 7 minutes) to confirm the presence of blood parasites. However, their presence was also confirmed by preparing blood smears from whole blood stained with Giemsa or azur-eosin-methylene blue. After examination of blood parasites, hosts were sacrificed for isolation of helminth parasites. Parasites were observed in the liver and were removed with the help of brush and collected in normal saline. The parasites were fixed in 10% formalin, pressed and stained in Borax Carmine as per routine techniques.

Prevalence and intensity were calculated according to Bush *et al.* (1997).

$$\text{Prevalence \%} = \frac{\text{Total no. of infected rats X 100}}{\text{No. of rats examined}}$$

Mean Intensity = *T. lewisi* : Total no. of parasites/1000 RBC
C. fasciolaris: No. of parasites/ host

Biochemical studies

Experimental groups

Female rats were divided arbitrarily into the following groups for making biochemical estimations.

Group I Non Pregnant

Ia: Uninfected . Ib: *T. lewisi* infected
Ic: *C. fasciolaris* infected Id: *T. lewisi* + C. *fasciolaris* infected

Group II Pregnant

IIa: Uninfected. IIb: *T. lewisi* infected
IIc: *C. fasciolaris* infected . IId: *T. lewisi* + *C. fasciolaris* infected

Weight groups

Biochemical results were calculated in the following weight groups:

Weight group A: 50-100gm
Weight group B: 100-150gm
Weight group C: 150-200gm

Biochemical estimations

For biochemical estimations, rats were anaesthetized in slight chloroform by prompting them to enter a glass mason jar and were held in the jar till unconscious as determined by failure to respond to gentle tactile stimuli add blood samples collected into sterile tubes with the help of sterile syringe. The samples were allowed to stand for 10 minutes at room temperature and then serum was separated with a Remi Centrifuge run at 3000 rpm for 15 minutes and collected in another vial. The supernatant (straw coloured serum) was carefully removed with pasteur pipette and stored at -20°C frozen until analyzed. Concentrations of glucose, cholesterol and serum protein were determined by using "Ruby" Brand of Snijders autoanalyser with the help of Autospan and Cogen laboratory kits. Haemoglobin was estimated by Sahli's method.

Statistical analysis

The group mean ± SE (standard error) calculated for each analyte and value of significance (P values) were calculated by SPSS software. Data obtained was analysed using analysis of variance (ANOVA) to determine level of significance. Values of $P<0.05$ were considered to be statistically significant.

Results and Discussion

The blood of *Rattus rattus* was infected with a monophyletic group of unicellular parasitic protozoa and was identified as *Trypanosoma* (*Herpatosoma*) *lewisi* Kent, 1880. The liver parenchyma was infected with creamish white, fluid filled encapsulated cysts morphologically consistent with the cestode, *C. fasciolaris* (broad necked feline tapeworm) the larval stage *of Taenia taeniaeformis*. It commonly reaches the liver of rats (intermediate hosts) through contaminated water or food of infected cat faeces. Final hosts are carnivores of the families Felidae, Canidae and Mustelidae including domestic cats and dogs.

Parasite Profile

Type Host	:	*Rattus rattus* Linnaeus, 1758
Type Locality	:	Rohilkhand, Bareilly (U.P.), India (28.35ºN, 79.42ºE)
Prevalence	:	*T. lewisi* 12.40%, *C. fasciolaris* 46.70%
Intensity	:	*T. lewisi* 14 parasite/1000 RBC
		C. fasciolaris 2-4 parasites/liver/host

Biochemical estimations

Glucose

Glucose concentration in *R. rattus* infected with *Trypanosoma* (Group Ib) showed insignificant changes ($P>0.05$) (Table 1). Both the weight categories (A and B) showed marginal falls of 0.33% and 0.43% respectively (Fig. 1). In rats infected with *C. fasciolaris* (Group Ic), glucose value in all three weight categories was significantly lower ($P<0.05$) as compared to that of Group Ia. The value in Ic group of all weight categories (A, B and C) showed 21.94%, 21.19% and 24.85% fall respectively. Thus, rats of heavier weight showed greater fall as compared to low-weight ones. The value of glucose in rats having mixed infection was significantly ($P<0.05$) lower as compared to control rats. Weight categories B and C, showed 25.97% and 28.47% fall respectively**,** whereas, mixed infection was not found in rats of weight category A.

