SUMMARY AND CONCLUSIONS
Adrenal gland is an important endocrine gland which secretes hormones concerned with carbohydrate metabolism, balance of electrolytes in the blood, maintenance of the volume of circulatory blood, control of sexual maturity and regulation of extracellular fluid (ECF) volume. Bilateral removal of the adrenal produces a series of metabolic disturbances, which are identical with those appearing in patients with Addison's disease such as extreme muscular weakness, a variable degree of hypoglycemia, gastrointestinal disturbance, reduced blood pressure and body temperature. The most common cause of adrenal deficiency is due to polyendocrine deficiency syndrome or autoimmune adrenal insufficiency. The symptoms of the syndrome are hypoparathyroidism, diabetes mellitus and other inheritable diseases such as an autosomal recessive pattern in Sibships, the dominant pattern appearing in multiple generations of an affected family. The hyperfunction of adrenal gland leads to Cushing's syndrome primarily due to pituitary abnormality. The symptoms of the disease is obesity, reproductive dysfunction, depression, moon face, osteoporosis etc. The aim of the present work is to understand the physiological symptoms of Addison's disease through the study made on adrenalectomy in rats, and the effect of time and sex on it. During adrenal deficiency growth ceases in young animals and older animals generally lose their weight. Similar to Addison's disease, adrenalectomized animals are unable to tolerate stress of any type such as exposure to trauma, cold, heat,
toxins, infections, fasting, forced exercise etc., and they are likely to prone fatal.

2. Several reports though are available on the general impact of adrenalectomy, very little information is available specifically on the hepatic and reproductive tissues which are the important sites of growth and development. Liver being the metabolic tissue which controls all the general and reproductive functions of the body of an organism, studies are needed on this metabolic tissue in order to understand the impact of adrenalectomy on general metabolism; and as the reproductive tissue functions are directly connected to the adrenal gland secretions much work need to undertake on these tissues of adrenalectomized animals in order to understand coherently the implication of Addison’s disease. Further, no comparative studies are reported on the impact of adrenalectomy of male and female animals for the understanding of the differential effects of adrenalectomy, if any, on sex of the animal. Hence, the present study is taken up to observe the impact of adrenalectomy on the reproductive tissues like epididymis, penis and testis in males and uterus, vagina and ovary in female and on the liver of both the sexes of the albino rats at biochemical and histological levels. In addition, haematological and hormonal levels studies are also made for correlation.

3. The selection of albino rats is based on its ability of survival, its withstanding capacity in a fairly wide range of stress conditions and its maintenance and handling is quiet comfortable for study. Healthy Wistar strain
male and female albino rats (*Rattus norvegicus albinus*) of the age of 120 days and body weight 220 gms have been selected for the present study. The rat colony was maintained in laboratory at 28 °C at 12 hours of light and 12 hours of darkness. Rats were divided into 3 groups, each group consisted of 12 individuals, of this six were males and the remaining six were females. First group of rats were called as shamoperated (SO) in them the adrenal glands were kept intact and considered as controls. The second and third group of rats were bilaterally adrenalectomized (ADX) by the dorsal approach in a single stage of operation as followed by Stith *et al.*, (1989), and these two groups of animals were considered as experimentals. The second group of experimentals was maintained for 15 days and the third group for 30 days. After the stipulated time, both the SO and ADX rats were killed by survical dislocation and immediately the tissues of them, the liver, epididymis, penis and testis in males and the liver, uterus, vagina and ovary in female rats were isolated for biochemical and histological investigations. The blood was drawn for haematological and hormonal assays.

4. The levels of blood glucose, tissue glucose and glycogen decreased and with an increase in the levels of lactate and pyruvate in all the organs of both male and female ADX rats than the SO rats at both the days of study i.e., day 15 and day 30. It indicated the diversion of glucose into the glycolytic pathway of cells of the liver and reproductive organs. The lowering of glycogen reserves in response to adrenal hormone insufficiency might be due
to stepped up glycogen break down through glylcogenolysis and glycolysis and/or decreased glycogenesis. Increase in the levels of pyruvate and lactate indicated the activation of energetically less efficient anaerobic glycolysis resulting in the accumulation of lactic acid. The SDH activity exhibited a decrease with an increase in the activities of LDH and G-6-Pase in the organs of ADX rats at both the experimental days in male and female than the SO rats. These shifts suggested suppression of oxidative metabolism and elevation of anaerobic glycolysis in the organs in order to meet the energy requirements after adrenalectomy. The gradual decrease in SDH and elevation in LDH, G-6-Pase activities are resulted in slow accumulation of pyruvate and lactate in the organs of both sex groups. The magnitude of these changes increased with the increase in the duration of adrenalectomy, as well the effect at any day was more in female rats than in males. It indicated a progressive prevalence of anaerobic glycolysis and suppression of oxidative metabolism with the increase in the duration of adrenalectomy; and female rats are more sensitive to adrenalectomy than the males.

5. Changes in protein metabolism can be considered as one of the important diagnostic tools in evaluating the hormonal levels after adrenalectomized animal. Total, soluble and structural proteins decreased in the hepatic and reproductive organs of male and female ADX rats at both the days of experimentation i.e., 15 and 30 days, indicated the decrease in the weight of these organs. It is due to increased protease activity and the
insufficiency of cortisol, which in turn might have increased the free amino acid levels in the hepatic and reproductive organs of male and female ADX rats. The increase in the activity of proteases could be due to the damage caused to lysosomal membranes and also due to cellular destruction and decreased protein synthetic potentials. In addition, the increase in protease activity and amino acid levels indicated the turn over of proteins for metabolic reorganization and for incorporation of amino acids into TCA cycle as evidenced by the increase in AAT and AlAT activities to combat the energy crisis during ADX stress. The degree of proteolysis increased over the duration of experimentation and was also greater in females than in males.