Pregnant rats infected with *Trypanosoma* (Group IIb) showed greater fall in glucose concentration as compared to the values of uninfected pregnant rats. Pregnant infected rats of weight categories B and C showed 38.01% and 6.66% fall respectively. Only one pregnant rat infected with *C. fasciolaris* (Group IIc) was obtained which belonged to weight category B and showed 15.26% fall in the glucose value. One pregnant rat belonging to weight category C was found to be infected with *T. lewisi* and *C. fasciolaris* which showed similar pattern of fall in the glucose value (32.85% fall). The value declined by 38% in *Trypanosoma*-infected pregnant rats whereas, it declined by 32% in mixed infection indicating that trypanosomes were more involved in glucose metabolism dwelling in the blood itself as compared to the cestode parasite in the liver.

Small ruminants are fully susceptible to trypanosomiasis (Adah *et al.*, 1993, Ogunsanmi *et al.*, 1994) and the economic impact of the disease on these animals has been shown to be substantial (Luckins, 1992). Serum biochemical changes

during trypanosomiasis have been reported in goats (Fiennes *et al.*, 1946) and sheep (Anosa and Isoun, 1976). These changes have been extensively reviewed in human and animal trypanosomiasis by Anosa (1988).

Linton (1930) found that blood glucose level of *Trypanosoma* -infected rats remained normal. He further reported that blood sugar of *T. equiperdum*- infected rats remained unchanged. During the present findings, insignificant change in blood glucose level was recorded in Group Ib but significant decrease in glucose level was recorded in *C. fasciolaris* infected (Ic) rats in all three weight categories. Similarly, significant decrease in blood glucose level was observed in rats having mixed infection (Id) and all the weight categories of infected groups of pregnant rats.

Igbokwe *et al.* (1998) observed blood glucose in experimentally induced *T. brucei*-infected fasting rats and found a significant increase from 2.73± 0.14 mmol/l on day 0 p.i. (post infection) to 6.21± 0.16 mmol/l and 5.93± 0.33 mmol/l on day 5 and 8 p.i. respectively. However, the values had decreased in most of the rats on day 11 p.i.

Blood glucose concentration in mammals is tightly regulated, reflecting a trade-off mainly between two opposite hormones: glucagons and insulin, produced by α and β cells respectively in the islets of Langerhans of the pancreas (Opazo *et al.*, 2004) therefore, its role in body metabolism is self evident. It has been proposed that bloodstream forms of some trypanosomes scavenge blood glucose as a source of energy (Chaudhary *et al.*, 2006). This may partly contribute to the development of hypoglycemia, observed in some *Trypanosoma*-infected animals. In the present study, the hypoglycaemic condition could be manifested because of increased catabolism of lipids in order to meet some strategic energy needs in the body of the host animals.

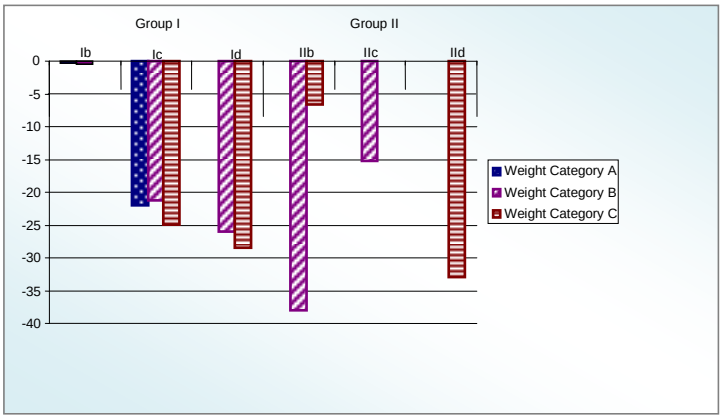

Fig. 1. Percentage change in glucose content of parasitized non-pregnant and pregnant rats.