6. The increased activity of GDH in the hepatic and reproductive organs of male and female ADX rats could lead to increased production of glutamate may be to meet the energy demands under ADX stress. The increase in AAT and AlAT activities also favours in greater production of glutamate, which in turn elevates the GDH activity.

7. In protein metabolism, ammonia production and its detoxification are the major events. An elevation was observed in ammonia levels in the hepatic and reproductive organs of both the sexes of ADX rats suggested increased deamination reactions due to tissue proteolysis. It is known that profuse ammonia production also take place through the operation of purine nucleotide cycle. Increased levels of urea suggested the conversion of part of ammonia formed during trans-deamination reaction to less toxic substance by elevating
the urea cycle enzymes. The degree of increase was, however, greater in female ADX rats, probably due to the rapid operation of urea cycle. But, in both the sex groups the production of ammonia as well as its conversion into less toxic urea increased from day 15 to day 30.

8. The decreased level of RBC count, Hb concentration and PCV values in the blood of ADX rats suggested a possible damage to red cells due to ribosomal abnormality and decreased protein synthesis. This results in anaemia in ADX rats; the intensity of it increased with the duration. The low PCV values could be correlated to the decreased RBC production. The red cell indicators like MCV, MCH and MCHC are dependant on the RBC count, Hb concentration and PCV values. A fall in MCV values in both sex groups of the ADX rats may be due to the decrease in red cell count and PCV values. A slight increase in MCH and MCHC values indicated a reciprocal relationship with RBC, Hb and PCV values. In contrast, the increase in total WBC count indicated hypoadrenal crisis or initiation of infection of the tissues; to combat it there is little production of WBC. The degree of decrease or increase was greater in female ADX rats over to male ADX rats which in turn suggested that the female ADX rats were prone to more stress to adrenalectomy resulted in a greater degree of anaemia as supported by lower number of RBC count.

9. The levels of testosterone and estrogen decreased in male and female ADX rats respectively than the SO rats indicated an increase in the levels of ACTH consequent with the decrease in the levels of LH, estrogen and
This gradually resulted in the regression of the reproductive organs thereby a decrease was noticed in their weight. The degree of decrease was greater at day 30 than at day 15 in both the sexes of ADX rats suggested greater increase in the level of ACTH in circulation with the duration of adrenalectomy. In between the two sex groups the percent decrease of testosterone was more at day 15 of male ADX rats than the decrease of estrogen in females, where as an opposite trend was observed at day 30, indicated greater regression of ovarian activity in female ADX rats on prolonged experimentation leading to more suppression of estrogen production.

10. Histological changes in tissues like liver, epididymis, penis and testis in male rats and liver, uterus, vagina and ovary in female rats after adrenalectomy provided support to the shifts in protein metabolism and hormonal levels observed at day 15 and at day 30. The liver of males exhibited a mild degree of cellular damage at day 15, which included destruction of hepatocytes with darkened nuclei. Severe cytoplasmic and nuclear damage was observed at day 30. In the liver of female ADX rats most of the nuclei of hepatocytes faded and shrunken while karyolysis were also observed in few nuclei. The intensity of the above changes was more in female ADX rats than the male ADX rats indicated more protein degradation due to the structural disruption and disintegration by the adrenalectomy.
11. Cessation of spermatogenesis with necrotic spermatogonia, atrophy of leydig cells and the loss of the cellular identity of the germinal epithelium was seen in the seminiferous tubules of testis in ADX rats on day 15. The diameter of the tubules decreased and spermatogenesis was completely arrested at 30 days of ADX rats. Similarly certain pathological changes were observed in epididymis and penis like the decrease in tubular diameter and overlapping of three erectile tissues of penis with irregular arrangement and congestion of areolar tissue. All these changes in the organs of male ADX rats conformed the decrease in their protein levels due to perturbation in hormonal levels and decreased weight in testis, penis and epididymis. Degenerative changes were also observed in female reproductive organs under ADX stress. The ovary exhibited on day 15 the absence of all the stages of developing follicles and on day 30 the breakage of the membrane of oocytes and reduced number of granulosa cells. A mild atrophy was noticed in the cells of all the layers of uterus and clear-cut blood clots were seen in the uterine glands of endometrium with the increase in vacuolization. The vagina exhibited on day 15, less number of leucocytes; and the elastic fibres of muscularis mostly disappeared on day 30 of ADX rats. All these changes confirmed the decreased weight in female reproductive organs and suppression of ovarian steroidogenesis due to decreased ovarian activity after adrenalectomy. On the whole, the impact of adrenalectomy in rats was dependent on the duration of experimentation, specificity of the organ and sex of the animal.
12. The biochemical, haematological, hormonal and histological changes observed in ADX rats revealed that in the patients suffering from Addison's disease there is the possibility of gradual decrease in their weight, suppression of oxidative metabolism, increased proteolysis, ammonia toxicity, decreased RBC count, increased infectivity, suppression of reproductive ability and regression of the structural organization of reproductive organs. These changes may gradually lead to the severe metabolic instability and failure of homeostasis. The intensity of them may increase with the increase in the duration of the disease; and the female individual may become more sensitive to this disease than the males. However, further experimental studies at molecular level are required to arrive definite conclusions.