Cholesterol

The changes in the cholesterol value of *Trypanosoma*-infected rats was insignificant (P>0.05) in Group Ib (weight categories A and B) (Table 1). The cholesterol content of rats of Group Ib (weight category A) showed 3.04% rise whereas, weight category B showed a 6.67% fall in the cholesterol values (Fig. 2). In all weight categories of Group Ic (*C. fasciolaris*-infected rats), no significant increase or decrease in cholesterol values was noticed. 0.46% fall in cholesterol was recorded in rats (Group Ic) of weight category A.

Prevalence of *T. lewisi* and *C. fasciolaris* (Group Id) was not observed in rats of weight category A, whereas, in rats belonging to weight categories B and C, cholesterol concentration fell showing 0.24% and 3.28% fall respectively. Thus the change in cholesterol values in rats of weight categories B and C was insignificant (P>0.05).

Pregnant rats of weight categories B of Group IIb showed 12.46% fall, whereas, weight category C showed 5.40% fall. Pregnant rats infected with *C. fasciolaris* (Group IIc) showed insignificant change (P>0.05). Rats of weight category B, showed a fall of 25.32%. On the other hand, rats belonging to weight category C was free from *C. fasciolaris* infection. The pregnant (Group IId) rat belonging to weight category C was infected with *T. lewisi* and *C. fasciolaris* and showed a fall of 12.16%.

Cholesterol is the building block for cell membranes and it is essential in the formation of bile (which subsequently aids in the digestion of fats), vitamin D, other steroids and hormones such as progesterone, testosterone and estrogen. Low density lipoprotein (LDL) is the major cholesterol carrier in the blood and is responsible for transporting cholesterol from the liver to organs and tissues of the body. High density lipoprotein (HDL) on the other hand is responsible for carrying cholesterol from various organs and tissues to the liver for recycling or degradation.

Diehl and Risby (1974) observed serum cholesterol value in experimentally infected rabbits with *T. gambiense* and found that cholesterol value increased progressively with time, attaining a 3 to 4-fold increase over control values nearing the terminal stage of infection. Choubey *et al.* (1978) found that cholesterol value of female rats (*Rattus rattus arborious*) was lower as compared to male and the values increased with increase in body weight. Our findings are contrary in relation to infected rats where the cholesterol values decrease more sharply in heavier rats. Studies of Katunga-Rwakishaya *et al.* (1997) have indicated that plasma cholesterol levels decrease during trypanosome infection in sheep. Similarly, decreases in serum cholesterol level with increase in body weight have been observed in weight category C of *Trypanosoma*-infected pregnant rats (IIb) and all infected groups of pregnant rats.

TABLE 1. Biochemical estimations of non pregnant and pregnant *Rattus rattus* under normal and parasitized conditions.

Biochemical parameters	Weight categories	Group I (Non pregnant)				Group II (Pregnant)			
		Group Ia	Group Ib	Group Ic	Group Id	Group IIa	Group IIb	Group IIc	Group IId
Glucose mg/dl	A	95.44± 5.74	95.12± 2.68	74.50± 3.73	-	-	-	-	-
	B	93.21± 1.75	92.80± 1.27	73.45± 1.17	69.00± 6.02	65.50± 6.50	40.60± 0.24	55.50± 2.50	-
	C	100.39± 2.10	-	75.44± 1.81	71.80± 0.37	84.00± 5.00	78.40± 0.40	-	56.40± 0.40
Cholesterol mg/dl	A	71.33± 5.22	73.50± 7.19	71.00± 5.12	-	-		-	-
	B	73.51± 1.68	68.60± 7.91	73.07± 1.13	73.33± 1.20	77.00± 5.00	67.40± 0.24	57.50± 3.5	-
	C	73.00± 1.81	-	73.20± 1.89	70.60± 0.24	74.00± 3.00	70.00± 0.44	-	65.00± 0.31
Protein g/dl	A	6.46± 0.28	7.60± 1.11	5.91± 0.56	-	-	-	-	-
	B	7.00± 0.13	6.92± 0.34	6.84± 0.15	8.06± 1.12	6.85± 2.05	8.12± 0.03	5.30± 0.10	-
	C	6.78± 0.19	-	6.51± 0.16	7.36± 0.11	7.65± 0.86	7.60± 0.04	-	6.76± 0.05
Haemoglobin g/dl	A	12.78± 0.40	10.80± 0.14	9.25± 0.21	-	-	-	-	-
	B	12.43± 0.22	10.92± 0.28	9.19± 0.10	9.46± 0.26	12.40± 0.02	11.52± 0.03	9.30± 0.50	-
	C	13.13± 0.21	-	9.47± 0.17	8.32± 0.03	13.00± 0.35	10.82± 0.03	-	10.42± 0.03

Group Ia: Uninfected rats
Group Ib: *Trypanosoma* infected rats
Group Ic: *Cysticercus fasciolaris* infected rats
Group Id: *Trypanosoma* and *C. fasciolaris* infected rats
Group IIa: Uninfected pregnant rats
Group IIb: *Trypanosoma* infected pregnant rats
Group IIc: *C. fasciolaris* infected pregnant rats
Group IId: *Trypanosoma* and *C. fasciolaris* infected pregnant rats

In the observations of Waner and Nyska (1994), serum cholesterol concentration levels did not change at any level of fasting periods as compared to the non fasting control group. Insignificant change ($P>0.05$) in serum cholesterol concentration in *C. fasciolaris* infected (Ic) and rats having dual infection (Id) were recorded in the present studies in all weight categories, whereas, insignificant rise in cholesterol level was observed in *Trypanosoma* -infected (Ib) rats belonging to weight category A.

T. congolense infection caused significant decreases in the serum values of total cholesterol triglycerides, HDL cholesterol and LDL-cholesterol in all sheep

of the infected group (Adamu *et al.* 2008). The mean p.i. value of total serum cholesterol was reported to be significantly (P<0.05) lower in the infected group than that in the control group by the authors. These results are also in conformity with those of Wellde *et al.* (1989) and Katunguka-Rwakishaya *et al.* (1992) in *T. rhodesiense* infection of cattle and *T. congolense* infection of sheep. However, the observations of Adamu *et al.* (2008) differ from those of Diehl & Risby (1974) and Rouzer & Cerami (1980) in *T. gambiense* and *T. brucei* infected rabbits, respectively.

Blood-stream forms of trypanosomes, which are unable to synthesize cholesterol, are known to require it along with phospholipids and total lipids for synthesis of their membranes and growth (Black & Vanderweed, 1989, Hue *et al.*, 1990, Katunguka-Rwakishaya *et al.*, 1991, Green *et al.*, 2003 and Nok *et al.*, 2003). Further, the findings of Adamu *et al.* (2008) infer that the continuous removal and utilization, from the blood-stream these molecules could partly be responsible for the lowered levels of lipids and total cholesterol in serum. Impaired synthesis and subsequent release of cholesterol from the liver could also be a contributory factor in decreasing the serum levels of total cholesterol observed in the trypanosome-infected animals. This may be due to the pathological changes occurring as a consequence of trypanosome infection in the liver (Logan-Henfrey *et al.*, 1992). Impaired synthesis of cholesterol in the liver could also be the result of insufficient hepatocellular respiration due to hypoxia, reproductive endocrine disorders that could impair the reproductive performance of the trypanosome-infected animals (Adamu *et al.*, 2008). Igbokwe *et al.* (2008) observed serum and hepatic lipid levels in rats infected with *T. brucei* and found that the total serum cholesterol, HDL, and LDL cholesterol concentrations decreased (P <0.0.05) when compared to the control values on days 7 and 11 p.i.

It has been reported by Armando *et al.* (2007) that *C. fasciolaris* is mainly localized to the liver of rodents and develops neoplasm in the liver and the infected rats exhibit mild decrease in serum cholesterol concentration. It is possible that *C. fasciolaris*- induced immunosupression plays a contributory role in decreasing the biochemical values of the infected rats.

Infection with *T. brucei*, was followed by a decrease in the serum concentration of cholesterol in the infected group. The difference between the post-infection mean serum cholesterol concentration in the infected group and that in the control pigs was reported to be significant (P < 0.05). Further, the findings infer that *T. brucei* infection of pigs causes significant decrease in the serum levels of cholesterol similar to that of *T. congolense* in infected sheep (Adamu *et al.*, 2008, 2009).

The role of changes in the serum concentrations of lipid and cholesterol in the pathophysiology of some of the disorders reported in trypanosome-infected animals can be better appreciated when the functions of such lipids and cholesterol in the mammalian physiology are taken into cognizance. Cholesterol

is produced in the liver as well as it is supplied to the body in human and animal diets. Cholesterol is essential in the formation of bile, vitamin D, other steroids and hormones such as progesterone, testosterone and estrogen. LDL-cholesterol is the major cholesterol carrier in the blood and is responsible for transporting cholesterol from the liver to organs and tissues of the body. HDL-cholesterol on the other hand is responsible for carrying cholesterol from various organs and tissues to the liver for recycling or degradation.

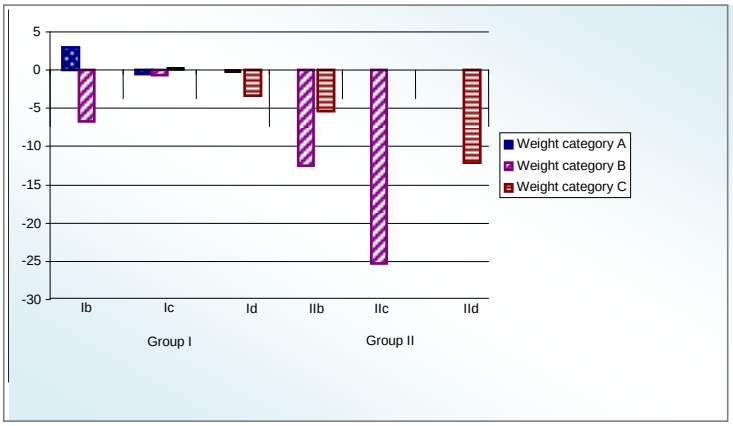

Fig. 2. Percentage change in cholesterol content of parasitized non-pregnant and pregnant rats

Total Serum Protein

Total serum protein (TSP) values of rats infected with *T. lewisi* (Group Ib) showed 17.64% rise (weight category A) as compared to the control (Group Ia) whereas, rats belonging to weight category B showed 1.14% fall as compared to the controls (Fig. 3). All the weight groups of *C. fasciolaris*-infected rats (Group Ic) showed insignificant (P>0.05) fall in the values of TSP as compared to uninfected rats. 8.51% fall was observed in weight category A. The weight categories B and C (Group Ic) showed a similar trend, however, the fall was low (2.28% and 3.98% respectively). TSP concentration in rats infected with *T. lewisi* and *C. fasciolaris* (Group Id) also showed insignificant change (P>0.05). Values in weight categories B and C increased marginally as shown in Table 1.

Pregnant rats of weight categories B and C of Group IIb showed 18.54% rise and an insignificant fall of 0.65% respectively in their TSP values. Weight category B of *C. fasciolaris*-infected pregnant rats (Group IIc) showed 22.62% fall whereas, the parasitized pregnant rat of Group IIb (weight category C) showed a 11.63% fall.

Popov and Ivanov (1975) examined changes in total protein content and protein fractions during the process of ontogeny in male and female rats and found that the total protein content in the blood serum in both sexes increased corresponding to their age. Choubey *et al.* (1978) inferred that protein value in the female rats was higher than that of males and these values increased with the increase in body weight. However, in the present studies, insignificant decrease in protein values were recorded in *Trypanosoma*-infected rats corresponding to the increase in body weight.

Although the majority of internal parasites rely heavily upon carbohydrate metabolism as their basic source of energy, a few examples are known in which protein and amino acid metabolism achieve some degree of importance, particularly under emergency conditions. Incubation *in vitro*, in the absence of carbohydrates leads to increased production of ammonia in the number of parasites including trypanosomes suggesting active protein metabolism. Other protozoan parasites including trypanosomes and *Plasmodium* can also utilize amino acids for energy metabolism. The total protein may be normal, increased or decreased in African trypanosomiasis (Anosa, 1988). It is very interesting to note that due to the infection of parasites, the level of serum globulin increases, however, serum albumin decreases leading to the fall in albumin/ globulin ratio of the infected animals. In the present observation, increase in serum protein may be due to increase in gamma- globulin level. Hyper gammaglobulinaemia in African trypanosomosis is usually associated with the increase in immunoglobin M (IgM) level which is a consistent finding in trypanosomosis of man and animals (Anosa, 1988). Mean serum IgM levels in rhodesian sleeping sickness were similarly elevated to nearly three folds than those of control population.

It is also possible that increase in globulin level might have been due to enhanced antibody production. Hyperglobulinaemia generally dominates on hypoalbulinaemia in the infected animals showing a marginal increase in the protein level of the infected rats.

Elevations in the TSP of monkeys after infection support earlier observations in *T. rhodesiense* infection of man (Wellde Chumo *et al.*, 1989) and cattle (Wellde Reardon *et al.*, 1989) and in goats infected with *T. congolense* (Witola and Lovelace, 1997, Ndoutamia *et al.*, 2002). Ahmed *et al.* (2004) reported that the mean of total serum proteins in the normal camels was 7.381 ± 0.048 g/dl whereas, the corresponding value in haemoparasitized group was 6.831 ± 0.270 g/dl. The haemoparasitic infection had a significant ($P \leq 0.05$) effect on the TSP. The comparison of the results of serum protein levels led to the conclusion that sera of camels with haemoparasites have a lower mean TSP than non-infected camels. Similar results were reported by Safwat and El-Abdin (1982). The change in protein value probably corresponds to the degenerative changes in the haemoparasitized organs. This is contrary to the findings of Jatkar *et al.* (1973) Boid *et al.* (1980) and Ahmed *et al.* (2004) who reported higher serum protein values in trypanosome-infected camels than in the non-infected ones. Findings of the present investigation agree with those of the above authors where significant

increase in serum protein level in weight category A of *Trypanosoma*-infected (Group Ib) rats was reported as compared to the uninfected ones. High protein concentrations observed in the infected rats are in agreement with previous reports (Orhue *et al.*, 2005 and Ekanem &Yusuf, 2008).

Highly significant increase in (TSP) and globulin ($P<0.05$) in infected animals as compared with controls was also observed by Abenga and Anosa (2005). The increase in TSP may have been due to increased release of tissue specific enzymes and other intracellular proteins secondary to parasite-induced cell membrane disruption. These findings are in accordance to that of the present investigation where increase in TSP was observed in weight category B of rats carrying dual infection (Group Id), *Trypanosoma*- infected pregnant rats (Group IIb) and weight category A of *Trypanosoma*- infected (Group Ib) rats due to hyperglobinaemia. On the other hand, the decline in TSP observed in groups IIc and IId would evidently be due to hypoalbulinaemia.

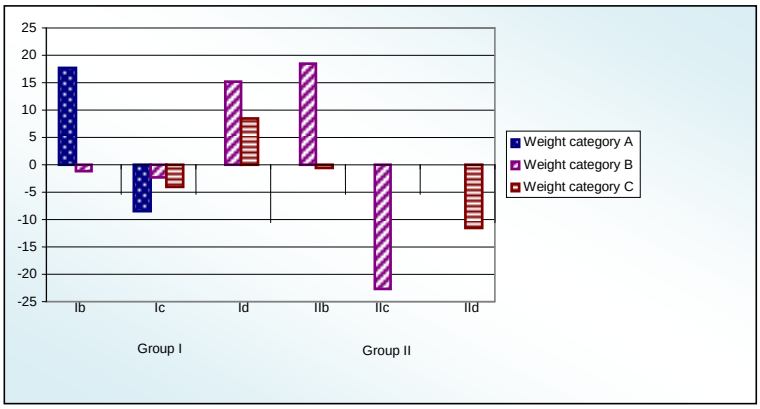

Fig. 3. Percentage change in serum protein content of parasitized non-pregnant and pregnant rats

Haemoglobin

All the weight categories of parasitized rats of all groups showed highly significant ($P<0.05$) fall in haemoglobin level. *Trypanosoma*-infected rats (Group Ib) belonging to weight categories A and B showed a decline in haemoglobin values. The haemoglobin percentage in uninfected rats (Group Ia) belonging to both the weight categories A and B reduced showing 15.49% and 12.14% fall respectively (Table 1). In Group Ic (*C. fasciolaris* infected rats), the haemoglobin percentage followed the same pattern as above, showing a significant ($P<0.05$) decrease in values in all three weight categories. In Group Id (*T. lewisi* and *C. fasciolaris*-infected rats), again similar variations were observed. Weight categories B and C showed 23.89% and 36.63% fall respectively (Fig. 4).

Pregnant rats of Group IIb and Group IIc again showed a declined pattern in the haemoglobin percentage. Haemoglobin values declined in *Trypanosoma*-infected rats belonging to weight categories B (Group IIb) showing a 7.09% and 16.76% fall respectively. Similarly, haemoglobin values fell (25.00%) in pregnant rats infected with *C. fasciolaris* (Group IIc) belonging to weight category B (Group IIa). Results in pregnant rats of Group IId (*T. lewisi* and *C. fasciolaris*-infected pregnant rats) again fall on the same pattern, where weight category C (Group IIa) values showed 19.84% fall in haemoglobin concentration.

The function of carrying and delivering of oxygen for cell survival is carried out by haemoglobin, a conjugated protein. The global importance of this respiratory pigment was stressed by Harris and Kellermeyer (1970) due to its physiological necessity. Much work on haemoglobin has been done in humans, however, such type of information in rodent blood is inadequate. Gupta and Gupta (1986) and Rauthan *et al.* (1995) observed the impact of haemoparasites in fish blood. Heavier depletion in haemoglobin values under mixed or concurrent infectivity in *Bufo* parasitized by *Hepatozoon*, *Trypanosoma* and *Icosiella* microfilariae was observed by Gangwar (2001).

In relation to host sex and size, Choubey *et al.* (1978) found that haemoglobin concentration was higher in female rats as compared to males and this value increased with increase in size. However, in the present findings, all the weight categories (A, B and C) of uninfected rats of non pregnant (Group I) and pregnant (Group II) had almost similar haemoglobin values.

Okochi (2003) observed very low concentration of haemoglobin (7.2 g/l) in *Trypanosoma*-infected rats. The present findings fall on similar lines where significant decrease in haemoglobin values have been observed in *Trypanosoma*- infected rats, *C. fasciolaris* infected rats and rats carrying dual infection of *T. lewisi* and *C. fasciolaris*. These results are true for rats belonging to all weight categories.

Nadim and Soliman, (1967) recorded the pathogenic potential of trypanosomes manifested by a decrease in haemoglobin. Nadim & Soliman (1967) and Jatkar & Mohan (1971) held similar observations for *T. evansi* and Ahmed *et al.* (2004) also reported a decrease in the mean value of haemoglobin in infected camels. The present findings follow suit by showing decreased values of haemoglobin in the infected groups indicating anemia.

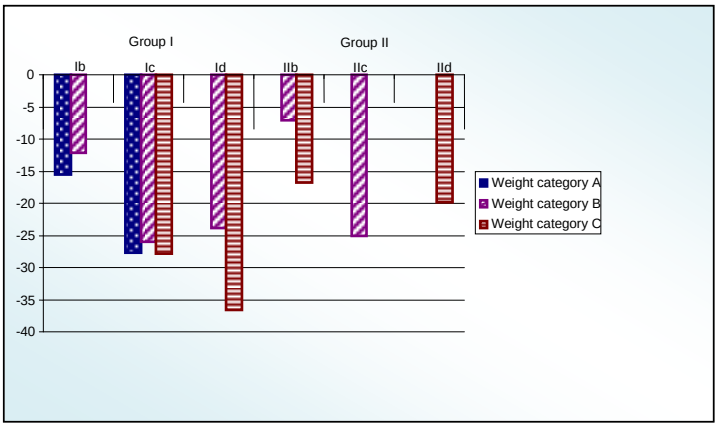

Fig. 4. Percentage change in haemoglobin content of parasitized non-pregnant and pregnant rats.

Evidence suggests that etiology of anemia is multifactorial and haemolysis and haemodilution and disorder and/ or noncompensatory erythropoieses are some of the mechanisms proposed for the manifestation (Jenkins and Facre, 1985). de Rijk *et al.* (2002) attributed anemia to the increase in normal plasma volume termed as haemodilution that occurs during pregnancy.

It has also been proposed that *Trypanosoma*-infection precipitates increased red blood cell destruction which results in anemia (Ekanem & Yusuf, 2008 and Akanji *et al.*, 2009). Severity of anemia usually reflects the intensity and duration of parasitaemia as shown by Saleh *et al.* (2009) who also reported acute anemia in trypanosomiasis due to proliferation of the parasites. It has also been reported that infection with trypanosomes may cause increased susceptibility of red blood cell membrane to oxidative damage probably due to reduced glutathione on the surface of red blood cell (Akanji *et al.*, 2009) culminating in severe anemia.

The presence of blood parasites is responsible for the breakdown of red blood cells leading to anemia. Concurrently, the haemoglobin level would also decline as observed in the present case. Similar results due to the presence of the larval cestode would suggest its probable association with red blood cells / haemoglobin metabolism and hence play a contributory role for the ensuing anemia under pregnant and non-pregnant conditions of the host.

The clinical picture as shown above reflects the manifestations of the parasites, *T. lewisi* and *C. fasciolaris* in the host, *R. rattus*. The observations under two physiological stages of the female rat shows that the parasites in pregnant rats are more intricately associated with the clinical chemistry of the host. The findings can be utilized as an important diagnostic tool for assessing the metabolic changes incurred during parasitism and pregnancy.

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التغيرات الاكلينيكية والدموية للفئران الحوامل و غير الحوامل تحت تأثير الطفيليات

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لقد تم فصل المصل من الفئران البرية غير الحوامل (مجموعة ١) والحوامل (مجموعة٢) و التى تتبع أوزان مختلفة (مجموعة أ: ٥٠-١٠٠ جرام) , (مجموعة ب: ١٠٠-١٥٠جرام) , (مجموعة ج: ١٥٠-٢٠٠ جرام) و المعداه و غير المعداه بالتريبانوسوما .

لقد تم تقسيم المجموعة ١ و ٢ الى ٤ مجاميع و تشمل : أ- فئران غير معداه , ب- فئران معداه بالتريبانوسوما لويزى و ج- فئران معداه بالسيستيسركس فاشيولاريز .

أظهرت النتائج نقصا معنويا للجلوكوز (٣٨٪) فى الفئران الحوامل من المجموعة ٢ب (مجموعة ب طبقا للوزن) لم يحدث تغير معنوى للكولسترول فى المجموعة ٢ج (مجموعة ب طبقا للوزن) حيث شمل الانخفاض ٢٥٪ فقط , لم تحدث تغيرات معنوية فى بروتين المصل الكلى فى جميع المجاميع ؛ ما عدا ٣ب (مجموعة ب طبقا للوزن) والذى تزايد بنسبة ١٨٪ و ٢ج و الذى انخفض ٪ . لم يحدث تغير معنوى للهيموجلوبين للفئران المعداه و التى أظهرت ٣٦٪ انخفاضا فى المجموعة ٢ب (مجموعة ب طبقا للوزن). وبهذا يمكن القول بأن الجلوكوز و الهيموجلوبين هم أكثر المؤشرات التى تتأثر بالتطفل وأن الفئران الحوامل الأكبر سنا ووزنا و كذلك الفئران المعداه بأكثر من طفيل قد أظهرت تغيرات أكثر فى مكونات الدم.